Prolonging and Boosting NMR Signals with Long-lived States and Catalytic Polarization Transfer

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry in the Graduate School of Duke University

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ABSTRACT

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Abstract

Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) are powerful and versatile tools that are being broadly and intensively used. Their application ranges from molecular structure determination to medical imaging diagnosis. Though NMR and MRI have great advantages such as high chemical environment sensitivity and non-invasive imaging ability with deep penetration, current magnetic resonance (MR) methodologies have fundamental limitations: they mainly use proton as signal source and provide primarily structural information. There are great challenges using MR to obtain metabolic information: for the life supporting elements carbon and nitrogen, their MR non-silent isotopes are in extremely low concentration and the signal they yield is extremely weak under thermal conditions. In addition, their short-lived signal gives us very limited time window to perform complicated experiments. Thus, it is of great significance to obtain long-lived and enhanced NMR and MRI signals for $^{13}$C and $^{15}$N. Doing so would not only augment information for in vitro chemical analysis, but also make it possible for metabolism monitoring, disease early diagnosis and treatment assessment.

This dissertation will first show how we can make the signals long-lived using molecules with specific structures and sophisticated NMR pulses. Then, based on the concept of the long-lived state, it will demonstrate how we can obtain both enhanced and
long-lived signals for $^{13}\text{C}$ and $^{15}\text{N}$ spin-pairs with the development of a novel hyperpolarization technique. This technique is robust, cost-efficient and straight-forward to operate comparing to other existing hyperpolarization methods. Though extra endeavor is needed to develop this hyperpolarization technique towards $\textit{in vivo}$ applications, numerical simulations and experimental results readily show that it can be improved and applied for $\textit{in vitro}$ chemistry analysis.
Dedication

This dissertation is dedicated to my family, for their unconditional love and support.
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1. Introduction

Nuclear magnetic resonance (NMR) has been a powerful and versatile tool for organic chemistry because it provides molecularly specific information. The one-dimensional spectra and two-dimensional correlation spectroscopies can be used for composition analysis, concentration estimation and molecular structure determination [1]. On the other hand, magnetic resonance imaging (MRI), whose signal originates following the same fundamental principles as NMR, has been broadly and intensively used as one of the key biomedical imaging systems to non-invasively provide vital information for medical diagnosis or preclinical researches [2]. However, though NMR and MRI play such important roles in medical care and research, there are critical limitations restricting further applications of these systems. In this chapter, I will briefly introduce the basic principles of NMR and MRI, illustrates their fundamental limitations and outline the contents of this dissertation.

1.1 Overview of NMR and MRI

The signal of NMR originates from the nuclear spin angular momentum, which is an intrinsic property of the atomic nucleus. The nuclear spin angular momentum is quantized and can have integer integral (i.e., 0, 1, 2, ..., such nuclei are bosons) or half-integer integral values (i.e., 1/2, 3/2, 5/2, ..., such nuclei are fermions). For a nucleus with spin angular momentum $S$, there are $2S+1$ sublevels $M$, ranging from $-S$ to $S$. Without the presence of quadrupole coupling, these sublevels are degenerate with no external
magnetic field, i.e., all the sublevel will have the same energy. Application of an external magnetic field will break the degeneracy, splitting these sublevels to different energy levels. This phenomenon is called nuclear Zeeman splitting and the corresponding states are called Zeeman states, which will be more introduced in next chapter. For example, the most widely and intensively used nucleus in NMR and MRI, $^1$H, is a spin-1/2 nucleus. Its spin angular momentum has two sublevels: $M = 1/2$, namely the spin-up or the $\alpha$ state; and $M = -1/2$, namely the spin-down or the $\beta$ state. The “up” and “down” merely mean that the direction of the spin angular momentum, i.e. the spin polarization, is well defined along the magnetic field and it is whether in the same or opposite direction of the field. Since they are well defined, the states are also known as eigenstates. Conventionally, in the Cartesian coordinate system the magnetic field is along the $z$ direction.

Besides the spin angular momentum, the nucleus also has the magnetic moment as an intrinsic property and it is given by:

$$\mu = \gamma S$$  \hspace{1cm} (1.1)\nonumber

where $\mu$ is the magnetic moment and $S$ is the spin angular momentum. Note that here they are expressed as quantum mechanical operators. The gyromagnetic ratio, $\gamma$, is nucleus specific and could have either plus or minus sign, determining whether the magnetic moment will be in the same or opposite direction of the spin polarization direction. Similar as the macroscopic magnetism, the microscopic magnetic moment interacts with the magnetic field $B$ and the magnetic energy is:
The dot product and the negative sign indicate that, if the magnetic moment is aligned with the magnetic field the energy will be the lowest and if they are anti-aligned, the energy will be the highest. So, when a magnetic field is present, for a spin-1/2 nucleus with positive $\gamma$ the spin-up state will have lower energy and the spin-down state will have higher energy. In addition, it is clearly seen from equations 1.1 and 1.2 that, the energy difference is larger for the nuclear Zeeman splitting with higher gyromagnetic ratio and stronger magnetic field.

Figure 1: Nuclear Zeeman splitting of $^{1}\text{H}$, $^{13}\text{C}$ and $^{15}\text{N}$. Since the gyromagnetic ratio of $^{1}\text{H}$ is about 4 times larger than $^{13}\text{C}$ and 10 times larger than $^{15}\text{N}$, the energy differences of the Zeeman splitting are also shown proportionally. Note that the high and low energy states of $^{15}\text{N}$ are inverted contrast to the other two nuclei because it has negative gyromagnetic ratio.

Now let us look at a more classical picture. With the absence of an external magnetic field, for an ensemble of $^{1}\text{H}$ spins (such as a tube of water) at thermal equilibrium the spin polarizations are uniformly distributed (i.e., they point to all possible directions,
note that it is not only the “up” or “down” direction is allowed) so the net magnetic moment is very close to 0. Upon application of a magnetic field, all the spins begin to execute the precession motion around the field under the effect of both the magnetic moment and its spin angular momentum. The frequency of the precession motion is called the nuclear Larmor frequency, which is given by:

\[ \omega_0 = -\gamma B_0 \]  

Besides the applied \( B_0 \) field, each nuclear spin also experiences the microscopic magnetic field coming from other sources, such as the surrounding electrons and its neighboring nuclear spins. If the spins are in different chemical environment, they will experience different local magnetic fields and have different Larmor frequencies. These frequencies are therefore called chemical shift frequencies. In addition, these local fields also rapidly fluctuate both in direction and magnitude because of the thermal motion of all the molecules. Though small, it is the fluctuation that causes the precession motion’s axis to wander around, playing an essential role to generate the NMR or MRI signal: in the long-time term, the spin polarization isotropy will gradually be broken, with more spin polarizations orienting to the same direction as the main field, which has lower magnetic energy, and less orienting to the opposite, which has higher magnetic energy. The population difference is determined by the Boltzmann distribution. The longitudinal net magnetization is hence formed, being the difference between the spin-up and spin-down polarized magnetic moments at thermal equilibrium.
The longitudinal magnetization buildup under thermal conditions is an exponential process, with the exponential time constant called $T_1$ relaxation time. Typically, for $^1$H it is around seconds, for $^{13}$C and $^{15}$N, it could range from tens of seconds to minute. $T_1$ is usually measured using the pulse sequence shown in Fig. 2.

Figure 2: An inversion recovery experiment to measure the $T_1$ relaxation constant. The first $180^\circ$ pulse flips the magnetization to the $-z$ direction. During the waiting time $\tau$, the magnetization will gradually recover and by recording the signal at different time points using the $90^\circ$ pulse, we can have the curve fitting for the $T_1$ relaxation constant. The time between each experiment, which is to allow the $z$ magnetization to fully recover, is usually referred as the repetition time (TR).

To detect this magnetization, a $90^\circ$ pulse rotates the magnetization to the xy plane so it begins to precess around the $z$ axis at the rate of the Larmor frequency. Through Faraday’s law of induction, the precession of the transverse magnetization will induce an oscillating current in the detection coil, generating the signal. Finally, Fourier transform this time domain oscillating signal will give us the NMR spectrum, showing different chemical shift frequencies of nuclear spins in different chemical environments. For MRI,
the principles are the same, except that it requires the sampling of chemical shift frequencies in two or three dimensions by using gradients and performing 2D Fourier transform to get an image, instead of a spectrum.

Similar as the $T_1$ time constant, a $T_2$ constant is used to characterize the transverse magnetization relaxation process, which is also an exponential decay and is caused by the thermal fluctuations of the environment. Due to the fluctuating fields each spin will have slightly different precessional frequency thus the initially highly synchronized precession motions of the spins will gradually lose synchrony, causing decay of the macroscopic transverse magnetization. This decay is irreversible and the spectrum will be broadened due to this exponential decay, so this broadening effect is called homogenous broadening. On the other hand, if the external magnetic field is not homogenous (such as instrument imperfection and applied gradient), the transverse magnetization will lose phase more quickly with a $T_2^*$ relaxation constant, and the spectrum will be more broadened. Such effect is called inhomogeneous broadening and could be refocused by a spin echo.

![Spin echo and gradient echo experiments](image)

**Figure 3:** Spin echo and gradient echo experiments. (A) The spin echo pulse sequence that measures the $T_2$ relaxation constant. If the magnetic field is not homogenous, the transverse magnetization will quickly lose precession synchrony and decay exponentially with time constant $T_2^*$. However, application of a $180^\circ$ pulse will flip the phase of the spin precessions, making them to regain synchrony after the
same amount of time between the 90° and 180° pulses. Recording the echo signal by using different waiting time $\tau$ will yield the $T_2$ relaxation constant. (B) The gradient echo that are often used in MRI to show the $T_2^*$ contrast of different tissues. The region that experiences stronger magnetic field distortion will have faster $T_2^*$ relaxation, thus by using longer gradient time, that specific region will have lower signal intensity. For both spin echo and gradient echo experiments, the time between the excitation pulse and the echo, $2\tau$, is usually referred as echo time (TE).

The relaxation time constants are often tissue specific. For example, in brain the white matter has the shortest $T_1$ time since it is mainly composed of fat, the relatively more rigid structure makes the $^1H$ spin magnetic moment to align with the field more quickly; while the cerebrospinal fluid (CSF), which is mainly composed of water, has the longest $T_1$ because it has more homogenous structure and more freely moving water molecules. Taking advantage of these different relaxation properties different structures of the brain could be highlighted.

![Image](http://fmri.ucsd.edu)

**Figure 4:** [Adapted from http://fmri.ucsd.edu] Brain MRI images using different relaxation contrasts. On the left, the $T_1$ weighted image is acquired using short TR and short spin echo TE time, where only the white matter could fully build up its $z$ magnetization due to its short $T_1$ thus is the brightest. In the middle, the $T_2$ weighted image is acquired using long TR and long spin echo TE time, where the cerebrospinal fluid (CSF) signal relaxes the slowest thus have the brightest region. On
the right, the image is acquired using short TR and long gradient echo TE time, which generates the $T_2^*$ relaxation contrast as previously explained.

### 1.2 Limitations of current systems

Though magnetic resonance (MR) has great advantages over other analysis and imaging methods, there are fundamental limitations for most of the current systems, severely restricting its further applications.

The limitations are mainly concerned with two basic properties of the MR signal: the signal intensity and its lifetime. As introduced in last section, the signal strength depends on the polarization difference of the sample spins, i.e., the population difference of the two eigenstates. Based on the Boltzmann distribution, the population difference is determined by the energy difference. For spin-1/2 nuclei, the energy levels and their difference of the Zeeman splitting are:

\[
E_\alpha = \frac{1}{2} \hbar \omega_0 = -\frac{1}{2} \hbar \gamma B_0 \\
E_\beta = -\frac{1}{2} \hbar \omega_0 = \frac{1}{2} \hbar \gamma B_0 \\
\Delta E = |\hbar \omega_0| = \hbar \gamma B_0
\]  

(1.5a)

At a magnetic field of 8.45 T, that would be around $2.4 \times 10^{-25}$ J, which is about 4 orders smaller than the available thermal energy at room temperature ($k_B T$ is around $4.1 \times 10^{-21}$ J), so the population difference is quite small at thermal equilibrium. Furthermore, the ratio between the difference and the ensemble population, which is proportional to the net magnetization, is:
\[ M \propto \frac{N_\alpha - N_\beta}{N_\alpha + N_\beta} = \tanh \left( \frac{\hbar \gamma B_0}{2k_B T} \right) \approx \frac{\hbar \gamma B_0}{2k_B T} \] (1.5b)

Plug in the numbers the ratio is \( \sim 3 \times 10^{-5} \), this extremely small number means that only 3 out of \( 10^5 \) atomic nuclei contribute to the MR signal.

Though the ratio is small, for \(^1\text{H}\), the signal is not too weak because \(^1\text{H}\) possesses huge advantages that other nuclei do not have: it has much higher gyromagnetic ratio than other spins, which is about 4 times larger than \(^{13}\text{C}\) and 10 times larger than \(^{15}\text{N}\). On the other hand, it has large natural abundance, which is nearly 100\%, meaning that almost all the natural hydrogen atoms are the magnetic sensitive isotope \(^1\text{H}\). However, the natural abundance of \(^{13}\text{C}\) and \(^{15}\text{N}\) are much lower and they are only about 1.1\% and 0.37\%.

Though for NMR experiments we can specifically enrich the \(^{13}\text{C}\) or \(^{15}\text{N}\) isotope to obtain spectra with decent signal, the enrichment is extremely expensive and is not always available for our target molecules. Based on these reasons, most of the MR spectra and nearly all the imaging methods only use \(^1\text{H}\), yielding mainly \(^1\text{H}\) based chemical information or showing primarily structural images of the body. As fundamental elements of the earth and composing life, to obtain information of \(^{13}\text{C}\) and \(^{15}\text{N}\) from their special chemical environments or perform functional imaging using these elements, such as metabolism monitoring or drug effect assessment, is challenging.

On the other hand, the short-lived lifetime of MR signal puts another limitation for it to be further applied. As mentioned, the signal lifetime is determined by the \( T_1 \) and \( T_2 \) time constants, which are usually on the order of seconds or up to minute for \(^{13}\text{C}\) and
$^{15}$N. With such short lifetimes, it is difficult to use MR for the longer-time scale applications, such as chemical reaction monitoring and metabolism tracking as mentioned. So, besides the signal enhancement, to prolong the signal lifetime is also of great significance.

1.3 Overview of this dissertation

This dissertation is focused to resolve these two limitations and advance MR for more extensive applications. To enhance the signal, the basic route is to increase spin polarization difference and such methods are in general called “hyperpolarization” [3]. Based on equation 1.5b, there are already several approaches, such as using stronger magnetic field, or cooling the sample to lower temperature. These methods are to increase the Zeeman splitting energy difference thus increase the population difference, though effective, they are not practical and the effect is limited. I will show that how we could achieve hyperpolarization using other more practical and more efficient methods.
Figure 5: [From Ref. 3] Concept of hyperpolarization. At thermal equilibrium (left), the population difference is minuscule so the generated signal is weak and noisy. Hyperpolarization (right) is to change the population distribution and by enlarging their difference we can obtain much stronger and sharper MR signal. Note that the energy levels do not change and the population distribution could also be reverted.

To prolong the signal lifetime, there has been quite a few researches using the singlet state [4], which is immune to the magnetic dipole interaction so the signal “stored” in this state could achieve much longer lifetime. During our research, we found that the singlet state was closely related with the hyperpolarization method we were developing. This is quite exciting because we can have both significantly enhanced and prolonged signal at the same time by simply performing the hyperpolarization procedure.

Therefore, in next chapter and chapter 3, I will give further background introduction and discussion about the singlet state, which lies the foundation for this dissertation. Specifically, I will demonstrate an approach to prolong the $^1$H signal life time using a method we have extended.
In chapters 4, 5 and 6, I will mainly discuss about the work I have done or involved using the novel hyperpolarization method, with different experimental data sets. Particularly, I will demonstrate the very recent advances we have achieved for the novel hyperpolarization method.

In chapter 7, I will show some potential applications for this method, such as applying our hyperpolarization method for correlation spectroscopy and bio-molecule structure monitoring. Finally, chapter 8 is the conclusion of this dissertation.
2. Background

In this chapter, I will introduce several basic concepts closely related to my research of prolonging and boosting NMR signals. These concepts also lay the foundation for the modern NMR researches, which have high potential to be developed towards preclinical and clinical MRI studies and applications.

2.1 Zeeman and singlet-triplet states

One fundamental concept that will be used in the entire dissertation is the singlet and triplet state basis, or singlet and triplet states. In this section I will start from the conventional Zeeman basis and eigenstates, then explain the concept of the singlet-triplet basis.

2.1.1 Zeeman eigenstates

For a certain nucleus, the spin angular momentum operators, such as $\hat{I}_x$, $\hat{I}_y$ and $\hat{I}_z$, represent the nuclear spin angular momentum components along the corresponding Cartesian axes. Traditionally, take the $\hat{I}_z$ operator (which is the direction of the main magnetic field), it is shown that the eigenstates of $\hat{I}_z$ could be specified by two quantum numbers: $S$, the total quantum number, which defines the total square angular momentum; and $M$, the azimuthal or the $z$ quantum number, which defines the angular momentum along the $z$ axis. As mentioned in last chapter the $z$ quantum number ranges
from -S to S, with the only step of 1 (i.e., $M = -S, -S+1, \ldots, S-1, S$). These eigenstates of $\hat{I}_z$, written as $|S,M\rangle$, are called Zeeman eigenstates and they obey the eigenequation:

$$\hat{I}_z |S,M\rangle = M |S,M\rangle$$ (2.1)

The Zeeman states are orthogonal to each other and form an eigenbasis, namely the Zeeman basis, which can be used as the basis for matrix representation. Particularly for a spin-1/2 nucleus, it has two Zeeman eigenstates, $|\alpha\rangle$ and $|\beta\rangle$, and the eigenequations are:

$$|\alpha\rangle = \left| \frac{1}{2}, \frac{1}{2} \right\rangle, \quad |\beta\rangle = \left| \frac{1}{2}, -\frac{1}{2} \right\rangle$$

$$\hat{I}_z |\alpha\rangle = \frac{1}{2} |\alpha\rangle, \quad \hat{I}_z |\beta\rangle = -\frac{1}{2} |\beta\rangle$$ (2.2)

Using the Zeeman basis $\{|\alpha\rangle, |\beta\rangle\}$, based on equation 2.2 and the orthonormality condition, the four matrix elements of $\hat{I}_z$ operator are:

$$\langle \alpha | \hat{I}_z | \alpha \rangle = \frac{1}{2}, \quad \langle \alpha | \hat{I}_z | \beta \rangle = 0, \quad \langle \beta | \hat{I}_z | \alpha \rangle = 0, \quad \langle \beta | \hat{I}_z | \beta \rangle = -\frac{1}{2}$$

On the other hand, since the raising and lowering operators are defined as [5]:

$$\hat{I}_+ = \hat{I}_x + i\hat{I}_y, \quad \hat{I}_- = \hat{I}_x - i\hat{I}_y$$

We could have:

$$\hat{I}_x = (\hat{I}_+ + \hat{I}_-)/2, \quad \hat{I}_y = (\hat{I}_+ - \hat{I}_-)/2i$$

Similarly, the matrix elements of $\hat{I}_x$ and $\hat{I}_y$ operators are:
\[
\langle \alpha | \hat{I}_z | \alpha \rangle = \langle \alpha | \frac{\hat{I}_x + \hat{I}_y}{2} | \alpha \rangle = 0, \quad \langle \alpha | \hat{I}_z | \beta \rangle = \frac{1}{2}, \quad \langle \beta | \hat{I}_z | \alpha \rangle = \frac{1}{2}, \quad \langle \beta | \hat{I}_z | \beta \rangle = 0
\]

\[
\langle \alpha | \hat{I}_z | \alpha \rangle = \langle \alpha | \frac{\hat{I}_x - \hat{I}_y}{2i} | \alpha \rangle = 0, \quad \langle \alpha | \hat{I}_z | \beta \rangle = \frac{1}{2i}, \quad \langle \beta | \hat{I}_z | \alpha \rangle = -\frac{1}{2i}, \quad \langle \beta | \hat{I}_z | \beta \rangle = 0
\]

Written in matrix, they are:

\[
\begin{pmatrix}
\hat{I}_x &=& \frac{1}{2} \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}, \\
\hat{I}_y &=& \frac{1}{2i} \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix}, \\
\hat{I}_z &=& \frac{1}{2} \begin{pmatrix} 1 & 0 \\ 0 & -1 \end{pmatrix}
\end{pmatrix} \tag{2.3}
\]

Now consider a pair of coupled spin-1/2 nuclei, spin 1 and 2. Since there are two states for each spin, the coupled spins will have 4 different Zeeman product states, they are \(|\alpha\alpha\rangle\), \(|\alpha\beta\rangle\), \(|\beta\alpha\rangle\) and \(|\beta\beta\rangle\). Suppose the gyromagnetic ratio, \(\gamma\), of the two spins are positive. If spin 1 has smaller chemical shift \((\delta_1 < \delta_2)\), it will have larger chemically shifted Larmor frequency \((\omega_1 > \omega_2, \text{ since } \omega = -\gamma B_0 \delta)\), thus the energy levels of the four Zeeman product states from low to high are \(|\alpha\alpha\rangle\), \(|\alpha\beta\rangle\), \(|\beta\alpha\rangle\), \(|\beta\beta\rangle\), as shown in Fig. 6.

![Figure 6: Zeeman product states and their energy level differences, without considering the J-coupling between the two spins. Energy difference between the outer two states is the sum of the Larmor frequencies of the two spins, while the energy difference between the inner two states is the difference between the two spins’ Larmor frequencies. In addition, the transition energy between \(|\alpha\alpha\rangle\) and \(|\beta\alpha\rangle\) is the same as between \(|\alpha\beta\rangle\) and \(|\beta\beta\rangle\), which is proportional to \(\omega_1\); the transition](image)

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Energy between $|\alpha\alpha\rangle$ and $|\alpha\beta\rangle$ is the same as between $|\beta\alpha\rangle$ and $|\beta\beta\rangle$, which is proportional to $\omega$. 

Energy levels of the Zeeman product states could also be seen from the Hamiltonian. For the coupled spin-1/2 nuclei, consider about the chemical shift, $J$-coupling ($J_{12}$) and secular dipole-dipole coupling ($d_{12}$) interactions, the Hamiltonian of the spin system in Zeeman basis is:

$$H_{\text{Zeeman}} = \omega_1 \hat{I}_{1z} + \omega_2 \hat{I}_{2z} + 2\pi J_{12} \hat{I}_1 \cdot \hat{I}_2 + d_{12} \left(3\hat{I}_{1z} \hat{I}_{2z} - \hat{I}_1 \cdot \hat{I}_2\right)$$

$$= \frac{1}{2} \begin{pmatrix}
\omega_1 + \omega_2 + \pi J_{12} + d_{12} & 0 & 0 \\
0 & \Delta\omega - \pi J_{12} - d_{12} & 2\pi J_{12} - d_{12} \\
0 & 2\pi J_{12} - d_{12} & -\Delta\omega - \pi J_{12} - d_{12} \\
0 & 0 & -\omega_1 - \omega_2 + \pi J_{12} + d_{12}
\end{pmatrix}$$

(2.4)

where $\Delta\omega = \omega_1 - \omega_2$.

This matrix is not diagonal, which means that the Zeeman product states are not eigenstates of the Hamiltonian. However, if the chemical shift difference of the two spins is much larger than the $J$-coupling and the dipole-dipole coupling ($\Delta\omega \gg \pi J_{12} - d_{12}/2$), the two spins are addressed as weakly coupled (usually in isotropic liquids the intramolecular dipole-dipole coupling will average to 0, thus $d_{12}=0$). Based on the secular approximation, the off-diagonal elements could be ignored and the Zeeman product states can be considered as eigenstates of the Hamiltonian. This is usually the case for heteronuclear spins or homonuclear spins of asymmetric molecules at high magnetic field.
2.1.2 Singlet-triplet states

Although Zeeman product states could be considered as eigenstates for weakly coupled spins, however, when the chemical shift difference is comparable to the $J$-coupling ($0 < \Delta \omega \leq J_{12}$, near equivalent spins), or is equal to 0 ($\Delta \omega = 0$, equivalent spins), secular approximation is not valid and the off-diagonal elements cannot be discarded. The spins are called strongly coupled and the Zeeman product states are no longer eigenstates. To find the eigenstates of the Hamiltonian, first we may rewrite equation 2.4 by defining $\omega_1 = \omega_2 = \omega_0$, i.e., the two spins are chemically equivalent, then the matrix expression is:

$$H_{\text{Zeeman}} = \frac{1}{2} \begin{pmatrix} 2\omega_0 + \pi J_{12} + d_{12} & 0 & 0 & 0 \\ 0 & -\pi J_{12} - d_{12} & 2\pi J_{12} - d_{12} & 0 \\ 0 & 2\pi J_{12} - d_{12} & -\pi J_{12} - d_{12} & 0 \\ 0 & 0 & 0 & -2\omega_0 + \pi J_{12} + d_{12} \end{pmatrix} \quad (2.5)$$

By looking at the normalized eigenvectors, we can find the diagonalizing basis for equation 2.5, and the basis is:

$$ZeemanST = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 1/\sqrt{2} & 0 & 1/\sqrt{2} \\ 0 & 1/\sqrt{2} & 0 & -1/\sqrt{2} \\ 0 & 0 & 1 & 0 \end{pmatrix} \quad (2.6)$$

Columns of the diagonalizing basis (equation 2.6) indicate the new eigenstates, which are combinations of the original Zeeman product states. They are:
\[ |T_{+1}\rangle = |\alpha \alpha\rangle \]
\[ |T_{0}\rangle = \left( |\alpha \beta\rangle + |\beta \alpha\rangle \right)/\sqrt{2} \]
\[ |T_{-1}\rangle = |\beta \beta\rangle \]
\[ |S\rangle = \left( |\alpha \beta\rangle - |\beta \alpha\rangle \right)/\sqrt{2} \tag{2.7} \]

The first three states are like the states of a spin-1 nucleus: the total angular momentum quantum number is 1 and it has three \( z \) quantum numbers ranging from -1 to 1. Together they are called the “triplet states”. The rest has only one state with total angular momentum equals 0 and is called the “singlet state”. Since the singlet state has no net spin angular momentum, it is magnetically silent and gives no NMR signal (similar as \(^{12}\)C which is NMR silent). Thus, for a pair of magnetically equivalent spins, only the (-1)-coherences inside the triplet manifold give rise to NMR signal.

Using equation 2.7 as the diagonalizing basis, the original Hamiltonian, \( H_{\text{Zeeman}} \), can be transformed to:

\[
H_{ST} = \text{Zeeman}_{ST}^{-1}.H_{\text{Zeeman}}.\text{Zeeman}_{ST}
\]

\[
= \frac{1}{2} \begin{pmatrix}
2\omega_0 + \pi J_{12} + d_{12} & 0 & 0 & 0 \\
0 & \pi J_{12} - 2d_{12} & 0 & 0 \\
0 & 0 & -2\omega_0 + \pi J_{12} + d_{12} & 0 \\
0 & 0 & 0 & -3\pi J_{12}
\end{pmatrix} \tag{2.8}
\]

From both the eigenstates and the diagonalized Hamiltonian, we can see that the singlet state has fundamentally different properties from the other states. First, it is antisymmetric in terms of interchanging the two spins \( (|S_{(0)(2)}\rangle = -|S_{(2)(0)}\rangle) \) while other states are symmetric \( (|T_{(0)(2)}\rangle = |T_{(2)(0)}\rangle) \). The interconversion between singlet and triplet states are
therefore forbidden thus for the ensemble due to intermolecular interactions, this interconversion is usually extremely slow. Second, the antisymmetric property also makes the singlet state “immune” to dipole-dipole interactions, which could also be seen from the last entry of equation 2.8. Diagonal elements of the Hamiltonian determine the energy levels of each state. While the dipole-dipole coupling affects the energy of the triplet states, it does not affect the singlet state. The singlet state is not affected by the intramolecular dipole-dipole interaction, which is a strong relaxation mechanism limiting the $T_1$ lifetime of NMR signals. Thus, the special properties of the singlet state make it a great “agent” to store MR signals, which could have a much longer lifetime than $T_1$. As will be introduced later, para-hydrogen, a rotational spin isomer of hydrogen, is indeed such an agent in pure singlet state.

Although the singlet state has such great property to store NMR signals and make them long-lived, at the same time it is challenging to access this state because it is magnetically silent, no RF pulses could directly convert population into the singlet state. One immediate solution is to refer to the chemical inequivalence. The singlet-triplet eigenstates are found by defining that chemical shifts of the two spins are the same. However, taking one step back, if the two spins are chemically inequivalent and we still perform the transformation using the same singlet-triplet basis, an extra term of $\Delta \omega = \omega_1 - \omega_2$ will rise at off-diagonal in the transformed Hamiltonian, that is:
From equation 2.9 we can see that, the previously “disconnected” singlet state now suddenly is “connected” with the $|T_0\rangle$ state. This connection gives a very convenient tool to access the singlet state: using the chemical shift difference $\Delta \omega$ we can now load population into the singlet state from the triplet by RF pulses. Although now the Hamiltonian is not diagonal and the singlet state is not a “true” eigenstate, given that the chemical shift difference is small (i.e., the two spins are nearly equivalent), the singlet state could still be approximated as an eigenstate and it is well preserved. As discussed in detail in later chapters, there are numerous methods to access the singlet spin order and the singlet lifetime could be orders longer than $T_1$, which yields exciting potential to use NMR or MRI for longer time-scale purposes.

In addition, another useful way to view the singlet state is to look at the density operator, which is:

$$\rho_s = |S\rangle \langle S|$$

$$= \frac{1}{2} (|\alpha\beta\rangle - |\beta\alpha\rangle)(\langle \alpha\beta| - \langle \beta\alpha|)$$

$$= \frac{1}{2} (|\alpha\beta\rangle \langle \alpha\beta| - |\alpha\beta\rangle \langle \beta\alpha| - |\beta\alpha\rangle \langle \alpha\beta| + |\beta\alpha\rangle \langle \beta\alpha|)$$

$$= \frac{1}{2} (I_{1}^{a}I_{2}^{\beta} - I_{1}^{a}I_{2}^{\beta} - I_{1}^{\beta}I_{2}^{-} + I_{1}^{\beta}I_{2}^{a})$$

(2.10)
The first and the fourth terms in the bracket are the projection operators of $|\alpha\rangle$ and $|\beta\rangle$, the second and third terms are called zero-quantum coherences (ZQ) because the total coherence order is 0 by rising the $z$ quantum number of the first spin and at the same time lowering the second one or vice versa. The ZQ part is essentially the sum of $x$ and $y$ angular momentum product operators, which is:

$$ZQ = 2\left(I_{1x}I_{2x} + I_{1y}I_{2y}\right)$$  \hspace{1cm} (2.11)

Expressed in spin angular momentum operators, the density operator can be further written as:

$$\rho_s = \frac{1}{2}\left(\frac{1}{2}E - 2I_{1z}I_{2z} - ZQ\right)$$  \hspace{1cm} (2.12)

$$= -I_1 \cdot I_2 + \frac{1}{4}E$$

where $E$ is the identity matrix. So, population of the singlet state exists in the total angular momentum product of the two spins. On the other hand, the density operator of the middle triplet state is:

$$\rho_{\alpha\beta} = |T_0\rangle\langle T_0|$$

$$= \frac{1}{2}\left(|\alpha\beta\rangle + |\beta\alpha\rangle\right)(\langle \alpha\beta\rangle + \langle \beta\alpha\rangle)$$

$$= \frac{1}{2}\left(I_{1}^{\alpha}I_{2}^{\beta} + ZQ + I_{1}^{\beta}I_{2}^{\alpha}\right)$$  \hspace{1cm} (2.13)

$$= \frac{1}{2}\left(\frac{1}{2}E - 2I_{1z}I_{2z} + ZQ\right)$$

Equation 2.12 and 2.13 are similar except the sign of the ZQ term, so the zero-quantum coherence can be expressed as:
From equation 2.14 we can see that the zero-quantum coherences, which can be created by RF pulses, contains the singlet population thus have the potential to access to it. This will be discussed in further detail in the next chapter.

2.2 Para-hydrogen and para-hydrogen induced polarization (PHIP)

Having introduced the singlet-triplet states, one important hyperpolarization method, para-hydrogen induced polarization, or PHIP, needs to be particularly discussed in this section because it uses para-hydrogen, which is exactly the singlet spin order of hydrogen nuclei, to achieve the goal of hyperpolarization. Since the concept of singlet spin order and hyperpolarization is closely related with each other in this method, a clear understanding of para-hydrogen and PHIP will lead to better perception of the following chapters.

2.2.1 Concept of para-hydrogen

Protons are half-integer spins (spin-1/2) so they are fermions. Based on Pauli principle, the total wavefunction (including motional and spin wavefunctions) of an assemble of particles must be antisymmetric (change sign) under the position interchange of any pair of identical fermions. The total wavefunction of the diatomic hydrogen is:

\[ \psi_{total} = \psi_E \cdot \psi_V \cdot \psi_T \cdot \psi_R \cdot \psi_N \]  

(2.15)
where E, V, T, R represent the motional modes of electronic, vibrational, translational and rotational wavefunctions respectively, and N represent the nuclear spin wavefunction. Imagine the molecule is rotated by π, which interchanges the position of the two protons. However, this rotation at the same time has several other effects as well. Translational and vibrational wavefunctions are straightforward to analyze. Since the translational motion could be completely unconfined and the rotation will not alter the motional direction, ψ_T could be considered symmetric for all cases. The ground vibrational state (\( v = 0 \)) accommodates for majority of molecules at normal temperature, recalling the harmonic oscillator solutions, then the vibrational wavefunction \( ψ_V \) is also symmetric under that rotation. The symmetry of the electronic motional wavefunction (i.e., only the spatial wavefunction of electrons are considered here), \( ψ_E \), needs more analysis. Since both the electrons occupy the 1σ_g (the subscript g means the overall parity of the orbital is even) orbital for the ground state hydrogen (\( ^1Σ^+ \)), such electron configuration gives both inversion and reflection symmetry. Thus, although the electron spatial wavefunction is rotated by π, an inversion followed by a reflection operation about the plane that contains the internuclear axis yields the original wavefunction, i.e., the π rotation does not change \( ψ_E \) and it is symmetric under the rotation. Now, only \( ψ_R \) and \( ψ_N \) are left to be discussed.

For the rotational wavefunction \( ψ_R \), conclusion could be drawn from the spherical harmonics \( Y_{lm} \). Interchange the two protons, or equivalently, the π rotation, is to convert
the polar coordinates \((\theta, \varphi)\) of the spherical harmonics to \((\pi - \theta, \varphi + \pi)\). Plug in the new coordinates we will find that rotational wavefunctions with even rotational quantum numbers (including 0) will be symmetric, while those with odd numbers will be antisymmetric. More concisely, interchange the two protons has an effect of multiplying \(\psi_R\) by \((-1)^J\), where \(J\) is the quantum number of the current rotational state. The parity of the rotational wavefunction leads to the symmetry requirement for the last dimension, the nuclear spin states \(\psi_N\). We already know that there are four states for the paired nuclei, namely the three triplet states with total spin angular momentum of 1, and one singlet state with 0 total spin angular momentum as discussed in last section. In terms of interchange the nuclei, the triplet states are symmetric and the singlet state is antisymmetric. Thus, to satisfy Pauli principle, the total wavefunction \(\psi_{\text{total}}\) has two allowed combinations and the diatomic hydrogen gas has two species: one with even rotational states \((J = 0, 2, 4, \ldots)\) and antisymmetric singlet nuclear spin states, is called “\(\text{para-}\)hydrogen”; the other with odd rotational states \((J = 1, 3, 5, \ldots)\) and symmetric triplet nuclear spin states, is called “\(\text{ortho-}\)hydrogen”. Para- and \(\text{ortho-}\)hydrogen are usually called spin-rotation isomers, or spin isomers for short.

Since there are three triplet states and one singlet state, at thermal equilibrium and high temperature (e.g., room temperature), we would expect \(\text{ortho-}\)hydrogen to have a 3 times larger population than \(\text{para-}\)hydrogen in a hydrogen gas sample. In other words, all four spin states would have equal population of 25%. Particularly, concentration of \(\text{para-}\)
hydrogen as function of temperature could be calculated using the partition function, and

the population ratio between \textit{para}- and \textit{ortho}-hydrogen is [6]:

\[
\frac{N_{\text{para}}}{N_{\text{ortho}}} = \frac{\sum_{J=\text{even}} (2J+1) \exp\left[-J(J+1)\frac{\theta_R}{T}\right]}{3 \sum_{J=\text{odd}} (2J+1) \exp\left[-J(J+1)\frac{\theta_R}{T}\right]}
\]

\[
= \frac{1 + 5 \exp\left(-6\frac{\theta_R}{T}\right) + 9 \exp\left(-20\frac{\theta_R}{T}\right) + \ldots}{3\left[3 \exp\left(-2\frac{\theta_R}{T}\right) + 7 \exp\left(-12\frac{\theta_R}{T}\right) + \ldots\right]}
\]

where \( J \) is the rotational quantum number, \( \theta_R \) is the ratio between rotational constant \( B \) and Planck constant \( k_B \) (for dihydrogen, \( \theta_R \approx 87.6 \) K so the rotational energy difference between \( J = 0 \) and 1 states is \( 2B \approx 175 \) \( k_B T \), which is huge and provides easy access separating the states). Now it is clear that under high temperature approximation, at room temperature (e.g., 300 K) \textit{para}-hydrogen will compose ~25\% for a hydrogen gas sample. Concentration of \textit{para}-hydrogen with temperature ranging from 20 to 400 K is plotted in Fig.7.
Figure 7: Para-hydrogen concentration in dihydrogen sample as function of temperature. The labeled points are typical temperature points with corresponding para-H\(_2\) percentage. At boiling point of dihydrogen, almost all hydrogen is in para form. Liquid N\(_2\) temperature (77 K) gives around 50% para-hydrogen, and at room temperature para-hydrogen consists about 25% of hydrogen. The hyperpolarization experiments done in our lab usually uses 92% enriched para-H\(_2\), which is generated at 38 K.

Though para-hydrogen can be easily enriched due to the large energy difference between the rotational states, converting ortho-hydrogen to para-hydrogen could be quite slow due to their symmetry difference as mentioned in last section. Nonetheless, the long waiting time could be circumvented by introducing paramagnetic catalyst, such as iron oxide (rust); or just using charcoal. Using rust or charcoal, the nuclear spin can be randomly oriented, thus under low temperature favoring the anti-alignment of the nuclear spins and enriching para-hydrogen.
2.2.2 PASADENA and ALTADENA

With the concept of para-hydrogen, now I would like to introduce para-hydrogen induced polarization (PHIP), which takes advantage of the pure state of such spin isomer. The general process of PHIP is that with hydrogenation catalyst, para-hydrogen will be added to the double or triple bond of the target molecules, which breaks the symmetry of para-hydrogen and generates greatly amplified signal from the added protons. PHIP was first introduced as the term PASADENA (para-hydrogen and synthesis allow dramatically enhanced nuclear alignment) by Bowers and Weitekamp [7]. Later, another form of hyperpolarization, ALTADENA (adiabatic longitudinal transport after dissociation engenders net alignment) was introduced by Pravica and Weitekamp [8]. Since both methods involve using para-hydrogen and hydrogenation of the target molecule, they are both categorized as PHIP in general.

Processes of PASADENA and ALTADENA are very similar, the key difference is the magnetic field where hydrogenation happens for the target molecule using para-hydrogen. For PASADENA hydrogenation occurs inside the detecting magnet, which usually has strong magnetic field (the original experiment was done in a 4.7 T magnet). While for ALTADENA, hydrogenation happens outside the magnet and the sample is then adiabatically transferred into the magnet for signal detection. Magnetic field for hydrogenation is the earth field, which is ~50 μT, much smaller than the magnet. The difference of hydrogenation fields yields significantly different hyperpolarization result:
in PASANEDA, the amplified signals are antiphase for each added $^1$H spin, and the net alignment would be 0 at each chemical shift site; in ALTADENA, each $^1$H has only one line in the spectrum and the net alignment is not 0.

Difference of PASADENA and ALTADENA could be explained by how the para-hydrogen magnetic equivalence is broken in different cases. Para-hydrogen is the pure singlet state of $^1$H pair, which is $|S⟩ = (|αβ⟩ − |βα⟩)/\sqrt{2}$. For PASADENA scenario, since hydrogenation happens at high magnetic field, once the para-hydrogen is added to the target, usually because of the asymmetric structure of the product molecule, magnetic equivalence of the $^1$H spin pair from para-hydrogen is immediately broken and the two protons have large chemical shift difference. As introduced in last section, under such condition the protons are weakly coupled and singlet-triplet states are not eigenstates of their Hamiltonian, however the Zeeman product states could be considered as eigenstates. Thus, the original population of the singlet spin order could be treated as equally occupied populations in $|αβ⟩$ and $|βα⟩$ states, which are both significantly higher than those in thermal equilibrium. Transitions from these two over-populated states to $|αα⟩$ and $|ββ⟩$ states will occur, leading to hyperpolarized antiphase lines at each $^1$H chemical shift position.

On the other hand, for ALTADENA scenario, the hydrogenation happens at low magnetic field. Though the product molecule may have asymmetric structure, because of the low magnetic field the two protons fall into the nearly equivalent regime and the
singlet-triplet states could still be approximated as the eigenstates. Adiabatically transfer of the sample to high field will transfer the singlet eigenstate to the Zeeman product eigenstate with energy continuity, i.e., only the state with lower energy related with the singlet state will be over-populated. In the case $\omega_1 > \omega_2$, $|\alpha\beta\rangle$ will have lower energy and only this state will be over-populated, thus in the spectrum there will be only two lines representing the population transitions for each individual nucleus. If $\omega_1 < \omega_2$, then $|\beta\alpha\rangle$ will be the only over-populated state and the spectrum is $180^\circ$ inverted. The spectra of different situations are illustrated in Fig.8 [9].

Figure 8: [From Ref. 9] Spectra of two coupled spins of different PHIP situations. (A) The standard NMR spectrum acquired at thermal equilibrium, in-phase doublet of doublet is shown, with the splitting of $J$-coupling between 1, 2 and 3, 4. (B) Spectrum of PASADENA. Since the middle two states are over-populated, coherences 1 and 3 switch directions and their lines in the spectrum are inverted by $180^\circ$, the net alignment is 0. (C) Spectrum of ALTADENA, only coherences 2 and 3 are
significantly amplified since only $|\alpha\beta\rangle$ is over-populated. Again, line 3 is inverted by $180^\circ$.

To better understand the ALTADENA process, energy levels and population of different states at different stages are shown in Fig. 9.

Figure 9: [From Ref. 8] Energy levels and population transfer during ALTADENA. (A) Para-hydrogen stage. With no magnetic field, the three triplet states are degenerate, while the singlet state of para-hydrogen is $175 \text{k}_B T$ lower. (B) Hydrogenation stage. Once the hydrogenation finishes at low field, because of the small chemical shift difference of the two protons, the triplet states are no longer degenerate. However, since they are strongly coupled, the singlet and triplet states are close to the eigenstates. (C) Adiabatically transfer the sample to high field for detection. (D) Detection stage. Since the transfer is slowly enough, the large population of the original singlet state will follow the energy path to the $|\alpha\beta\rangle$ state, generating the spectrum shown in Fig. 8.

In the initial para-hydrogen preparation stage, hydrogen is highly populated in the singlet state and the three triplet states are energy degenerated with no magnetic field. Note that the energy gap between singlet and triplet states is determined from the rotational state...
energy as mentioned earlier. Then for the hydrogenation stage, para-hydrogen is added to the target molecule and the magnetic field is still weak. Because of the magnetic field the triplet states are not energy degenerate. However, since the field is extremely small the singlet-triplet states could still be considered as eigenstates. Furthermore, since the rotational state is irrelevant after hydrogenation, the energy gap between singlet and triplet states is determined by the $J$-coupling of the added protons instead of the rotational constant. As the magnetic field adiabatically increases, the energy gap between the $|T_-\rangle$ and $|T_+\rangle$ states increases much faster ($\propto \Sigma \omega_{12}$) than that between the $|T_0\rangle$ and $|S\rangle$ states ($\propto \Delta \omega_{12}$). Because of energy continuity, population of the singlet state will be transferred to the approximated singlet state. Finally, for the detection stage, magnetic field increases to the maximum (inside the magnet) and Zeeman product states are the eigenstates. The large singlet population is only preserved in $|\alpha \beta\rangle$ state and it will yield antiphase alignment, as shown in Fig.8.

Besides the energy level diagram, again we can look at the density operators. As shown in equation 2.12, density operator of the singlet state of para-hydrogen can be expressed as the total angular momentum product $I_1 \cdot I_2$. Similarly, if we express the density operator of $|\alpha \beta\rangle$ (or $|\beta \alpha\rangle$, which is similar with only sign difference), that is:
\[ \rho_{\alpha\beta} = |\alpha\beta\rangle\langle\alpha\beta| = I_{1\alpha}I_{2\beta} = \left( \frac{1}{2} E + I_{1z} \right) \left( \frac{1}{2} E - I_{2z} \right) = \frac{1}{4} E + \frac{1}{2} I_{1z} - \frac{1}{2} I_{2z} - I_{1z}I_{2z} \]  

(2.17)

From this density operator, we can also see why ALTADENA gives spectrum in Fig. 7. Since population of the singlet state is preserved only in the \( |\alpha\beta\rangle \) state following energy continuity, after the sample is adiabatically transferred into the magnet, the density operator is transferred from \( I_{1} \cdot I_{2} \) to \( I_{1z} - I_{2z} \) and this gives one line for each spin with 180° phase difference.

Interestingly, this \( I_{1} \cdot I_{2} \) to \( I_{1z} - I_{2z} \) transition gives another way for accessing the singlet state and this involves adiabatically switching the magnetic field. The process can be viewed as an inverse process of ALTADENA. We can flip the polarization of one spin by RF pulses in the magnet to prepare \( I_{1z} - I_{2z} \), then by adiabatically lowering the magnetic field for the sample (shuttling the sample out of the magnet), following the same route \( I_{1z} - I_{2z} \) will be transferred to \( I_{1} \cdot I_{2} \), which contains the singlet population as shown in equation 2.12. This singlet accessing method will also be discussed in detail in next chapter.

Having reviewed the concepts of singlet-triplet states, para- and ortho-hydrogen, we can see that there is close relationship between these concepts and hyperpolarization. My research is closely related with these concepts and methods, and in future chapters I
will show how we can combine singlet together with hyperpolarization and expand the research and application to broader dimensions.

2.3 Other hyperpolarization methods

Though PHIP could achieve good polarization results, major drawbacks exist. First is the requirement of unsaturated bond for hydrogenation and consequently the alteration of the hyperpolarized substrates. Since the hydrogenation is hard to control in vivo, this also makes it unpractical for broader biomedical MR imaging applications except for lung imaging, which uses hyperpolarized ethylene or propane generated from PHIP. Besides PHIP, several other methods exist to achieve high nuclear spin polarization levels. These methods are usually categorized as two types: one is within the nuclear thermal polarization range, such as using higher magnetic field (also known as brute force polarization), or using specific RF pulse sequences to elevate heteronuclear spin polarization. These pulse sequences include INEPT [1], [10], [11], DEPT [1], [12], which transfer higher polarization of nuclear spins with higher gyromagnetic ratio (such as $^1$H) to insensitive spins with lower gyromagnetic ratios (such as $^{13}$C, $^{15}$N). The other type of polarization methods, however, exceeds the limit of nuclear thermal polarization which is determined by Boltzmann distribution. Including PHIP, there are dynamic nuclear polarization (DNP), optical pumping and signal amplification by revisable exchange (SABRE). The last one is the general form of the hyperpolarization method I have been
working with. In this background chapter, I will give a brief introduction of the latter type of polarization techniques, namely DNP and optical pumping.

### 2.3.1 Dynamic nuclear polarization (DNP)

Dynamic nuclear polarization is a broad class of hyperpolarization methods that were first introduced to solid NMR [13] then made its use in liquid NMR through the invention of dissolution DNP [14]–[16]. For both solid and solution samples, the hyperpolarization mechanism is similar: DNP utilizes the high polarization levels of unpaired electrons (nearly to 100% at high field and low temperature because of its much larger gyromagnetic ratio) and transfers the electron polarization to target nuclear spins (such as $^1$H, $^{13}$C, $^{15}$N, $^{29}$Si) via the electron-nuclear dipolar coupling. By irradiating continuous microwave around the electron resonance frequency ($\omega = \omega_e \pm \omega_n$), we can induce the double-quantum (with $\omega = \omega_e + \omega_n$) or zero-quantum (with $\omega = \omega_e - \omega_n$) transition of the electron-nucleus spin pair, which is the “solid effect”: the flip of one electron spin will simultaneously force the flip of a neighboring nuclear spin (shown in Fig. 9). The transitions are usually addressed as forbidden transitions because with strong magnetic field approximation, the non-secular dipolar interaction part between the electron and the nucleus could be dropped from the spin-pair Hamiltonian. However, though the interaction is weak, they play the essential role of generating the nuclear polarization; in addition, that’s also the reason why DNP usually takes quite a long time for the polarization to build up (up to hours) [13]. Another point needs to be noticed is
that, to permit the hyperpolarization build-up for the nuclear spins, the thermal relaxation of electrons must be much faster than the relaxation of nucleus, and the nuclear $T_1$ relaxation must be long enough. These conditions are usually satisfied with the relatively strong magnetic field ($\sim 3.35$ T) and cryogenic temperature (liquid Helium, $\sim 1.4$ K). Using DNP, $\sim 37\%$ polarization for $^{13}$C and $\sim 8\%$ for $^{15}$N could be achieved [14].

Since the introduction of dissolution DNP, this method has been intensively used in preclinical researches, especially for MR imaging to track bio-chemical reactions or metabolisms using specific bio-markers. Currently $^{13}$C labelled pyruvate is the most broadly used bio-marker because of its well-studied glycolytic metabolism and the
relatively long $T_1$ relaxation time (~ 1 min) of the hyperpolarized $^{13}$C in solution at room temperature. It has been demonstrated that DNP hyperpolarized $^{13}$C-pyruvate has the potential for early prostate cancer detection [17] and cancer progression monitoring [18], as shown in Fig. 11 and 12.

**Figure 11:** [From Ref. 17] In-human study of using DNP hyperpolarized $^{13}$C-pyruvate to detect prostate cancer. Shown on the left, in cancer cells pyruvate will be converted to lactate by the enzyme LDH (lactate dehydrogenase) thus showing specific lactate line in the 13C spectrum due to the different chemical environment of lactate carbon-13. While in normal cells this line is absent. On the right are the MR images using conventional $T_2$ contrast (top right) and its super-positioned image with the lactate/pyruvate metabolism information (bottom right). We can see that while $T_2$ contrast can show the cancer site on the left of the image (arrow pointed dark region), it fails to detect the cancer site on the right. However, using the hyperpolarized $^{13}$C metabolism signal, cancer site that is not obvious by structural imaging emerges.
2.3.2 Optical pumping

The other polarization method that exceeds the nuclear thermal equilibrium limitation is optical pumping and this is mainly used to hyperpolarize nuclear spins of the noble gas, which are used for lung imaging. Briefly, two mechanisms exist to polarize the noble gas. One is called spin exchange optical pumping (SEOP) [19][20], [21], which polarizes both $^3$He and $^{129}$Xe (both are spin-1/2 nuclei). In this mechanism electrons of the alkali metal (such as rubidium) vapor gas is first hyperpolarized by using circularly polarized laser light and it could reach almost complete polarization [22]. Then via spin exchange accomplished by the weak dipole-dipole interaction, polarization of the metal
electrons could be transferred to the noble gas nuclear spins. The other mechanism is through metastability exchange optical pumping (MEOP) [23], [24] and it polarizes only $^3$He. Instead of using alkali metal, metastable state of $^3$He electrons are first polarized using laser light, then via strong spin coupling provided by the exchange with the ground-state nuclear spin, the entire ground-state nuclear system can be hyperpolarized. However, due to limited availability of $^3$He, currently most of the medical imaging researches use $^{129}$Xe as the polarizing target. Shown in Fig. 13, hyperpolarized $^{129}$Xe could yield good spatial resolution of human lung MR imaging and can be used to detect lung abnormalities [19].

![Figure 13: Optical pumping hyperpolarization process and a hyperpolarized $^{129}$Xe MR lung imaging.](image)

On the left is the process of SEOP, where the electrons of alkali metal are first polarized by laser light, the polarization is then transferred to the noble gas nucleus via dipolar coupling. On the right is a typical application of such polarization method, where the hyperpolarized $^{129}$Xe can be used for lung imaging with good resolution, providing important diagnosing information for lung diseases, for example the diagnosis of chronic obstructive pulmonary disease (COPD).
Though both DNP and SEOP could achieve great hyperpolarization levels and they have obtained numerous accomplishments in pre-clinical or clinical researches, both hyperpolarization techniques suffer essential obstacles limiting their further developments or applications. One of the most challenging problems is the short polarization lifetime, which rises a fundamental difficulty to apply DNP or SEOP for functional and metabolic MRI. Though $^{13}$C-pyruvate could reach $T_1$ lifetime up to 1 minute, for most of the potential bio-markers their polarization lifetimes are on the order of tens of seconds. The short polarization lifetime makes most of the metabolism tracking or monitoring tasks impossible given the complex and time-consuming operational procedures including sample transferring, injection, in vivo circulation and reaction. Similar for SEOP, though the polarization lifetime could be much longer for $^{129}$Xe (tens of hours) for storage [20], nevertheless the in vivo lifetime is under 1 minute because of the strong relaxation effect of paramagnetic spices such as O$_2$ and iron of hemoglobin. Second is the high expense. DNP is quite expensive including both the instrumentation and usage cost, which consumes large amount of liquid helium. $^3$He for optical pumping is formidable expensive given its extremely small natural abundance (0.000137%) and can only be obtained by radioactive tritium decay. These are essentially the problems this thesis will address: to develop more general and accessible hyperpolarization methods with long-lived polarization lifetimes. We will see that in the next chapter, my research in the singlet states provides a possible way to extend the lifetime of the polarized signal by
orders. On the other hand, my research in the other method of SABRE related hyperpolarization, achieves both goals of amplified signal and long-lived polarization lifetime, and at the same time is much cheaper than the techniques introduced in this section.
3. Accessing long-lived states

In this chapter, I will first introduce several ways that are used to access the long-lived singlet states of coupled nuclear spin pairs in section 2.1. These methods include physically switching the static magnetic field used in the experiment, chemically changing the target compound, or using specific pulse sequences. Then I will discuss the novel method that also provides access to the singlet state, which is to use deuterium, a quadrupole nucleus, to substitute the hydrogen at specific positions in a target molecule. Deuteration breaks the symmetry of the molecule and makes the singlet state RF pulse accessible. Finally, I will discuss the experimental and theoretical results of accessing $^1$H singlet via $^2$H coupling in my project using trans deuterated ethylene.

3.1 Methods to access long-lived singlet states

3.1.1 Field shuttling

Introduced by Levitt et. al. [25], [26], field shuttling was first demonstrated that it could effectively store the singlet state of spin pairs lacking intrinsic equivalence, e.g., 2,3-dibromothiophene (shown in Fig. 15). In this example, the singlet state of the proton spin pair was first created at high magnetic field inside the magnet (~9.4 T) by applying two consecutive hard RF pulses. The sample was then adiabatically transferred to low field. At low magnetic field (~ 20 mT), the originally large chemical shift difference between the two proton spins ($\Delta \nu_{\text{high}} \approx 244$ Hz) became much smaller ($\Delta \nu_{\text{low}} \approx 0.5$ Hz) and the spin pair was strongly coupled ($\Delta \nu_{\text{low}} \ll J_{\text{HH}} \approx 5.7$ Hz). In this strongly coupled regime, as discussed
in the previous chapter, the singlet state is an eigenstate of the spin Hamiltonian and is immune to the dipole-dipole spin interaction. With this immunity, the singlet state will have a much longer relaxation lifetime, $T_s$, than the spin-lattice relaxation time, $T_1$. In this example, the singlet lifetime is $\sim 120$ s, which is about 7 times longer than its $T_1$ lifetime. Nonetheless, after storing the singlet state at low magnetic field for certain amount of time, $\tau_{LF}$, the sample is transported back adiabatically to the high field, where the equivalence is broken again and antiphase signal of the singlet state is read out.

---

**Figure 14:** [Adapted from Ref. 26] Field shuttling procedure for singlet state creation, storage and detection. (A) Sequence of the high and low magnetic fields used. The sample is transported from high magnetic field, $B_{\text{high}}$, to low magnetic field, $B_{\text{low}}$, and vice versa, in a time $\tau_{\text{transport}}$, where $1/J_{HH} < \tau_{\text{transport}} < T_1$ to satisfy the adiabatic process. (B) Pulse sequence to create and detect the signal originated from the singlet state. (C) The spin state populations at different point of the experiment: for (1, 2, 5),

clockwise from the top the energy levels are $|\beta\beta\rangle$, $|\alpha\beta\rangle$, $|\alpha\alpha\rangle$, $|\beta\alpha\rangle$; for (3, 4), the upper three are triplet states and the lower one is the singlet state.

As shown in Fig. 14, the initial populations of the spins at high field are at thermal equilibrium, with the highest energy level $|\beta\beta\rangle$ having slightly depleted population (empty balls) while the lowest energy level $|\alpha\alpha\rangle$ slightly over-populated (filled balls). The difference of their populations depends on Boltzmann distribution. The consecutive 90-degree pulses in block A break the thermal equilibrium and creates population difference between the states $|\beta\alpha\rangle$ and $|\alpha\beta\rangle$. The first adiabatic transportation could be considered as an inverse process of ALTADENA, where the population of $|\alpha\beta\rangle$ is locked into the singlet state following the slow decrease of magnetic field, however the population of $|\beta\alpha\rangle$ equilibrates among the three energetically degenerate triple states. The singlet remains as the eigenstate at low field and relaxes at a low rate, yielding a long-lived lifetime for this spin order; then the second adiabatic transportation is similar to the ALTADENA process, as discussed in the previous chapter, population in the singlet state arrives only at the $|\alpha\beta\rangle$ level and generates antiphase signal. Applying the pulse sequence in block B, the signal centered at only one of the two $^1\text{H}$ chemical shift positions will be detected in antiphase, as shown in Fig. 15.
Figure 15: [Adapted from Ref. 26] Signal after storing the $^1$H spin pair lacking intrinsic equivalence in singlet state at low magnetic field. (A) Thermal spectrum of 2,3-dibromothiophene. (B) Spectrum generated using the A+ pulse block in Fig. 14. (C) Spectrum generated using the A- pulse block in Fig. 14, with the phase of the second 90° pulse switched from x to -x.

3.1.2 Strong spin locking

Besides magnetic field cycling, another method to explore the singlet state of weakly coupled spins at high field in liquid solution is first to create the singlet spin order using a specific combination of hard RF pulses, then store the singlet using a strong spin locking continuous wave (CW) pulse [27]. This idea can be clearly illustrated with Fig. 16.
Figure 16: [Adapted from Ref. 27] Pulse sequence creating and storing the singlet spin order using spin locking field. Labelled are important time points for different states. Thermal equilibrium is achieved at time point (1), at time point (2) singlet spin order is created and it is stored till time point (3) for signal read out.

Starting from time point 1, thermal equilibrium is established at high field and the density operator is proportional to:

\[ \rho_1 = I_{1z} + I_{2z} \]

The first 90° x pulse converts the population into coherence:

\[ \rho_a = -I_{1y} - I_{2y} \]

With \( \tau_1 = 1/4J_{12} \) and \( \tau_2 = 1/4J_{12} + \pi / \Delta \omega \), where \( J_{12} \) is the \( J \)-coupling between spin 1 and 2, and \( \Delta \omega \) is their Larmor frequency difference at high field, from time point a to b the pulse sequence can be seen as a spin echo. Since the spins are weakly coupled, \( \Delta \omega \) is much larger than \( J_{12} \). Under \( J \)-coupling evolution, the density operator at b is:

\[ \rho_b = -I_{1x}I_{2z} - I_{1z}I_{2x} \]

Assuming the carrier frequency of the RF pulse is in the middle of spin 1 and 2’s Larmor frequency, after the offset evolution of time \( \pi / \Delta \omega \), the density operator at c is:

\[ \rho_c = -I_{1y}I_{2z} + I_{1z}I_{2y} \]
Applying the 90° y pulse, the density operator at d is:

\[ \rho_d = -I_{1y}I_{2x} + I_{1x}I_{2y} \]

Finally, after evolution time \( \tau_3 = \pi / 2\Delta \omega \), the density operator at (2) is:

\[ \rho_2 = I_{1x}I_{2x} + I_{1y}I_{2y} \]

Expressing with shift operators, the density operator is rewritten as:

\[ \rho_2 = -(I_1^zI_2^z + I_1^-I_2^+) \]

The density operator now can be seen exactly as a zero-quantum (ZQ) coherence, which contains the singlet state population, as discussed in previous chapter.

\[ \rho_2 = |S_0\rangle\langle S_0| - |T_0\rangle\langle T_0| \]

By applying the strong spin locking field, the chemical shift difference between the 2 spins could be considered efficiently suppressed (no chemical shift evolution) thus the singlet is an eigenstate of the nuclear spin Hamiltonian, which is protected from intramolecular dipole-dipole interaction by symmetry and will have a much longer relaxation lifetime. For the example illustrated here in Fig. 17, the singlet lifetime is \(~140\) s, which is about 18 times longer than its \(T_1\) lifetime.

Signal from the singlet state could then be detected by turning off the spin locking CW pulse. Once the strong field is removed, the spins return to inequivalence and the evolution delay will generate new double-quantum (DQ) coherence terms:

\[ \rho_e = -I_{1x}I_{2y} + I_{1y}I_{2x} \]
The last $90^\circ$ x pulse converts the DQ coherences into antiphase single-quantum (SQ) coherences, which are detectable by NMR:

$$\rho_f = -I_{1z}I_{2z} + I_{1x}I_{2x}$$

Since there is a “−” for the first SQ coherence term, the antiphase signal of spin 1 is flipped comparing to spin 2, as is shown below for the example of 2-chloroacrylonitrile.

![Figure 17](image)

**Figure 17:** [From Ref. 27] The antiphase signal coming from the singlet states of the two $^1H$ spins, using the example of 2-chloroacrylonitrile. The signals at the two proton sites are flipped. Depending on the phase of the first $90^\circ$ pulse, the spectra in (a) and (b) have $180^\circ$ phase difference.

### 3.1.3 Chemical conversion

In addition to accessing the singlet state using magnetic field and pulse manipulations, the singlet spin order could also be prepared using chemistry [28]. One example would be $^{13}$C enriched diacetyl, [CH$_3$($^{13}$C=O)($^{13}$C=O)CH$_3$], which could go through interchange with its monohydrate form, CH$_3$($^{13}$C=O)[$^{13}$C(OH):CH$_3$], and
depending on the solvent it is dissolved in, the interchange equilibrium could shift to either site.

![Image](image.png)

**Figure 18:** [From Ref. 28] The interchange process of diacetyl. The middle two carbon sites are enriched with $^{13}\text{C}$, indicated by the asterisks. In water solution, the monohydrate is the major species and the two $^{13}\text{C}$ spins are not equivalent; while in acetone solution diacetyl is the major species and the two $^{13}\text{C}$ spins are equivalent, which is critical to preserve the singlet spin order.

In an aqueous solution, the monohydrate is the major species and the two $^{13}\text{C}$ spins are not equivalent. The large chemical shift difference (~8000 Hz at 7T) makes the spins weakly coupled and the singlet state is accessible by exchanging the populations in $|\alpha\alpha\rangle$ and $|\beta\beta\rangle$ with those in $|\alpha\beta\rangle$ and $|\beta\alpha\rangle$, or equivalently, by selectively flipping the magnetization of one spin by $180^\circ$ (e.g., $I_{1z} - I_{2z}$) using RF pulses. Once the singlet spin order is prepared, large amount of acetone is injected into the original solution to inhibiting the hydration, thus the diacetyl is preserved and the singlet state is locked into the equivalent $^{13}\text{C}$ spins. Protected by the singlet, $^{13}\text{C}$ signals will have much longer lifetime and are ready for read out simply by injecting water to make the carbons inequivalent again through hydration.
3.2 Accessing the singlet state using novel pulse sequences

All the methods discussed above have shown great capability of accessing to the singlet state for the inequivalent spins, where the “inequivalence” provides entry to the disconnected state. However, if we choose the structure of molecules that the target spins are chemically equivalent, i.e., they have the same chemical shift, novel methods using sophisticated pulse sequences to access the singlet state emerge.

3.2.1 The MSM pulse sequence

The acronym MSM stands for “magnetization - singlet - magnetization”, and the pulse sequence consists of two parts: M2S and S2M, meaning “magnetization to singlet” and “singlet to magnetization” respectively [26], [29]. The first part converts the magnetization of the target spins to singlet; after storage time, the second part, which is just the reversed version of part 1, convert the singlet back to magnetization for signal detection. The pulse sequence is shown in Fig. 19.
Figure 19: [From Ref. 29] Illustration of the M2S pulse sequence with population and coherence transformations. The [90°180°90°] blocks shown in the middle is composite 180° pulses to overcome pulse imperfection. The population difference between $|T_{+1}\rangle$ and $|T_{-1}\rangle$ is finally converted to the population difference between $|S_0\rangle$ and $|T_0\rangle$ states.

This pulse sequence was first introduced by Levitt et al. [26] for near equivalent spins, i.e., spins that had small chemical shift difference ($\Delta\nu_{ij} = \nu_1 - \nu_2$) comparable to their $J$-coupling thus the spins were in the strong coupling regime. Since the spins are strongly coupled, it is not possible to resolve the individual chemical shift frequencies thus the selective inversion method will not work. However, the M2S pulse sequence, which circumvents the difficulty of chemical shift resolvability, could achieve this goal by considering the middle triplet and singlet states in the Bloch sphere as a two-level system.
As shown in Fig. 19, at thermal equilibrium, the initial density matrix is the population difference between \( |T_{+1}\rangle \) and \( |T_{-1}\rangle \):

\[
\rho_0 = I_{iz} + I_{zz} = |\alpha\alpha\rangle\langle\alpha\alpha| - |\beta\beta\rangle\langle\beta\beta| = |T_{+1}\rangle\langle T_{+1}| - |T_{-1}\rangle\langle T_{-1}|
\]

The population difference is converted to coherences within the triplet manifold by the first 90° y pulse:

\[
\rho_1 = I_{iz} + I_{zz} = \frac{1}{2} (I_{iz} + I_{iz} + I_{zz} + I_{zz}) = \frac{1}{\sqrt{2}} \left( |T_{+1}\rangle\langle T_0| + |T_{-1}\rangle\langle T_0| + |T_0\rangle\langle T_{+1}| + |T_0\rangle\langle T_{-1}| \right)
\]

In the Bloch sphere, the first train of composite 180° pulses behaves as one 180° pulse, accumulating the small evolution caused by the small chemical shift difference between the spins. The \( |T_0\rangle \) state is exchanged with the \( |S_0\rangle \) state, generating the coherences connecting \( |S_0\rangle \) with \( |T_{+1}\rangle \) and \( |T_{-1}\rangle \) respectively. The density matrix now is:

\[
\rho_3 = \frac{i}{\sqrt{2}} \left( |T_{+1}\rangle\langle S_0| + |T_{-1}\rangle\langle S_0| - |S_0\rangle\langle T_{+1}| - |S_0\rangle\langle T_{-1}| \right)
\]

\[
= \frac{i}{2} \left[ (I_{zz} - I_{zz}) - (I_{z+} - I_{z-}) \right]
\]

\[
= I_{1y} - I_{2y}
\]

Note that the coherences at this point is equivalent to \( I_{1y} - I_{2y} \), so the phase of the next 90° pulse is critical, and a 90° x pulse converts it to \( I_{iz} - I_{zz} \), which is just like a selective inversion and now they are the coherences connecting \( |S_0\rangle \) and \( |T_0\rangle \):

\[
\rho_4 = I_{iz} - I_{zz} = |\alpha\beta\rangle\langle\beta\alpha| - |\beta\alpha\rangle\langle\alpha\beta| = |T_0\rangle\langle S_0| + |S_0\rangle\langle T_0|
\]
Since the energy difference between the triplet and singlet states is $2\pi J_{12}$, the evolution changes the phase of the singlet-triplet coherences:

$$\rho_S = i(-|T_0\rangle\langle S_0| + |S_0\rangle\langle T_0|)$$

Finally, the second composite 180° pulse train behaving as a 90° pulse in the Bloch sphere, converts the singlet-triple coherence into their population difference and the density matrix is:

$$\rho_6 = |S_0\rangle\langle S_0| - |T_0\rangle\langle T_0|$$

The last density matrix could be rewritten as:

$$\rho_6 = |S_0\rangle\langle S_0| - \frac{1}{3}(|T_{13}\rangle\langle T_{13}| + |T_0\rangle\langle T_0| + |T_{11}\rangle\langle T_{11}|) + \frac{1}{3}(|T_{13}\rangle\langle T_{13}| - 2|T_0\rangle\langle T_0| + |T_{11}\rangle\langle T_{11}|)$$

The first part is the difference between the singlet and averaged triplet population, which will have a long singlet lifetime referred as $T_S$; while the second part is a non-uniform population distribution in the triplet states and it will have a much shorter lifetime. Using the exactly reversed pulse sequence, S2M, signals from the first part could be retrieved after the storage time and by following the reversed order of the analysis, the retrieved signals at the end are in-phase as $I_{1x} + I_{2x}$. Note that the first and the second part have two different lifetimes, they will contribute two different relaxation constants for the measured signal as a function of the storage time, which will be a bi-exponential decay curve.
Several examples have been demonstrated using the M2S sequence to access singlet, such as $^{15}\text{N}_2\text{O}$ [26], and protons on the same carbon site of peptide [29]. However, since the two spins are intrinsically inequivalent, the small chemical shift difference will slowly mix the singlet with the triplet thus reduce the singlet lifetime, which is not a favorable situation.

### 3.2.2 MSM pulse sequence for chemical equivalent spins

Introduced by Warren et. al. [30], the state-mixing problem mentioned above could be solved by using spins that are chemically but non-magnetically equivalent spin pairs, i.e., the two spin pairs form an AA'XX' spin system. As introduced in the previous chapter, the same letters stand for the same spin and the two spin pairs are far away from each other in terms of their resonant frequencies, with the $J$-coupling differences breaking the symmetry. Such spin pairs give several advantages: firstly, since the target spins have the same chemical shift frequency, the singlet state is an eigenstate and will be preserved after the M2S pulse sequence; secondly, this method is still suitable for a broad range of molecules with the required symmetric structure; finally, the singlet-triplet interchange between two different spin pairs could be extended that we store the signal of spins with higher sensitivity, e.g., $^1\text{H}$, to spins with longer singlet lifetime, e.g., $^{13}\text{C}$ or $^{15}\text{N}$, and retrieve the signal after the storage, thus preserving the signal sensitivity and longevity simultaneously [31].
The analysis of coherence pathway and population change is similar with that in the one spin pair case, however, instead of only 4 states, there are now 16 energy states, with combinations of singlet and triplet states between the 2 spin pairs. Although the states space is much larger than the one spin pair case, the combined singlet-singlet state, i.e., $|SS\rangle = \frac{(\alpha\beta - \beta\alpha)}{\sqrt{2}}(\alpha\beta - \beta\alpha)_x \rangle / 2$, has the same overall inversion symmetry as the triplet-triplet state, i.e., $|T_0T_0\rangle = \frac{(\alpha\beta + \alpha\beta)}{\sqrt{2}}(\alpha\beta + \alpha\beta)_x \rangle / 2$. Again, this could be considered as a two-level system and more precisely, as already shown by Pople and Bernstein et al. [32], [33], the Hamiltonian for this two-level subspace is:

$$H = \begin{pmatrix} |T_0T_0\rangle & |SS\rangle \\ \\
0 & -\frac{1}{2}(J_{AX} - J_{AX'})\end{pmatrix}$$

This could be an analogy of the one spin situation: though the spin is in a strong static $B_0$ magnetic field, given that a much weaker $B_1$ field changes phase at the correct resonant frequency, irradiation of the weak $B_1$ perturbation field along $x$ could drag the spin by $180^\circ$. The Hamiltonian is expressed as below:

$$H = \begin{pmatrix} |\beta\rangle & |\alpha\rangle \\ \\
\frac{1}{2}\gamma B_0 & \frac{1}{2}\gamma B_1\end{pmatrix}$$

(3.1)
Here, $\gamma$ is the gyromagnetic ratio of the spin, and $|\beta\rangle, |\alpha\rangle$ are the eigenstates of $I_z$. Comparing equation (3.1) and (3.2), we can see that the off-diagonal small $J$-coupling difference, $\Delta J_{AX} = J_{AX} - J_{AX'}$, has the same role as the weak $B_1$ irradiation. Using the M2S pulse sequence by irradiating on spin A, $\Delta J_{AX}$ changes sign after each composite 180° pulse, which is similar as the evolution of the one spin pair case in the Bloch sphere. The $|T_0T_0\rangle$ and $|SS\rangle$ states are exchanged and $\Delta J_{AX}$ provides the handle to load population onto the singlet states of both spin pairs from their triplet states. The comparison between the one spin pair, two spin pairs and one single spin cases are shown in Fig. 20.
Figure 20: [Adapted from Ref. 29&30] State evolution process during M2S. (A) Comparison of the state evolution between one spin-pair and two spin-pairs using the M2S pulse sequence. (B) For the 2-level subsystems in both cases, they are very similar to the one spin case that is diverted from the z axis, which has the strong $B_0$ field, using very weak on-resonance oscillating $B_1$ field.

\[
\Omega = \sqrt{J_{12}^2 + \Delta \nu_{12}^2}
\]

\[
\theta = \arctan(\Delta \nu_{12} / J_{12})
\]

\[
\tau = 1 / (2\Omega)
\]

\[
n = \pi / (2\theta)
\]
On the other hand, in the two-spin-pair situation, another pair of states with
different symmetry could also be exploited to access the long-lived singlet state. They are
\[ |T_0S\rangle = \frac{1}{\sqrt{2}} (|\alpha\beta + \beta\alpha\rangle_x |\alpha\beta - \beta\alpha\rangle_x) \]
and
\[ |ST_0\rangle = \frac{1}{\sqrt{2}} (|\alpha\beta - \beta\alpha\rangle_x |\alpha\beta + \beta\alpha\rangle_x) \]
states, which are antisymmetric and are disconnected from the inversion symmetric group.
However, since these two states belong to the same symmetry group, similarly they are
connected by the small off-diagonal term \( \Delta J_{AX} \), and the Hamiltonian is below [32], [33]:

\[
H = \langle T_0S \rangle - \frac{1}{2} (J_{AA} + J_{XX}) |\alpha\beta + \beta\alpha\rangle_x |\alpha\beta - \beta\alpha\rangle_x
\]

The resonance condition is slightly different from the singlet-singlet and triplet-
triplet exchange case, since the effective Hamiltonian now is
\( \Omega = \sqrt{(J_{AA} - J_{XX})^2 + \Delta J_{AX}^2} \)
and the evolution angle before each 180° pulse is
\[ \theta = 2 \times \arctan \left[ \frac{\Delta J_{AX}}{J_{AA} - J_{XX}} \right] \].
However, if the target spin for singlet is \(^{13}\text{C}\) (spin A) and the auxiliary spin is \(^1\text{H}\) (spin X),
usually the \( J \)-coupling between the carbons will be much larger than that between the
protons, so the \( J_{XX} \) term could be ignored and the resonance conditions will be similar as
the other symmetry group.

3.2.3 SLIC pulse sequence for near equivalent spin pair and chemically
equivalent spin pairs

Till now, all the discussed methods have achieved good results preparing and
manipulating the singlet state, however, all the methods have their obstacles, and
particularly, they are not practical for clinical or MR imaging applications. Transporting
the patient in and out the magnet quickly will cause nausea and dizziness, and both the
strong spin locking and MSM methods will dissipate huge amount of energy into the
patient, which tremendously exceeds the safety limit of energy dissipation. By the
International Electrotechnical Commission (IEC), the current specific absorption rate
(SAR) limit is 1.5 W/kg averaged in any 15 minutes period (i.e., 1.35 kJ/kg in 15 min).
However, the strong spin locking using nutation frequency of 3500 Hz for 100 s would
deposit energy of 13.8 MJ/kg, ~10,000 times more than the limit; the composite hard 180°
pulses used in MSM has nutation frequency of ~25000 Hz, applying in total 192 such
pulses for both M2S and S2M would deposit 54 kJ/kg, 40 times more.

A different pulse sequence, called spin locking induced crossing (SLIC) [34], solves
the problems stated above and is by far the most practical approach to apply long-lived
singlet states in MRI (energy dissipation in Ref. 34 is ~1.5 J/kg for creating and reading
out singlet only, well below the SAR limit). Fundamentally different from the strong spin
locking method, SLIC uses much weaker spin locking power (same as the $J$-coupling of
the target spins) to induce crossing between the singlet and triplet states, where again, the
small chemical shift difference ($\Delta \nu_{12}$) in the nearly equivalent spins’ case, or the out-of-
pair $J$-coupling difference ($\Delta J_{AX}$) in the chemically equivalent spin pairs’ case, is the
handle to perform the crossings.
First let us consider the case of two nearly equivalent spins. Suppose the chemical shifts of the two spins are $\omega_1$ and $\omega_2$, and their difference is $\Delta \omega_{12}$, with the singlet-triplet basis set, the Hamiltonian is:

$$
\begin{align*}
H &= \begin{pmatrix}
|T_{+1} \rangle & |T_0 \rangle & |T_{-1} \rangle & |S \rangle \\
\pi J / 2 + \frac{\omega_1 + \omega_2}{2} & 0 & 0 & 0 \\
0 & \pi J / 2 & 0 & \frac{\Delta \omega_{12}}{2} \\
0 & 0 & \pi J / 2 - \frac{\omega_1 + \omega_2}{2} & 0 \\
0 & \frac{\Delta \omega_{12}}{2} & 0 & -3\pi J / 2
\end{pmatrix}
\end{align*}
$$

Taking the rotating frame, if the spin locking pulse is irradiated at the middle frequency between $\omega_1$ and $\omega_2$, the $\omega_1 + \omega_2$ term could be considered as 0. On the other hand, with the spin locking irradiation of phase x and nutation frequency $\omega_n$, the Hamiltonian is:
This matrix is not diagonal either. However, if we firstly diagonalize the triplet subspace, we get:

\[
\begin{bmatrix}
\phi_{+1} & \phi_0 & \phi_{-1} \\
\phi_{+1} & & \\
\phi_0 & & \\
\phi_{-1} & & \\
\end{bmatrix}
\]

\[
\begin{bmatrix}
\frac{\pi J}{2} + \omega_n & 0 & 0 \\
0 & \frac{\pi J}{2} & 0 \\
0 & 0 & \frac{\pi J}{2} - \omega_n \\
\end{bmatrix}
\]

In this diagonalization, \( \phi_{+1} \), \( \phi_0 \) and \( \phi_{-1} \) are newly defined wave functions as combinations of \( |T_{+1}\rangle \), \( |T_0\rangle \) and \( |T_{-1}\rangle \). Their eigenvalues are listed on the diagonal, and the wave functions are:

\[
|\phi_{+1}\rangle = \frac{1}{2}(|T_{+1}\rangle + \sqrt{2}|T_0\rangle + |T_{-1}\rangle) = \frac{1}{2}(|\alpha\alpha\rangle + |\alpha\beta\rangle + |\beta\alpha\rangle + |\beta\beta\rangle)
\]

\[
|\phi_0\rangle = \frac{\sqrt{2}}{2}(|T_{+1}\rangle - |T_{-1}\rangle) = \frac{\sqrt{2}}{2}(|\alpha\alpha\rangle - |\beta\beta\rangle)
\]

\[
|\phi_{-1}\rangle = \frac{1}{2}(|T_{+1}\rangle - \sqrt{2}|T_0\rangle + |T_{-1}\rangle) = \frac{1}{2}(|\alpha\alpha\rangle - |\alpha\beta\rangle - |\beta\alpha\rangle + |\beta\beta\rangle)
\]
Using this new basis set, we can “partially” diagonalize the full triplet-singlet based Hamiltonian, $H_{SL}$, and the result is:

\[
\begin{bmatrix}
|\phi_{+1}\rangle & |\phi_0\rangle & |\phi_{-1}\rangle & |S\rangle \\
\langle \phi_{+1} | & \frac{\pi J}{2} + \omega_n & 0 & 0 & \frac{\Delta \omega_{12}}{2\sqrt{2}} \\
\langle \phi_0 | & 0 & \frac{\pi J}{2} & 0 & 0 \\
\langle \phi_{-1} | & 0 & 0 & \frac{\pi J}{2} - \omega_n & -\frac{\Delta \omega_{12}}{2\sqrt{2}} \\
\langle S | & -\frac{\Delta \omega_{12}}{2\sqrt{2}} & 0 & \frac{\Delta \omega_{12}}{2\sqrt{2}} & -\frac{3\pi J}{2}
\end{bmatrix}
\]

From equation (3.3), it is now clear that by choosing $\omega_n = 2\pi J$, the energy of states $|\phi_{-1}\rangle$ and $|S\rangle$ are equalized. At this energy level, the off-diagonal element $\frac{\Delta \omega_{12}}{2\sqrt{2}}$ becomes significant, and will drive the oscillation between $|\phi_{-1}\rangle$ and $|S\rangle$ back and forth. Again, we can focus on the 2-level subspace which connects $|\phi_{-1}\rangle$ and the singlet, the Hamiltonian is:

\[
H_{SL-2level} = \begin{bmatrix}
|\phi_{-1}\rangle & |S\rangle \\
\langle \phi_{-1} | & -\frac{3\pi J}{2} & -\frac{\Delta \omega_{12}}{2\sqrt{2}} \\
\langle S | & \frac{\Delta \omega_{12}}{2\sqrt{2}} & -\frac{3\pi J}{2}
\end{bmatrix}
\]

The oscillation frequency is $\frac{\Delta \omega_{12}}{\sqrt{2}}$, or $\frac{\Delta \omega_{12}}{2\pi \sqrt{2}}$ in unit of Hz. So the oscillating period is $2\pi \sqrt{2} / \Delta \omega_{12}$, to achieve the maximum transfer for the singlet state, the optimal
spin locking time should be half of the period, i.e., \( t_{\text{SL}} = \frac{\pi \sqrt{2}}{\Delta \omega_{12}} \), or \( t_{\text{SL}} = \frac{1}{(\sqrt{2} \Delta \nu_{12})} \)

if we plug in \( \Delta \omega_{12} = 2\pi \Delta \nu_{12} \).

To verify, we can start with the density matrix at thermal equilibrium:

\[
\rho_0 = I_{xx} + I_{zz} \quad ; \quad \text{after the 90°y pulse, it is converted to:}
\]

\[
\rho_1 = I_{xx} + I_{zz} = |T_0\rangle\langle T_1| + |T_{-1}\rangle\langle T_0| + |T_0\rangle\langle T_{-1}| + |T_{-1}\rangle\langle T_0|. 
\]

Expressed in matrix form, it is:

\[
\rho_1 = \begin{pmatrix}
\alpha\alpha & \alpha\beta & \beta\alpha & \beta\beta \\
\frac{1}{2} & 0 & \frac{1}{2} & 0 \\
\frac{1}{2} & 0 & 0 & \frac{1}{2} \\
0 & \frac{1}{2} & \frac{1}{2} & 0
\end{pmatrix}
\]

Currently the density matrix is expressed in Zeeman basis. From the previous process, we know that there is a \( \phi \) basis set based on the singlet-triplet basis, adapting \( \rho_1 \) using this \( \phi \) basis we have:

\[
\rho_1 = \begin{pmatrix}
\phi_{-1} & \phi_0 & \phi_{+1} & S \\
1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & -1 & 0 \\
0 & 0 & 0 & 0
\end{pmatrix}
\]

Now it is clear that after the 90° y pulse, there is population difference of \( |\phi_{-1}\rangle \) and \( |\phi_{+1}\rangle \) states. Since we are focusing on \( |\phi_{-1}\rangle \) and \( |S\rangle \) states, the subspace density matrix is:
This density matrix will evolve under the Hamiltonian, $H_{\text{SL-2level}}$, as defined in equation (3.4), so the time dependent density matrix is:

$$
\rho_{2\text{level}} = e^{-iH_{\text{SL-2level}}t} \rho_{2\text{level}} e^{iH_{\text{SL-2level}}t} = \begin{pmatrix} \langle \phi_1 \rangle & |S\rangle \\ |S\rangle & 0 \end{pmatrix}
$$

From equation (3.5) we can see that population oscillates between $|\phi_1\rangle$ and $|S\rangle$. To reach the maximum of singlet transfer, simply make the cosine term equal to -1, and the spin locking time $t_{\text{sl}}$ will be $t_{\text{sl}} = \frac{\sqrt{2} \pi}{\Delta \omega_{12}} = 1 / \left(\sqrt{2} \Delta \nu_{12}\right)$, which is the same as referring to the Hamiltonian matrix.

The situation for the chemically equivalent spins pairs, i.e., the AA’XX’ spin system, is similar to that for the nearly equivalent spins. Besides the four $\phi$ basis adapted states of AA’, there are four XX’ spin pair state, which are treated with the singlet-triplet basis set. With the combination of these states, again $|SS\rangle$ and $|\phi_1 T_0\rangle$ have the same symmetry and belong to the same group; while $|ST_0\rangle$ and $|\phi_1 S\rangle$ belong to the other with different symmetry properties. The submatrix Hamiltonians of the different groups are shown below:
Comparing with equation (3.3), $H_{SL}$ of the one spin pair case, the Hamiltonians listed above are quite similar and it is evident that the spin locking resonance conditions for AA’XX’ spin system is: $\omega_n = 2\pi(J_{AA} \pm J_{XX})$, if $J_{XX}$ is small enough to be neglected, then the resonance conditions will be degenerate and they are the same as the one spin-pair case. However, while the chemical shift difference drives the oscillation for the two nearly equivalent spins, here it is the out-of-pair $J$-coupling difference playing the same role, which is similar as in the M2S pulse sequence. The time to reach singlet is $t_{sl} = 1/\sqrt{2\Delta J_{AX}}$. 
To achieve better singlet transfer effect, one can manipulate the continuous wave with different pulse shapes (e.g., composite pulses or adiabatic pulse), nutation frequency functions or offset frequency functions [34]. However, these parameters are not tunable for M2S pulse sequence. Nonetheless, the “quality” (longer singlet lifetime) of the prepared singlet state using M2S can still be improved, by using a chemistry method, which will be introduced in the next section.

3.3 Deuterium substitution elongates singlet lifetime of chemically equivalent spin pairs

So far, we have discussed most of the methods to access the singlet state. For M2S and SLIC, both methods use the small chemical shift or out-of-pair $J$-coupling differences, which break the symmetry, as the handle to access to the singlet state. However, these handles also leave the singlet state not completely isolated, i.e., the singlet state could evolve into other states slowly and lose the ability to store the signal. Introduced by Claytor and Warren et. al. [35], one possible way to improve could be deuterium substitution for the protons, especially for the chemically equivalent spin pairs, for which the $J$-couplings play a critical role to create the singlet state.

Take the example of $^{13}$C enriched diacetylene, whose structure and $J$-coupling parameters are shown in Fig. 22, the two carbon-13 and two proton spins form the AA’XX spin system, with A for $^{13}$C and X for $^1$H. Determined by the symmetry of the molecule, it is evident that the carbons-13’s and protons are respectively chemically equivalent, while the $J$-couplings between the spins are not the same, which breaks the symmetry.
Figure 22: Structures and $J$-couplings of proton and deuterium substituted diacetylene (A) and diphenylacetylene (DPA) (B), with the target middle carbons isotope enriched.

Since the gyromagnetic ratio of deuterium is 6.5 times smaller than proton, i.e., $\gamma_{1H} / \gamma_{2D} = 6.5$, using deuterium to substitute proton will also decrease the $J$-coupling difference of the out-of-pair spins. In both examples, $\Delta J_{CH}$ is reduced by ~6.5 times, while $J_{CC}$ remains unchanged. This reduced $\Delta J_{CH}$ gives critical advantage for creating singlet spin order: with the smaller symmetry breaking agent, the singlet is “more disconnected” from other states, thus is more protected from relaxation and will have a longer lifetime.

As expected, experimental results showed that singlet lifetimes of both examples were extended by about 2 times longer ($T_\tau$-diacetylene increased from 49 s to 83 s, and $T_\tau$-DPA increased from 274 s to 479 s).

Further theoretical analysis showed why deuteration would promote the singlet state lifetime. Although the spin-1 nuclei bring much more complicated energy states, by writing the deuterium spins into coupled states, the analysis becomes straightforward:
there are 6 two-level subsystems that are connected by $\Delta J_{CD}$, 3 are in the symmetric group while the other 3 are in the antisymmetric group; and within each symmetry group there are two resonance frequencies.

The two deuterium spins are first combined as one spin-2 nucleus using the Clebsch-Gordan coefficients and $|F,M\rangle$ notation, where $F$ is the total angular momentum quantum number and $M$ is the z quantum number of deuterium. Since there are 3 spin states for a deuterium, we can use $p, z, \text{ and } m$ to represent $+1, 0, \text{ and } -1$ states of the spin. The combined $F = 2$ manifold is:

$$|2, +2\rangle = pp$$
$$|2, +1\rangle = \frac{1}{\sqrt{2}} (pz +zp)$$
$$|2, 0\rangle = \frac{1}{\sqrt{6}} (pm + 2zz +mp) \tag{3.6a}$$
$$|2, -1\rangle = \frac{1}{\sqrt{2}} (zm + mz)$$
$$|2, -2\rangle = mm$$

The $F = 1$ manifold is:

$$|1, +1\rangle = \frac{1}{\sqrt{2}} (pz -zp)$$
$$|1, 0\rangle = \frac{1}{\sqrt{2}} (pm -mp) \tag{3.6b}$$
$$|1, -1\rangle = \frac{1}{\sqrt{2}} (zm -mz)$$

and the $F = 0$ manifold is:
\[ |0,0\rangle = \frac{1}{\sqrt{3}} (pm - zz + mp) \quad (3.6c) \]

Now we can see that the combined states in \( F = 2 \) and \( F = 0 \) manifolds are all symmetric, while in \( F = 1 \) manifold they are all antisymmetric.

Next, since only the interaction Hamiltonian effects the singlet transition and the pulses are well on resonance of carbon-13, we can ignore the chemical shift Hamiltonian and the interaction Hamiltonian is:

\[
H_{\text{interaction}} = H_{AA} + H_{QQ} + H_{AQ} \\
= 2\pi J_{CC} I_1 I_2 + 2\pi J_{DD} S_1 S_2 + 2\pi J_{CD} (I_1 S_1 + I_2 S_2) + 2\pi J_{CD'} (I_1 S_2 + I_2 S_1) \quad (3.7a)
\]

Since the multi-bond deuterium-deuterium \( J \)-coupling, \( J_{DD} \), is small enough comparing to other terms (6.5\(^2\) times smaller than the usual \( ^1H\-^1H \) coupling), the \( H_{QQ} \) part could be neglected and equation (3.7a) reduces to:

\[
H_{\text{interaction}} = H_{AA} + H_{AQ} \\
= 2\pi J_{CC} I_1 I_2 + 2\pi J_{CD} (I_1 S_1 + I_2 S_2) + 2\pi J_{CD'} (I_1 S_2 + I_2 S_1) \quad (3.7b)
\]

Using the adapted basis together with the singlet-triplet basis set of the \( ^{13}\text{C} \) spins, the 6 two-level subsystems are listed below:

**Table 1: Two-level Subsystems of the AA'QQ' Spin System**

<table>
<thead>
<tr>
<th>Two-level Systems</th>
<th>( M_D )</th>
</tr>
</thead>
<tbody>
<tr>
<td>symmetric states</td>
<td></td>
</tr>
<tr>
<td>(</td>
<td>2, 1\rangle T_0 )</td>
</tr>
<tr>
<td>( \left[ \left(</td>
<td>2, 0\rangle + \sqrt{2}</td>
</tr>
<tr>
<td>$</td>
<td>2,-1\rangle_T_0$</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>$</td>
<td>1,1\rangle_T_0$</td>
</tr>
<tr>
<td>$</td>
<td>1,0\rangle_T_0$</td>
</tr>
<tr>
<td>$</td>
<td>1,-1\rangle_T_0$</td>
</tr>
</tbody>
</table>

For $M_D = \pm 1$ in each symmetry element, the resonance conditions are the same and for one example, the Hamiltonian submatrix is:

$$H_1 = T_0 \begin{pmatrix} |2,1\rangle_T_0 & |1,1\rangle_S \\ \langle 1,1| & \pi \Delta J_{CD} & \pi \Delta J_{CD} & -2 \pi J_{CC} \end{pmatrix}$$

So the resonance conditions within the $M_D = \pm 1$ cases are the same, however they are different for $M_D = 0$ cases, and one of the Hamiltonians is:

$$H_2 = T_0 \frac{1}{\sqrt{3}} \begin{pmatrix} \langle 2,0\rangle + \sqrt{2} \langle 0,0\rangle \right] T_0 & |1,0\rangle_S \\ \langle 1,0| & 0 & 2 \pi \Delta J_{CD} & 2 \pi \Delta J_{CD} & -2 \pi J_{CC} \end{pmatrix}$$

By examining the matrix elements, it is evident that in equation (2.8b) the transition frequency is two times larger than that in equation (2.8a), causing the resonance conditions to be different if using M2S pulse sequence (less inter-delay time, less number of 180° pulses). Furthermore, in equation (2.8b) we combine $|2,0\rangle$ and $|0,0\rangle$ states together because $J_{DD}$ is small enough and could be neglected, thus the original 3-level
system reduces to a 2-level system. If using SLIC pulse sequence, the situation here will be similar: while all these systems have the same nutation frequency for the CW pulse, which is $\omega_n = 2\pi J_{cc}$, the transition rate of the $M_D = 0$ case will still be 2 times larger than that of the $M_D = 1$ case.

3.4 Accessing long-lived singlet states of $^1H$ via $^2H$ coupling

Deuterium substitution opens another possibility to probe the singlet state of carbon-13. Although deuterium also brings quadrupolar interactions, inducing more complicated energy states, the quadrupolar interaction seems not a critical problem: singlet state of $^{13}C$ spins are well preserved and it has significantly longer lifetime than the normally protonated molecule.

The same strategy could be used to access the singlet states of chemically equivalent proton spin-pairs, such as ethylene. Originally, in the normal ethylene, all 4 protons are not only chemically equivalent, but also magnetically equivalent. Though each proton has 3 different $J$-couplings with other protons, all the spins have the same Larmor frequencies and cannot be distinguished. Introducing deuterium substitution breaks the magnetic equivalence, since deuterium has different Larmor frequency (6.5 times smaller than $^1H$). Also, the substitution on trans positions keeps the protons chemically equivalent, as well as the strong proton-proton coupling between them, thus forming an AA’QQ’ spin system that could be used to access the singlet state of the protons, as introduced in previous section.
It is interesting to note that, though the deuteration strategy has been proposed before [36], [37], the research shown in Ref. 36 did not particularly use the trans-deuterated ethylene and they only obtained limited lifetime, which was primarily due to the large amount of cis-deuterated ethylene synthesized during their experiment. But still, their method to measure a spin-isomer interconversion (could be the transition between $B_{1u}$ and $B_{2u}$ ethylene spin isomers with total angular momentum spin number equals 1) lifetime of the para-hydrogen enriched ethylene around 1000 s using chemical reaction to break the symmetry and unlock the signal is neat, since all proton spins are magnetically equivalent and there should be no interactions connecting the long-lived states of ethylene to the MR detectable states. In other words, states with different symmetry are never connected by the Hamiltonian.

In this section, I will show that long-lived states on $^1$H can be accessed by coupling to $^2$H; specifically, I studied trans-deuterated ethylene in solution, and showed that the singlet lifetime of the protons is ~30 times $T_1$ and can reach up to 117 s. The finding is particularly interesting for hyperpolarization storage, especially for para-hydrogen induced polarization (PHIP), which intrinsically produces proton singlets in closely related geometries when the protons are added to equivalent carbons such as DC≡CD or CDR=CRD.
Figure 23: (A) Ethylene with $J$-couplings shown. $J_n$ and $J_f$ are the near and far $J$-couplings between protons. (B) Trans-ethylene-$d_2$. $J$-couplings are shown in Hz. $J_{HH}$ in B is the same as in A; the near and far proton-deuterium scalar couplings are reduced by $(\gamma_D / \gamma_H)$, the deuterium-deuterium coupling is reduced by $(\gamma_D / \gamma_H)^2$.

3.4.1 Experimental setup and methods

Trans-ethylene-$d_2$ gas was delivered into a high-pressure NMR tube containing 500 μL per-deuterated hexane as solvent, with an initial tube pressure of ~150 psi. We measured the $^{13}$C (natural abundance) spectrum of sample and solvent to determine the (ethylene: hexane) ratio and found a signal ratio of 1:28. Hexane has density 0.65 g/ml and neat concentration is 7.54 M, so the concentration of ethylene was ~270 mM (7.54/28). Subsequent concentrations (i.e., equilibrium vapor pressure) were obtained by releasing a fraction of the gas. The remaining concentration was inferred by the relative amount of proton intensity of the ethylene peaks compared to the initial high-pressure proton measurement. Experiments were performed on our Bruker 8.45 T (360 MHz) spectrometer running Topspin 1.1. One specific note was that the deuterium lock needed to be turned off to avoid scrambling the spin states in our sample.
The M2S pulse sequence, discussed in previous section, was used to transfer population into the singlet state. To accurately measure the numerical values of the J-couplings was critical because it determined the resonance conditions of the M2S pulse sequence. These values were first estimated from the spectra and then refined by experimentally varying inter-pulse delay $\tau$ and 180° pulse number $n$ from which we could, in turn, get more accurate estimates of the J-coupling values.

Post processing and simulation were performed in MATLAB. The singlet decay (spectral extrema vs. $\tau$s) was fit to a bi-exponential decay. This allowed us to separately resolve any residual rapidly decaying triplet contribution from the long-lived state. Simulations of the spin system were performed using the SPINACH MATLAB package [38]–[40]. The outputs of a Gaussian09 simulation of trans-ethylene-$d_2$ molecular structure and NMR parameters were fed into SPINACH for NMR sequence simulation. The basis set of the density functional theory calculation used for the Gaussian09 simulation are gauge invariant atomic orbitals (GIAO) [41], hybrid functional (B3LYP) [42] and extension of Gaussian-type basis set (6-31G(df, 3p)) [43]. The $J$-couplings returned by Gaussian09 differed slightly from the couplings determined in experiment; GAUSSIAN output: $J_{HH} = 19.94$ Hz, $J_{HD} = 1.89$ Hz, $J_{HD'} = 0.35$ Hz, $J_{DD} = 0.47$ Hz; while the experimental values are: $J_{HH} = 19.10$ Hz, $J_{HD} = 1.73$ Hz, $J_{HD'} = 0.35$ Hz, $J_{DD} = 0.45$ Hz.
3.4.2 Experimental and simulation results

The resonance conditions of M2S are shown in Fig. 24, along with the simulated results of the singlet generated by M2S alone using the Gaussian09 calculated parameters, and the experimentally measured singlet after the full MSM sequence as both the inter-pulse delay and the number of pulses are swept. From both the simulation and experimental results, we can see that there are two distinct resonance conditions at $\tau = 12.6$ and 13.8 ms at the similar number of pulses (red and blue circles in simulation, black circles in experiment). The simulation results, however, is down shifted by around 0.6 ms, which is due to larger values of $J_{HH}$ and $J_{DD}$ are used. Also, in simulation there seems to be more resonance conditions (4 extra black circles) than the experiment, which will be discussed later in detail.

Figure 24: Resonance conditions of generating singlet as both the inter-pulse delay and number of pulses are swept. (A) Simulation results using only M2S pulse sequence and Gaussian09 output parameters. (B) Experimental signals after the whole MSM pulse sequence. Colored circles label the positions of resonance conditions.
We studied the singlet lifetime as a function of concentration by releasing the equilibrium vapor pressure of trans-ethylene-$d_2$ twice. The measurements of lifetime at a given pressure and resonance condition are aggregated and compared in Fig. 25 and Table 2. Though lifetime measurements under the resonance condition $\tau = 12.6$ ms was noisier than $\tau = 13.8$ ms, statistical test did not detect significant singlet lifetime difference under these two resonance conditions of different $\tau$. However, the singlet lifetimes of low pressure data were determined to be significantly different from the high and medium pressure data. The singlet lifetime was compared to the $T_1$ lifetime, and found to be ~30 times longer than $T_1$. (For low pressure experiments, the short-time component of the bi-exponential fit, $T_{\text{short}}$, was used to approximate the $T_1$ value).

![Figure 25: Singlet lifetime measurements of the $^1$H spin pair in trans-ethylene-$d_2$, at different pressure and resonance conditions.](image)

**Table 2: Aggregated $T_1$ and $T_S$ measurements of the $^1$H spin-pair of trans-ethylene-$d_2$**

<table>
<thead>
<tr>
<th></th>
<th>High Pressure ($\sim$ 150 psi, 270 mM)</th>
<th>Medium Pressure ($\sim$ 120 psi, 216 mM)</th>
<th>Low Pressure ($\sim$ 29 psi, 52 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$</td>
<td>1.06 ±0.02 s</td>
<td>1.53 ±0.02 s</td>
<td>N/A</td>
</tr>
<tr>
<td>$T_{\text{short}}$</td>
<td>2.44 ±0.44 s</td>
<td>2.94 ±0.12 s</td>
<td>5.59 ±0.32 s</td>
</tr>
<tr>
<td>$T_S @ \tau = 12.6$ ms</td>
<td>30.6 ±12.3 s</td>
<td>38.9 ±10.5 s</td>
<td>81.1 ±18.3 s</td>
</tr>
<tr>
<td>$T_S @ \tau = 13.8$ ms</td>
<td>30.6 ±10.7 s</td>
<td>44.2 ±3.38 s</td>
<td>117.1 ±9.80 s</td>
</tr>
<tr>
<td>$T_S / T_1 @ \tau = 12.6$ ms</td>
<td>28.9 ±11.6 s</td>
<td>25.4 ±6.87 s</td>
<td>14.5 ±3.38*</td>
</tr>
</tbody>
</table>
### 3.4.3 Discussion

Deuterium substitution of ethylene in trans position permits loading population into the proton pair singlet state and the it has a much longer singlet lifetime than $T_1$. To understand why this works, we need to go through the theoretical analysis below.

Like the deuterated diacetylene discussed in the previous section, the *trans*-ethylene-$d_2$ also forms an AA'QQ' spin system, however there are major differences. For diacetylene the $^{13}$C spins are already magnetically inequivalent and the singlet state is accessible; while for ethylene, without deuteration such states cannot be accessed by only using RF pulses, i.e., deuterium is required to break the intrinsic equivalence. Furthermore, in the diacetylene or DPA case, the multi-bond $^1$H-$^1$H coupling is already quite small and can be neglected ($J_{HH} \approx 0$) comparing to the strong $^{13}$C-$^{13}$C coupling, the even smaller $^2$D-$^2$D coupling will make no difference in terms of resonance conditions. However this is not the case for deuterated ethylene: the target spins’ coupling, $J_{HH}$, is not as strong comparing to the $J$-coupling difference which breaks the symmetry ($J_{HH} / \Delta J_{HD} \approx 14$, $J_{CC} / \Delta J_{CD} \approx 17$ for diacetylene, $J_{CC} / \Delta J_{CD} \approx 260$ for DPA); while the $^2$D-$^2$D coupling in ethylene, which is 0.45 Hz, is not small enough and cannot be neglected ($J_{DD} / \Delta J_{HD} \approx 0.3$, $J_{DD} / J_{HH} \approx 0.02$). Such relatively strong coupling of spin-1 nuclei could introduce interesting dynamics of spin order transitions.
The combined $|F, M\rangle$ basis of deuterium spins is the same as listed in equations (3.6 a, b and c). Since now $J_{dd}$ cannot be neglected, the interaction Hamiltonian is:

$$H_{interaction} = H_{AA} + H_{QQ} + H_{AQ}$$

$$= 2\pi J_{HH} I_1 I_2 + 2\pi J_{DD} S_1 S_2 + 2\pi J_{HD} (I_1 S_1 + I_2 S_2) + 2\pi J_{HD} (I_1 S_2 + I_2 S_1)$$

$$= 2\pi J_{HH} I_1 I_2 + 2\pi J_{DD} S_1 S_2$$

$$+ \pi (J_{HH} + J_{HD})(I_1 + I_2)(S_1 + S_2) + \pi (J_{HH} - J_{HD})(I_1 - I_2)(S_1 - S_2)$$

$$= H_{AA} + H_{QQ} + H_{\Sigma J} + H_{\Delta J}$$

(3.9)

where $J_{HD} + J_{HD'} = \Sigma J$ and $J_{HD} - J_{HD'} = \Delta J$.

In the combined angular momentum basis, $H_{AA}$, $H_{QQ}$ and $H_{\Sigma J}$ contribute along the diagonal because they always determine the eigenstates’ energy levels, i.e., eigenvalues. The eigenvalues for $H_{AA}$ are $\frac{\pi}{2} J_{AA}$ for $T_{1}$, $T_{0}$, $T_{1}$, and $-\frac{3\pi}{2} J_{AA}$ for $S$; similarly, the eigenvalues of $H_{QQ}$ are $2\pi J_{QQ}$ for the $F = 2$ states, $-2\pi J_{QQ}$ for the $F = 1$ states and $-4\pi J_{QQ}$ for the $F = 0$ state. On the other hand, $H_{\Sigma J}$ captures the change of $M$, the angular momentum quantum number. Since the Hamiltonian submatrices presented below describe state transitions with no changes of $M$, this term could be neglected. Finally, $H_{\Delta J}$ is the only term that contributes to the off-diagonal, since it is related with singlet-triplet coherences and will be the element that transfers states into singlet spin order.

Mathematical transformations give us the ability to quantify and understand the conversion of triplet states into long lived singlet in detail. Since only several individual
blocks are connected by the interaction Hamiltonian through the $H_{\Delta J}$ term in the chosen basis, we could simplify the matrices and focus only on the connected states.

Similar as diacetylene, we first find there are four 2-level sub-systems for $M_D = +1$ or -1, but only two are unique, they are:

$$H_1 = T_0 \langle 2,1 | \pi (J_{AA} + 2J_{QQ}) \pi \Delta J \pi \Delta J - \pi (J_{AA} + 2J_{QQ}) \pi \Delta J \pi \Delta J$$

and

$$H_2 = S \langle 2,1 | -\pi (J_{AA} - 2J_{QQ}) \pi \Delta J \pi \Delta J \pi (J_{AA} - 2J_{QQ})$$

Replacing $M = 1$ to -1 we will get the other two 2-level systems having the same transitions. For these subsystems, the resonance conditions transferring between the singlet and triplet states are:

$$\tau_1 = \frac{1}{2\sqrt{(J_{AA} + 2J_{QQ})^2 + (\Delta J)^2}}$$

$$n_1 = \pi / 2 \arctan \left( \frac{\Delta J}{J_{AA} + 2J_{QQ}} \right)$$

which is deduced from $H_1$. From $H_2$, the resonance condition is:
\[
\tau_2 = \frac{1}{2\sqrt{(J_{AA} - 2J_{QQ})^2 + (\Delta J)^2}}
\]
\[
n_2 = \pi \left( \frac{\Delta J}{2 \arctan \left( \frac{\Delta J}{J_{AA} - 2J_{QQ}} \right)} \right)
\]

Due to the relatively large value of \(J_{QQ}\), we see clearly that the resonance conditions for 2-level systems are split by this \(J\)-coupling term, the inter-pulse delay is slightly longer and the pulse number is a bit larger of \(H_2\) than \(H_1\), and they correspond well with the observed results. Another interesting point to note is that, instead of \(J_{AA} \pm J_{XX}\) in the AA’XX’ system, here it is \(J_{AA} \pm 2J_{QQ}\) for the AA’QQ’ system. The extra factor of 2 makes the resonance condition difference more obvious.

Besides the 2-level subsystems, there also exists two 3-level systems where \(M_D = 0\), the submatrices are:

\[
H_3 = \begin{pmatrix}
|2, 0\rangle T_0 & |1, 0\rangle S & |0, 0\rangle T_0 \\
T_0 \langle 2, 0| & \pi (J_{AA} + 2J_{QQ}) & 2\pi \frac{1}{\sqrt{3}} \Delta J & 0 \\
S \langle 1, 0| & 2\pi \frac{1}{\sqrt{3}} \Delta J & -\pi (J_{AA} + 2J_{QQ}) & 2\pi \sqrt{\frac{2}{3}} \Delta J \\
T_0 \langle 0, 0| & 0 & 2\pi \sqrt{\frac{2}{3}} \Delta J & \pi (J_{AA} - 4J_{QQ})
\end{pmatrix}
\]

and
Again since the $J_{QQ}$ term cannot be neglected, the $T_0 \langle 2,0 \rangle$ and $T_0 \langle 0,0 \rangle$ states, as well as the $S \langle 2,0 \rangle$ and $S \langle 0,0 \rangle$ states can no longer be merged and the resonance conditions are not degenerate, thus creating the 3-level systems, with 2 different resonance conditions in each of them. They are:

$$\tau_{3a} = \frac{1}{2 \sqrt{\left(J_{AA} + 2J_{QQ}\right)^2 + \left(2\Delta J / \sqrt{3}\right)^2}}$$

$$n_{3a} = \pi / \left(2 \arctan \left(\frac{2\Delta J / \sqrt{3}}{J_{AA} + 2J_{QQ}}\right)\right)$$

for the upper left two states transition of $H_3$ and

$$\tau_{3b} = \frac{1}{2 \sqrt{\left(J_{AA} - J_{QQ}\right)^2 + \left(2\sqrt{2}\Delta J / \sqrt{3}\right)^2}}$$

$$n_{3b} = \pi / \left(2 \arctan \left(\frac{2\sqrt{2}\Delta J / \sqrt{3}}{J_{AA} - J_{QQ}}\right)\right)$$

for the lower right two states transition of $H_3$.

For $H_4$, the resonance conditions are:
\[\tau_{4a} = \frac{1}{2\sqrt{(J_{AA} - 2J_{QQ})^2 + (2\Delta J / \sqrt{3})^2}}\]

\[n_{4a} = \pi \cdot \left(\frac{2 \text{ arctan} \left(\frac{2\Delta J / \sqrt{3}}{J_{AA} - 2J_{QQ}}\right)}{2}\right)\]

for the upper left two states transition of \(H_4\) and

\[\tau_{4b} = \frac{1}{2\sqrt{(J_{AA} + J_{QQ})^2 + (2\sqrt{2}\Delta J / \sqrt{3})^2}}\]

\[n_{4b} = \pi \cdot \left(\frac{2 \text{ arctan} \left(\frac{2\sqrt{2}\Delta J / \sqrt{3}}{J_{AA} + J_{QQ}}\right)}{2}\right)\]

for the lower right two states transition of \(H_4\).

The 4 resonance conditions from the 3-level systems are marked black in the simulation plot in Fig.24. All the experimental and theoretical resonance conditions are listed in the table below.

**Table 3: Experimentally optimal and theoretical resonance conditions of M2S for trans-ethylene-d₂**

<table>
<thead>
<tr>
<th></th>
<th>(\tau) (ms)</th>
<th>number of pulses</th>
<th>(\tau) (ms)</th>
<th>number of pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimentally</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>optimal:</td>
<td>12.6</td>
<td>18 or 24</td>
<td>13.8</td>
<td>18 or 24</td>
</tr>
<tr>
<td>Theoretical:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-level system</td>
<td>12.5</td>
<td>23</td>
<td>13.7</td>
<td>21</td>
</tr>
<tr>
<td>3-level system</td>
<td>12.5</td>
<td>20</td>
<td>13.3</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>12.7</td>
<td>14</td>
<td>13.7</td>
<td>18</td>
</tr>
</tbody>
</table>

Plugging in the \(J\)-coupling parameters, we see that the inter-pulse delay keeps nearly the same among different systems, and it is the more important parameter to transfer singlet state. As also seen from the experiments (Fig. 24), if the inter-pulse delay
is correct, changing the number of pules does not alter the measurements significantly, which could be due to the contribution of the 3-level systems. However, we still used the 2 resonance conditions that yielded the most signal to determine the $J$-coupling parameters of ethylene, which should be from the 2-level system and was largely consistent with Gaussian calculation.

3.4.4 Conclusion and possible applications of the ethylene singlet state

In conclusion, long lived singlet states have been observed on protons of deuterated ethylene gas. Substituting the trans-protons with deuterium, the singlet lifetime on protons is observed to be ~30 times longer than $T_1$. We extended the theory of the AA’QQ’ spin system to cover the case where $J_{QQ}$ is a significant fraction of $J_{AA}$. Series of measurements were done to explore the resonance conditions and the relationship between $T_s$ and equilibrium vapor pressure. Gaussian and SPINACH simulations of the spin system were also performed and found to agree with experimental work. Although in this project, M2S and MSM pulse sequences are mainly used, SLIC pulse sequence should yield theoretical consistent results. This geometry suggests a range of other interesting target systems, such as symmetric structures with deuterium atoms on opposite sides of unsaturated carbon bond (e.g. RDC=CDR) as motifs for storage of para-hydrogen induced polarization (PHIP) for longer lifetime than protonated molecules.
3.5 Conclusion

This chapter summarizes most of the methods recently developed to access the singlet state for variable situations: from largely inequivalent spins to chemically equivalent spins, from one spin pair to two spin pairs, from physical to chemical methods, and further extends the study of singlet state by discussing the states and transition dynamics using \textit{trans}-ethylene-$d_2$. Rigorous theoretical deduction and calculations are stated to help understand why and how these methods work to transfer the observable magnetization into the singlet states. Finally, the research of singlet state also leads us to an unexpected direction: using the theory, particularly about the AA’XX’ spin system to explore a novel method of hyperpolarization, i.e., signal amplification by reversible exchange (SABRE). This permits us not only to understand the new method, but also achieve great success expanding it towards much broader applications, which I will discuss in detail in following chapters.
4. Hyperpolarize long-lived states using \textit{para}-hydrogen

After introducing the long-lived state, \textit{para}-hydrogen and hyperpolarization, one can see that these three concepts are closely related with each other. Basically, we can consider \textit{para}-hydrogen itself as a “hyperpolarized long-lived agent” because the proton nuclear spins are in a pure singlet state. However, this agent itself cannot yield any NMR signal because it has zero magnetic momentum and is NMR silent. Nonetheless, \textit{para}-hydrogen has turned out to be a powerful means of hyperpolarization as discussed in chapter 2 for the PHIP techniques [9], [44]–[46].

The study about the transfer dynamics in AA’XX’ spin systems discussed in the last chapter led us to a deeper understanding of a novel hyperpolarization method involving \textit{para}-hydrogen, which is called SABRE (Signal Amplification by Reversible Exchange), a non-hydrogenating version of the PHIP methods. Furthermore, our study helps to develop the SABRE method to new dimensions: the hyperpolarized singlet state of \textit{para}-hydrogen can be used to induce magnetization or singlet order in a variety of substrates, as will be detailed in the following.

4.1 \textit{Signal amplification by reversible exchange} (SABRE)

Introduced by Adams \textit{et al}. [47], SABRE is a close variant of PHIP which also uses \textit{para}-hydrogen as the hyperpolarization source. Initially it was primarily used to polarize protons on target nitrogen containing aromatic heterocycles (pyridines, pyridazine, pyrazines, imidazole, triazoles etc.). The fundamental difference between PHIP and
SABRE is illustrated in Fig. 26: while the hydrogenation of unsaturated bonds performed in PHIP irreversibly alters the substrate structure, SABRE involves a transient interaction between \textit{para}-hydrogen and a substrate and it does not change the structure of the target molecule. However, this transient interaction is mediated by a transition metal catalyst and it requires coordinating the lone pair electrons on the substrates to attach to the transition metal catalyst, which can exchange hydrogen at the same time for SABRE to work.

In SABRE, the catalyst, \textit{para}-hydrogen and target substrate form a transient hydride complex in the methanol solution with a complex lifetime of around 40 ms [48], during which the hyperpolarization transfer happens at specific magnetic fields. The optimal magnetic field to polarize pyridine protons is around 65 G. Then the complex disassociates and goes into the next cycle. After a polarization build-up time of around 30
s, the sample is transferred quickly into the NMR instrument (1 T or 8.45 T NMR spectrometer) for signal detection and we can obtain largely amplified pyridine proton signal. Details of the catalyst and the polarization process are shown in Fig 27.

Figure 27: Illustration of the iridium catalyst activation (A - C) and pyridine proton polarization process using SABRE (D). In (E) we can see that the proton signal could be enhanced by ~1000 times.

SABRE has been an intriguing research topic since its introduction, but understanding the underlying spin dynamics leading to polarization was challenging. The SABRE process involves chemical dynamics, multiple exchange processes and the polarization source itself is NMR silent. Furthermore, the number of spins involved in the canonical examples (with three pyridine molecules acting as the substrate and a $^{31}$P containing catalyst) is on the order of 30. A massive breakthrough was simplification of
the spin system to an AA′BB′ system using a singlet-triplet basis. This simplification is based on the recognition that polarization transfer mainly proceeds in the equatorial plane of the catalyst, and the Hamiltonian of a C\textsubscript{2v} symmetry spin system can be used to account for the singlet transition dynamics in the same way as shown in last chapter.

Consider the case shown in Fig. 27. If for simplicity, we consider only one ortho-position proton (shown in blue in Fig. 28) from each pyridine, the two protons of \textit{para}-hydrogen and those two protons from each pyridine form an AA′XX′ spin system, where A and A′ stand for the \textit{para}-hydrogen derived hydride protons with identical chemical shift (-22 ppm), X and X′ stand for the pyridine protons having much larger, but also identical chemical shift (8.35 ppm, see Fig. 27e) because of the symmetry of the equatorial plane in the transient complex (Fig. 27c). Essentially, a square planar structure of these 4 protons is the result of this simplification, which is depicted on the left-hand side of Fig. 28. However, in this square planar symmetry the \textit{para}-hydrogen protons couple differently to the protons in the pyridine as depicted on the right-hand side of Fig. 28.
Figure 28: Structure (left) and $J$-coupling network of the AA’XX’ spin system (right) in the transient hydride complex. All 4 spins are protons, the \textit{para}-hydrogen derived $^1$H nuclei are shown in red, polarization transfer target $^1$H nuclei in blue.

When moving to low magnetic fields the field dependence of the frequency separation turns an AA’XX’ spin system into an AA’BB’ system, where letters close together in the alphabet denote that the frequency separation is small and is on the order of the $J$-coupling. As discussed in the last chapter, the $J$-coupling difference, $\Delta J_{AB} = J_{AB} - J_{AB'}$, breaks the symmetry and plays a crucial role in the singlet-triplet transition dynamics. As shown in the last section, the states of nuclear spin-pairs AA and BB in the Hamiltonian are now connected by an off-diagonal element proportional to $\Delta J_{AB}$. This element determines the rate of polarization flow. For the particular case of SABRE, \textit{para}-hydrogen enrichment defines the initial population of all states derived from \textit{para}-hydrogen, which is all AA’ state. However, the B spins were in thermal equilibrium, which means that there will be a population difference proportional to the \textit{para}-hydrogen enrichment acting as a driving force for hyperpolarizing specific spin states, when the energy difference between these states is matched (by providing the proper magnetic field). This is reminiscent of the
SLIC method discussed in the previous chapter, where the energy difference between the singlet state, $S$, and the basis adapted -1-triplet state, $\phi_{-1}$, had to be matched by continuous irradiation with a suitably chosen nutation frequency. The difference is that, while in SLIC population difference are very small, as defined by the Boltzmann factor $\gamma B/k_B T$, in SABRE the para-hydrogen enrichment defines the initial population as discussed before.

4.2 SABRE in shield enables alignment transfer to heteronuclei (SABRE-SHEATH)

A novel technique to hyperpolarize heteronuclei, such as $^{15}$N was introduced as SABRE-in shield enables alignment transfer to heteronuclei (SABRE-SHEATH) five years after the discovery of SABRE [49]. Theis, Warren and Chekmenev et. al. recognized that if $^{14}$N pyridine is replaced with $^{15}$N pyridine, one formally has the same AA’BB’ spin system discussed before. It is straightforward to recognize that, nitrogen-15 is hyperpolarized when the energy difference of the connected states is matched by choosing the correct magnetic field. From last chapter, we know that $J_{HH}$ and $J_{BB}$ are the diagonal Hamiltonian terms and they determine the energy levels of the singlet states; the chemical shift values $\pm v_A$ and $\pm v_B$, which are also on diagonal, determine the energy levels of triplet states and the plus or minus sign is dependent on the (+1) or (-1) state of the triplet.

It is important to remember that only the states of the same symmetry are connected. Suppose the para-hydrogen is 100% populated in singlet, and since the target spin pair is under thermal condition, they can be considered equally populated (each is 25%) for the 4 different states (1 singlet and 3 triplet). Start from the singlet-singlet state,
in the same symmetry group, this state will be connected to the states with net z spin angular momentum equals 0, i.e., to flip one spin pair up and the other one down, or vice versa, so the connected states are $|T_+^{A}T_-^{B}\rangle$ and $|T_-^{A}T_+^{B}\rangle$, whose energy levels are determined by $\pm(\nu_A - \nu_B) = \pm(\Delta \nu_{AB})$, or written in gyromagnetic ratios and the applied magnetic field, that is $\pm(\gamma_A - \gamma_B)B_{\text{match}}$. Thus, to match the energy difference between proton and nitrogen spins, it needs to satisfy:

$$J_{AA} + J_{BB} \approx \pm(\gamma_A - \gamma_B)B_{\text{match}} \quad (4.1)$$

Because of the exchange effect and the small $J_{BB}$ values, we can ignore the $J_{BB}$ term in finding the match conditions. An immediate point is seen that much lower magnetic field will be needed to polarize $^{15}$N, because the gyromagnetic ratio difference between proton and nitrogen is 36 MHz/T, accordingly we find the matching field to be ~0.4 μT (0.004 G). This is 100 times smaller than the earth magnetic field, which is about 0.5 G. A magnetic shield can be used to generate a field free region, which gives rise to the acronym SABRE-SHEATH. However, though a very low magnetic field is required, it is critical to understand that if the magnetic field is perfectly zero, there is no resonance frequency difference between different spin types anymore and no coherent polarization transfer should happen.

Apart from the symmetric group of the Hamiltonian, there are also off-diagonal elements allowing for evolution in between the anti-symmetric group. These are constituted by the coupled singlet-triplet and triplet-singlet states. The Hamiltonian will
create singlet state on the B spin pair, however, upon pyridine disassociation they will have equally distributed spin-up and spin-down thus no net polarization will be observed. Nonetheless, under the matching magnetic fields, the Hamiltonian will load populations out of $|T^B_{+1}\rangle$ or $|T^B_{-1}\rangle$ to the singlet state, thus depleting population of the original (+1) or (-1) triplet state of the B spin-pair. The matching condition is:

$$J_{AA} - J_{BB} = \pm (\gamma_A - \gamma_B) B_\text{match} \quad (4.2)$$

In SABRE, $J_{AA}$ is the coupling between the $para$-hydrogen derived hydrides ($-$(7-10 Hz)). The other coupling, $J_{BB}$, may be either the $J$-coupling between $^1$H nuclei in two different pyridine molecules, or the 2-bond $^{15}$N coupling over the Iridium center. When considering protons this is a 6-bond coupling, typically equals 0 Hz; when considering nitrogen-15, the small value of the gyromagnetic ratio of $^{15}$N renders this coupling unobservably small and $J_{NN} = 0$. Therefore, the resonance conditions in equations 4.1 and 4.2 degenerate to:

$$J_{AA} \approx \pm (\gamma_A - \gamma_B) B_\text{match} \quad (4.3)$$

From these considerations we can see that there is synergistic effect: population of the $|T^B_{-1}\rangle$ state is increased under the condition $J_{AA} \approx +\Delta \nu_{AB}$, while population of the $|T^B_{+1}\rangle$ state is depleted. Both two effects lead to the polarization of $|T^B_{-1}\rangle$ state. After dissociation this corresponds to enhanced polarization of the $\beta$ state. On the other hand, if the magnetic field preferential direction is inverted, we match the other resonance condition of $J_{AA} \approx$
−Δν_{AB}, accordingly after dissociation we obtain a hyperpolarized $\alpha$ state. This manifests itself as a 180° phase difference between spectra obtained by hyperpolarizing the spin system at the different matching conditions.

The magnetic field leading to efficient polarization buildup on $^1$H is 65 G, which is easily accessible by positioning the sample in the fringe field of a high field NMR magnet. For example, for the 8.45 T magnet in our laboratory, the 65 G stray field is found about 15 cm above the bore of the magnet.

The micro-Tesla field required to polarize nitrogen-15 is created inside a 3-layer mu-metal cylinder equipped with a small solenoid coil. The mu-metal is an extremely high susceptibility material and any space surrounded by it will be largely field free. The solenoid coil is used to obtain the required non-zero magnetic field. In our setup we use a coil with the number of turns $n_{\text{turn}} = 205$ and the length $l_{\text{solenoid}} = 0.42$ m, the magnetic field in the middle of the solenoid is given by $B = \left( \frac{U}{R} \times \frac{n_{\text{turn}}}{l_{\text{solenoid}}} \right) \times \mu_0$, where $U$ is the driving voltage, $R$ the resistivity and $\mu_0$ the permittivity of the vacuum. A simple calculation yields that a field of 0.6 $\mu$T is obtained if a series resistor of 11 kOhms and a supply voltage of 10 V is applied to the coil, corresponding to a current of 1 mA. The experimental setup is shown on the left of Fig. 29.
Figure 29: Apparatus to hyperpolarize $^{15}$N (left), the spin system (top right) and the process to detect hyperpolarized signal (bottom right). Right top shows the AA’BB’ spin system involving para-hydrogen and $^{15}$N nuclear spins, which is similar to the all proton system shown in Fig. 28.

4.3 Using SABRE-SHEATH to polarize long-lived states

So, we can use para-hydrogen to directly polarize a heteronuclear singlet when the required field is matched. To be able to observe that, we need non-equivalent positions in the high field, but a resonance frequency difference smaller than the $J$-coupling at low field. Chiral diazirines provides the proper molecule motif to approach this goal. [50].

4.3.1 $^{15}$N$_2$-diazirines present an opportunity to create magnetization and long-lived singlets on $^{15}$N spin-pairs

The structure of a $^{15}$N$_2$-diazirine and the spin system are shown in Fig. 30. In very low magnetic fields the two $^1$H’s from the para-hydrogen and the two $^{15}$N-nuclei of the diazirine moiety can be approximated as an AA’BB’ system. While the spin system is not symmetric and the $J$-coupling network is accordingly more complicated (there is now in
principle 4 different $J_{NH}$ couplings and a significant $J_{NN}$ coupling), there is no resonance frequency difference between nitrogen positions or the two hydrides in low field. There is, however, still a significant resonance frequency difference between $^1$H and $^{15}$N. If the diazirines has a chiral substituent reasonable close to the diazirine moiety the $^{15}$N positions have a significant resonance frequency difference $\Delta \nu_N$ in high fields. Therefore, the singlet and triplet states of the $^{15}$N spin-pair will be eigenstates in low magnetic fields when $\Delta \nu_N \ll J_{NN}$, but not in high fields.

![Figure 30: Structure and the spin system to polarize $^{15}$N$_2$-diazirine and the magnetic field dependency of hyperpolarized magnetization (blue) and singlet spin order (red). While the magnetization polarization is strongly field dependent, the singlet polarization stays nearly flat as the field elevates.](image)

If we now consider the Hamiltonian of an AA'BB' spin system, polarizing magnetization in a diazirine will require the same considerations as described in the previous section. However, the $J_{NN}$ coupling cannot be ignored and the two resonance conditions (Eqs. 4.1 and 4.2) may not be considered degenerate ($J_{HH} \approx -10$ Hz and $J_{NN} \approx -$
However, matching conditions are generally very broad as a result of the limited lifetime of the catalyst and the energy lifetime uncertainty. Therefore, the $J$-couplings are inherently affected by uncertainty and the same state will be polarized over a large range of magnetic fields. As shown in Fig. 30, significant levels of polarization are obtained even when sweeping the matching field relatively far away from the expected optimum of 5.75 mG.

To polarize the singlet spin order, the anti-symmetric group of the Hamiltonian needs to be considered. We are particularly interested in the conditions that connects the singlet state on the AA’ spin-pair with the singlet state on the BB’ spin-pair. We find that the energy levels are defined by $J_{NN}$ and $J_{HH}$, with no field dependent term. This means that the polarization transfer is, in principle, field independent. However, $J$-couplings are an intrinsic property of two coupled spins, so there is no handle to manipulate it. Nonetheless, in the case of a catalyst bound diazirine the $J_{HH}$ and $J_{NN}$ coupling happen to be very similar in magnitude and the resonance condition for singlet to singlet transfer is always fulfilled. Unlike magnetization, which is highly magnetic field dependent, singlet polarization is nearly field independent. This expectation based on theoretical considerations is clearly reflected in the experimental data shown in Fig. 30, where the singlet polarization is almost field independent over a large range of magnetic field (up to 12 $\mu$T). It is particularly interesting to note, that singlet polarization is more effective when magnetization is less effective and vice versa.
One more point needs to be clarified is, how to determine the fraction of signal originating from an initial singlet state, as all experimental spectra are superpositions of magnetization and singlet signal contributions. As already noted, a chemical shift difference between the nitrogen positions is required to break the symmetry of the singlet and render a singlet detectable. Upon adiabatic transfer to high magnetic field, if magnetization is polarized, the two chemically different $^{15}$N will show in-phase lines (i.e., $I_{1z} + I_{2z}$) in the pulse-acquire spectrum. However, if the singlet is hyperpolarized, as shown in chapter 2 in the ALTADENA process [50], adiabatically transfer the singlet from low field to high field will create single lines at each chemical shift position and the two lines are of 180° phase difference, i.e., $I_{1z} - I_{2z}$ is detected. Using the unique property of phase difference of magnetization and singlet components, the amount of signal from the magnetization and singlet individually could be expressed as:

\[
S_{\text{magnetization}} = \left( \frac{S_{\text{spin1}} + S_{\text{spin2}}}{2} \right)
\]

\[
S_{\text{singlet}} = \left( \frac{S_{\text{spin1}} - S_{\text{spin2}}}{2} \right)
\] (4.4)

This approach provides direct access to polarization levels of magnetization and singlet, enabled by the inequivalence of the $^{15}$N spin pair at high field. Spins with identical chemical shift do not evolve under a frequency different and accordingly S2M or SLIC [29], [34] pulse sequence, as well as a difference in J-couplings, are required to transform singlet into magnetization as discussed in last chapter.
After we have established how to accurately distinguish signal contribution from singlet and magnetization it was feasible to determine their characteristic relaxation constants, in the following referred to as lifetimes. By building up signal at a given field, followed by varied delay period and data analysis, we found that both 15N magnetization and singlet are long-lived at low magnetic fields. The $T_1$ (magnetization) lifetime of this diazirine compound is on the order of 6 min, and singlet lifetime $T_s$ is 23 min at 3 G. We note that such long lifetimes are of significance for developing hyperpolarized molecules in biochemical or biomedical applications.

### 4.3.2 SABRE-SHEATH to polarize $^{13}$C$_2$-pyridyl acetylene

We have seen from multiple examples [48], [50]–[53] that $^{15}$N can be readily hyperpolarized using SABRE-SHEATH, provided that a lone pair allowing for coordination to the catalyst exists. However, carbon is the backbone of all molecules sustaining life on earth. It is therefore extremely significant to investigate whether an approach exists that allows us to use SABRE-SHEATH to provide a rapid, continuous and easy way to hyperpolarize carbon-13, in particular, if both magnetization and singlet can be polarized. To our pleasure, the answer is affirmative.

In my research, I used SABRE-SHEATH to hyperpolarize magnetization as well as long-lived nuclear singlet states in carbon-13 spin pairs and found a magnetization lifetime $T_1$ of 12 s and a singlet lifetime $T_s$ of 2 min at 0.3 mT. Here it is important to note, that the current record of a long-lived singlet state is held by a $^{13}$C spin-pair.
(hyperpolarized by DNP, not SABRE-SHEATH) with Ts lifetime of more than one hour [54].

4.3.2.1 Materials and methods

For the presented experiments, we designed two molecules with various isotopic labeling schemes. We synthesized 1,2-(4-pyridyl) acetylene, with symmetric structure, and 1-phenyl-2-(4-pyridyl) acetylene, with asymmetric structure. For both, isotopomers with naturally abundant $^{13}$C, as well as doubly $^{13}$C labeled substrates at the triple bond are considered. Structures of the molecules and their magnetization polarization results are shown in Fig. 31. Furthermore, the standard precatalyst [IrCl(IMes)(COD)], (IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazole-2-ylidene; COD = cyclooctadiene, shown in previous section) is used.\(^{18-19}\) Substrate concentrations of 30 mM or 160 mM, and catalyst concentrations of 2 mM or 10 mM are used for the symmetric and asymmetric compounds respectively. The solvent was methanol-$d_4$, and the pre-catalyst was activated by bubbling \textit{para}$-\text{H}_2$ through the sample for 15 minutes at a pressure of 7 bar and a fractional \textit{para}-hydrogen enrichment of $\sim$85\%. Thereafter, hyperpolarization was performed according to the SABRE-SHEATH procedure [48]–[50]: the sample is exposed to \textit{para}$-\text{H}_2$ in a magnetically shielded environment outfitted with a small solenoid coil to obtain a controllable $\mu$T magnetic field. One minute of exposure to \textit{para}-H$_2$ is sufficient to equilibrate polarization. Subsequently, the sample is transferred manually as quickly as
possible (~8 s) to a Bruker 360 MHz (8.45T) magnet for read out. The manual transfer time of 8 s is relatively consistent, with variations of ~1 s.

As shown in Fig. 31, acetylene carbon spins as well as the aromatic bridge carbon spins are hyperpolarized. The enhancements are between 30 to 170-fold (corresponding to 0.02% to 0.12% polarization) compared to thermal signals acquired at 8.45 T. The molecules with $^{13}$C at natural abundance show 2-3 times higher enhancements compared to $^{13}$C enriched sites. This is likely due to faster $T_1$ relaxation in $^{13}$C$_2$ pairs as opposed to $T_1$ of isolated $^{13}$C spins. An additional cause may simply be the higher ratio of polarization source ($p$-H$_2$) to target spins in the naturally abundant case [55].
Figure 31: $^{13}$C spectra of naturally abundant (A&B) and $^{13}$C labelled (C&D) substrates used in experiments. A&C show results for the symmetrically substituted 1,2-2 pyridyl acetylene. B&D are from the asymmetrically substituted 1-phenyl-2-(4-pyridyl) acetylene. For the naturally abundant substrates the bridge carbons on the pyridyl rings (3, 4 in A, 3 in B) show significant enhancement, while the one on the benzene ring (4 in B) is only slightly hyperpolarized. The $^{13}$C-$^{13}$C coupling, $J_{CC}$, read from the line-splitting in panel D is 185 Hz.

4.3.2.2 Possible complex configurations during polarization transfer

The polarization transfer occurs in transient hydride complexes. Two possible energetically low polarization transfer complex (PTC) species, as well as other energetically higher species, are depicted in Fig. 31. The ground state energies were
determined by density functional theory calculations. Furthermore, the $^1$H-NMR spectra of the hyperpolarized hydrides bound to the Iridium center demonstrating the presence of at least two catalytic species (Fig. 32). In this first study, we were not able to detect hyperpolarized $^{13}$C signals from molecules bound to the Iridium molecules. Therefore, we rely on more indirect evidence coupled with $ab$ $initio$ calculations to determine likely the complex structures.
Figure 32: Two possible polarization transfer complexes and other possible complexes. Top: 3D models obtained after energy minimization in the all electron code FHI-aims. Bottom: Structural formulas of the PTCs for clarity. (A) The substrate is bound to all Ir binding sites via nitrogen. (B) One of the molecules’ triple bond binds to the iridium catalyst, which has a higher energy than the structure in A. DFT calculations reveal that the energy difference between the two proposed complexes is relatively small (0.09 eV). (C) Other structural possible complexes. However, since their energies are much higher, they are not considered responsible for polarization transfer.
Figure 33: $^1$H-NMR spectra of the asymmetric molecule. (A) Thermal $^1$H spectrum of the asymmetric molecule and its peak assignment, note that protons on position 2 and 3 are close to each other, and position 3 protons have little higher chemical shift than position 2. (B) Hyperpolarized (SABRE) $^1$H spectrum. para-H$_2$ is bubbled through the sample at 6.5 mT, an ~57 folds enhancements of position 1 and 2 protons are obtained. However, position 3 and 4 protons’ enhancements are not observed. (C) Spectrum acquired using 45° pulse immediately after stopping bubbling inside the magnet. The hydrides are observed and there are two major resonances with chemical shift difference around 0.2 ppm, as well as clear indications of minor species.

In the first complex model (Fig. 32A), all substrate molecules bind to the iridium center via nitrogen. This is the energetically lowest species identified by us. Here, spin orders diffuse from para-H$_2$ to the pyridyl protons first and finally transfer to the acetylenic carbons. In the second model (Fig. 32B), the catalyst binds with the triple bond and polarization is transferred directly to $^{13}$C sites.

The spectra displayed in Fig. 31 could quickly lead to the conclusion, that the active PTC must be the directly binding model (Fig. 32B), because we do not observe hyperpolarization from the ring carbons, other than from those in the bridge to the acetylene bond. Moreover, we observe hydrogenation, which most certainly requires binding of the triple bond to the iridium center. Hydrogenation rates depend on the ratio
of substrate to catalyst: at 3:1 hydrogenation completes in less than 30 minutes, however at above 15:1, hydrogenation takes more than 12 h. In a single SABRE experiment (with 1 min of bubbling) we estimate significantly less than 1% hydrogenation at the 15:1 ratio, which was used for most experiments. We even observe hyperpolarized hydrogenation products that display typical PASADENA type enhancements due to incorporation of \( \text{para-H}_2 \), as shown in Fig. 34.

![Figure 34: Hydrogenation of the asymmetric substrate and the PASADENA type enhancement of the hydrogenated acetylenic carbons.](image)

However, these conclusions may be premature. First, the PTC in of Fig. 32A is energetically lower. Furthermore, notice that all non-detected \(^{13}\text{C}\) spins are directly bound to protons. This leads to much faster \(^{13}\text{C}\) relaxation (a typical \( T_1 \) relaxation time for aromatic \(^{13}\text{C}\) directly bound to a proton is \(~5\) s \([56]\), whereas \( T_1 \) relaxation constants of the bridge carbons are found to be \(11(1)\) s and \( T_1 \) of the acetylenic carbons is \(12(0.5)\) s at low
fields) with two important consequences. First, the hyperpolarization buildup at these \(^{13}\)C sites will be much less efficient, and second, a small amount of hyperpolarization may quickly relax during the ~8 s sample transfer from polarization region into the magnet. In addition, as shown in Fig. 33, SABRE was performed under optimized condition for \(^1\)H polarization transfer at 6.5 mT, and this resulted in strong enhancement of the pyridyl ring protons, while enhancement of \(^{13}\)C were negligible and \(^1\)H enhancements on the distant phenyl ring were much smaller. A small chemical shift difference of ~0.2 ppm of the hydride peaks is observed, which would be much larger for the binding mode in Fig. 32B based on DFT calculation. Finally, I attempted to hyperpolarize diphenyl-13C2-acetylene (no ring nitrogens) in the SABRE-SHEATH mode and did not observe enhancements. All these considerations point to a strong contribution of the PTC shown in Fig. 32A.

### 4.3.2.3 Magnetic field dependent polarization

To help investigate the details of the polarization transfer complexes, another useful and interesting experiment to perform is the careful characterization of hyperpolarization transfer as a function of micro-Tesla field using the doubly \(^{13}\)C labeled molecules. As depicted in Fig. 35, the magnetic field was varied between -12 and +12 µT, accompanied by simulations of the hyperpolarization transfer process.
Figure 35: Field dependent hyperpolarization for the two substrates. Panel A shows (1) a hyperpolarized magnetization spectrum hyperpolarized at 0.17 µT (and acquired at 8.45 T) for the symmetric substrate and (2) its field dependence in the µT range. Panel B shows the experimental and simulated results of creating magnetization and singlet order for the asymmetric substrate, as function of magnetic field; (1) Magnetization spectrum hyperpolarized at 0.28 µT. (2) Experimental and (3) simulated field dependence for magnetization. (4) Singlet spectrum hyperpolarized at 6.2 µT. (5) Experimental and (6) simulated field dependence for singlet order. In (B3) and (B6), the highlighted points are the local maxima for polarization transfer labeled by analytically derived resonance conditions from careful inspection of the nuclear-spin Hamiltonian.

Different from the $^{15}$N$_2$-diazirine case, where the singlet polarization is field independent, we can now choose to directly polarize different states of the $^{13}$C pair, which are easily distinguishable by their spectra and the field dependence function.
Magnetization is easily detected from both molecules (Fig. 35 A1, B1), whereas singlet-order can only be detected immediately from the asymmetric 1-phenyl-2-(4-pyridyl) acetylene because the acetylenic carbons have a chemical shift difference (Fig. 31D). For this asymmetric compound, the acetylenic carbons are strongly coupled at low fields ($J_{\text{CC}}$ is $\sim185 \text{ Hz}$, whereas their chemical shift difference $\Delta \nu_C$ is less than $0.5 \text{ mHz}$). Upon transfer to the high field in the magnet ($8.45 \text{ T}$) for read out, the chemical shift difference becomes significantly larger than the $J_{\text{CC}}$ coupling ($\Delta \nu_C \sim770 \text{ Hz}$), the carbons are now weakly coupled, and the singlet state is no longer an eigenstate. As mentioned in last section and chapter 2, the sample transfer from low to high field transforms $I_1 \cdot I_2$ singlet order into detectable ($I_{1z} - I_{2z}$) which gives antiphase signals in a pulse acquire experiment, as shown in Fig. 35(B4). However, for the symmetric molecule, since the two carbons will remain symmetric at high field, the singlet state cannot be accessed immediately. In principle, access to the singlet can be accomplished by specialized pulse sequences such as singlet-to-magnetization (S2M) or SLIC, as mentioned earlier.

To analyze the polarization transfer mechanisms in detail, we can start from the spin system and its Hamiltonian. Consider a four-spin system consisting of two para-hydrogen derived hydrides and two $^{13}\text{C}$ spins of the acetylenic bond as depicted in Fig. 36. This represents the direct model, where the substrate is bound to iridium via the acetylenic bond.
Figure 36: The four-spin system of two $^1$H and two $^{13}$C spins, the iridium metal atom in the middle is omitted for simplicity. On the right is the rearranging terms for $\Delta J_{\text{near}}$, $\Delta J_{\text{far}}$, $\Sigma J_{\text{near}}$ and $\Sigma J_{\text{far}}$. Approximation of the $J$-coupling values (from both calculation and experimental results) are also shown.

Then Hamiltonian of this system can be expressed as:

$$H = v_H (I_{1H} + I_{2H}) + v_C (I_{1C} + I_{2C}) + J_{HH} I_{1H} I_{2H} + J_{CC} I_{1C} I_{2C} + J_{CH_{\text{near}}} I_{1H} I_{1C} + J'_{CH_{\text{near}}} I_{2H} I_{1C} + J_{CH_{\text{far}}} I_{1H} I_{2C} + J'_{CH_{\text{far}}} I_{1H} I_{2C}$$  

Introducing the CH-$J$-coupling terms (also illustrated in Fig. 36), the sum and the difference of the near and far $J_{CH}$ couplings can be written as:

$$\Sigma J_{\text{near}} = J_{CH_{\text{near}}} + J'_{CH_{\text{near}}}$$

$$\Delta J_{\text{near}} = J_{CH_{\text{near}}} - J'_{CH_{\text{near}}}$$

$$\Sigma J_{\text{far}} = J_{CH_{\text{far}}} + J'_{CH_{\text{far}}}$$

$$\Delta J_{\text{far}} = J_{CH_{\text{far}}} - J'_{CH_{\text{far}}}$$  

Using these definitions, the Hamiltonian is rearranged as:
\[ H = v^H (I^H_{1Hc} + I^H_{2Hc}) + v^C (I^C_{1cc} + I^C_{2cc}) \\
+ J_{HH} \bar{I}^H_{1H} \bar{I}^H_{2H} + J_{CC} \bar{I}^C_{1Cc} \bar{I}^C_{2Cc} \\
+ \frac{\Sigma J_{near}}{2}(\bar{I}^H_{1H} \bar{I}^C_{1Cc} + \bar{I}^H_{2H} \bar{I}^C_{2Cc}) + \frac{\Delta J_{near}}{2}(\bar{I}^H_{1H} \bar{I}^C_{1Cc} - \bar{I}^H_{2H} \bar{I}^C_{2Cc}) \tag{4.7} \\
+ \frac{\Sigma J_{far}}{2}(\bar{I}^H_{1H} \bar{I}^C_{2Cc} + \bar{I}^H_{2H} \bar{I}^C_{1Cc}) + \frac{\Delta J_{far}}{2}(\bar{I}^H_{1H} \bar{I}^C_{2Cc} - \bar{I}^H_{2H} \bar{I}^C_{1Cc}) \tag{4.8a} \]

As similar in the $^{15}$N pyridine and $^{15}$N$_2$-diazirine cases, using the singlet-triplet basis for both the hydride (from para-$\text{H}_2$) and the $^{13}$C pair, we can identify all possible 16 combinations of $^1\text{H}$ and $^{13}$C states, which can be sorted in two groups based on their symmetry, they are 10 symmetric states with respect of exchanging H$_1$ with H$_2$ and C$_1$ with C$_2$:

\[
S^H_0 S^C_0, \quad T^H_0 T^C_0 \\
T^H_+ T^C_-, \quad T^H_- T^C_+ \\
T^H_+ T^C_+, \quad T^H_- T^C_- \tag{4.8a} \\
T^H_0 T^C_+, \quad T^H_+ T^C_0 \\
T^H_0 T^C_-, \quad T^H_- T^C_0 \\
\]

And 6 antisymmetric states with respect of exchanging H$_1$ with H$_2$ and C$_1$ with C$_2$:

\[
S^H_0 T^C_0, \quad T^H_0 S^C_0 \\
S^H_0 T^C_+, \quad T^H_+ S^C_0 \\
S^H_0 T^C_-, \quad T^H_- S^C_0 \tag{4.8b} \\
\]

Again, since we are using highly enriched para-hydrogen, the initial states are the para-hydrogen derived singlet (100%) on the hydride proton pair and thermally equally populated states (25% for each) on the carbon pair, thus the initial states have the following populations:

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\[ p(S_0^H S_0^C) = p(S_0^H T_0^C) = p(S_0^H T_0^C) = p(S_0^H T_0^C) = 0.25 \]
\[ p(\text{other states}) = 0 \]

where there is 25% population on the symmetric singlet-singlet state, and 25% population for each of the anti-symmetric singlet-triplet states. Usually, in the structural symmetric AA'BB' cases, such as the $^{15}$N-pyridine situation in Fig. 29, the $\Delta J$ near and far terms are equal but opposite in sign while the $\Sigma J$ near and far terms are equal, that is:

\[ \Delta J_{\text{near}} = J_{\text{AB}} - J_{\text{A'B'}} = -(J_{\text{AB}} - J_{\text{A'B'}}) = -\Delta J_{\text{far}} \]
\[ \Sigma J_{\text{near}} = J_{\text{AB}} + J_{\text{A'B'}} = +(J_{\text{AB}} + J_{\text{A'B'}}) = +\Sigma J_{\text{far}} \]

Such “higher symmetry” will make the states in different groups totally disconnected because the $J$-coupling terms will cancel with each other when appearing on the off-diagonal (the effect is more clearly seen in equation 4.11). However, this is not the case for the acetylenic $^{13}$C spin pair, nor for the diazirine $^{15}$N spins either. For these spin pairs, the “lower symmetry” breaks the numerical equivalence of the $\Delta J$ near and far terms as well as the $\Sigma J$ terms thus connecting states that belong to different symmetry groups, one of the sub-blocks in the Hamiltonian is:

\[
\begin{pmatrix}
\langle S_0^H T_0^C \rangle & \langle T_0^H T_0^C \rangle \\
\langle S_0^H T_0^C \rangle & -V_C - J_{\text{HH}} & -\frac{\Delta J_{\text{far}} - \Delta J_{\text{near}}}{4} \\
\langle T_0^H T_0^C \rangle & -\frac{\Delta J_{\text{far}} - \Delta J_{\text{near}}}{4} & -V_H
\end{pmatrix}
\]

(4.11a)

When energy levels of the two states are equalized, population transition from $\langle S_0^H T_0^C \rangle$ to $\langle T_0^H T_0^C \rangle$ happens. This can be considered as depleting the $\langle T_0^C \rangle$ state and
creating hyperpolarized (+1) coherence, correspondingly, the complementary sub-block in the Hamiltonian is:

\[
\begin{pmatrix}
|S_0^H T_+^C\rangle \\
|T_+^H T_0^C\rangle
\end{pmatrix}
\begin{pmatrix}
\Delta J_{\text{far}} + \Delta J_{\text{near}} \\
4
\end{pmatrix}
\]

\[
\begin{pmatrix}
|S_0^H T_+^C\rangle \\
|T_+^H T_0^C\rangle
\end{pmatrix}
\begin{pmatrix}
\Delta J_{\text{far}} + \Delta J_{\text{near}} \\
4
\end{pmatrix}
\]  

thus, the resonance conditions are:

\[
\nu_H - \nu_C = \pm J_{\text{HH}} \quad (4.12)
\]

Since the chemical shift frequency \( \nu = \gamma B \), the magnetic field to match this resonance condition is:

\[
B = \frac{\Delta \nu}{\Delta \gamma} = \frac{\nu_H - \nu_C}{\gamma_H - \gamma_C} = \frac{\pm J_{\text{HH}}}{\gamma_H - \gamma_C} \quad (4.13)
\]

Using \( J_{\text{HH}} \approx -8 \text{ Hz}, \gamma_H = 42.577 \text{ Hz}/\mu\text{T}, \gamma_C = 10.705 \text{ Hz}/\mu\text{T} \), the magnetic fields are calculated as around \( \pm 0.25 \mu\text{T} \), which are close to the experimental results.

Equations 4.12 and 4.13 demonstrate the resonance conditions to polarize magnetization at low field by connecting states belong to different symmetry groups. On the other hand, within the symmetric group, the other connected states also provide possible magnetization polarization conditions. The sub-matrix is:
Correspondingly, to polarize the $|T_{-}^C\rangle$ or $|T_{+}^C\rangle$ magnetization, the resonance conditions are:

$$v_H - v_C = \pm \left( J_{CC} + J_{HH} \right) + \frac{\Sigma J_{near} + \Sigma J_{far}}{4}$$  \hspace{1cm} (4.15)$$

In this spin system, since the $\Sigma J$ term is around $7/4 = 1.75$ Hz, which is much smaller than the $J_{CC} + J_{HH}$ term of $185 - 8 = 173$ Hz, the sum of the $J$-coupling term could be dropped and the resonance conditions are simplified as:

$$v_H - v_C = \pm \left( J_{CC} + J_{HH} \right)$$  \hspace{1cm} (4.16)$$

Plugging in the numeric values, the magnetic fields are found around $\pm 5.4 \mu T$, which are also consistent with the experimental results. This result could also be understood intuitively: since the carbon singlet state is now involved, because of the strong carbon-carbon $J$-coupling, higher energy will be needed to match the energy level difference for the spins to flip, thus much higher magnetic field is needed.
Similarly, the singlet polarization conditions can be identified by examining the Hamiltonian matrix subsets that connect the hydride singlet and the singlet on carbons, they are:

\[
\begin{align*}
\left\langle S^H_0 T^+ \right\rangle & = -J_{HH} + v_C + \frac{\Delta J_{near} - \Delta J_{far}}{4}, \\
\left\langle T^H_+ S^C_0 \right\rangle & = -J_{CC} + v_H
\end{align*}
\]  

(4.17a)

and

\[
\begin{align*}
\left\langle S^H_0 T^- \right\rangle & = -J_{HH} - v_C + \frac{\Delta J_{near} - \Delta J_{far}}{4}, \\
\left\langle T^H_- S^C_0 \right\rangle & = -J_{CC} - v_H
\end{align*}
\]  

(4.17b)

So, the resonance conditions are:

\[v_H - v_C = \pm (J_{CC} - J_{HH})\]

The matching magnetic fields are around $\pm 6 \mu$T after plugging in the numerical values, which are close to the second magnetization polarization condition and match with the experimental results. A specific point to note is that, at high magnetic fields when the $^{13}$C pair singlet and triplet could be interchanged, creating the carbon $T_+$ or $T_-$ magnetization will at the same time deplete the carbon singlet $S_0$, which could be considered as an “inverse” hyperpolarization of the singlet state; and vice versa, creating the singlet $S_0$ will at the same time deplete $T_+$ or $T_-$, which is also an “inverse”
hyperpolarization of the magnetization. Thus, besides the resonance conditions directly derived above in the subsets of the Hamiltonian, the full resonance conditions to hyperpolarize magnetization and singlet are the same at high field ($\pm 5.4$ and $\pm 6 \mu T$), they are:

$$v_H - v_C = \pm (J_{CC} \pm J_{HH})$$

(4.18)

and the matching magnetic field is:

$$B = \frac{\Delta v}{\Delta \gamma} = \frac{v_H - v_C}{\gamma_H - \gamma_C} = \frac{\pm (J_{CC} \pm J_{HH})}{\gamma_H - \gamma_C}$$

(4.19)

The resonance conditions shown in equation 4.18 match exactly with the simulation results shown in Fig. 35., where we see 4 resonance conditions for both magnetization and singlet, including both positive and negative fields. However, this is not the case for the low field, where the carbon singlet is not involved at all. Since only the magnetization is created or depleted, which can also be seen from equation 4.11, it is exclusive to polarize magnetization at low fields.

Equations 4.11, 4.14 and 4.17 fully encompass the behavior observed in Fig. 35. In the low field region, maximum magnetization transfer is observed at $\sim \pm 0.34(0.1) \mu T$, whereas there is negligible singlet buildup. At slightly elevated fields, both magnetization and singlet have local maxima/minima at $\sim \pm 5.6(0.2) \mu T$ and $\sim \pm 6.4(0.2) \mu T$ (see Fig. 35B). These values are consistent with $J_{HH} \sim -10(3) \text{ Hz}$ and $J_{CC} \sim 190(5) \text{ Hz}$. $J_{CC}$ can also be
estimated from the hyperpolarized NMR spectrum of the free form where we find \( J_{cc} = 185 \text{ Hz} \) (in Fig.31 panel D).

### 4.3.2.4 Simulation of direct and indirect polarization transfer

As shown in Fig. 37, the simulation of direct polarization transfer shows great consistency with the theoretical derivation. Furthermore, I used simulation to detect if there were differences between the polarization transfer patterns using the two modes. I simulated the direct and indirect (by including the pyridyl protons) models and vary parameters of the \( J \)-coupling terms used in the simulation. However, as illustrated in Fig. 37, though only the positive fields were used in the simulation, it was found that the direct and indirect polarization transfer spin systems tend to give quite similar transition patterns for comparable \( J \)-coupling constants in the source (\( J_{HH} \)) and the target (\( J_{cc} \)) spins.
Figure 37: Matlab simulation using the Spinach package, for the indirect polarization transfer (A, where there are 8 spins in the system) and the direct polarization transfer (B, where there are 4 spins in the system). Simulation shows similar pattern of the magnetization and singlet for indirect or direct polarization transfer. On the other hand, the magnetic field at which the singlet peak appears is closely related with the $J_{CC}$ coupling: the stronger this coupling is, at higher field the singlet peak will appear.

As apparent from Fig. 37, in combination with the analytical expressions provided for the resonance conditions, the polarization profile is determined by two critical $J$-coupling parameters, $J_{HH}$ and $J_{CC}$. $J_{HH}$ determines the position of the first resonance condition for polarizing magnetization, it also controls the distance between the two resonance conditions at higher fields. Finally, $J_{CC}$ dictates the overall position of the higher field resonances. This is true irrespective of the hyperpolarization transfer pathway (direct or indirect).
In addition to the simulation of the polarization transfer at low field (from -12 to 12 µT), simulations at higher field (6.5 mT) where \(^1\)H hyperpolarization works best are also performed. However, all the simulations give 0 transfer to \(^{13}\)C, which is as expected because at such field there is no energy level matching between the para-hydrogen derived hydride \(^1\)H and the acetylenic \(^{13}\)C states. Although the polarization is transferred to pyridyl \(^1\)H, there is still no energy level matching between the pyridyl \(^1\)H’s and the \(^{13}\)C’s. This is also consistent with the experimental result, where the \(^{13}\)C polarization is negligible when bubbling at 6.5 mT.

By numerical simulations of the spin dynamics it is confirmed that the general behavior is largely independent of whether the polarization transfer is indirect or direct. However, the numerical value of \(J_{CC}\) strongly depends on the exact nature of the PTC. Again, we refer to the \textit{ab initio} calculations, which predict a \(J_{CC}\) of ~191 Hz for substrate bound via nitrogen (Fig. 32A) vs. a \(J_{CC}\) of ~120 Hz for substrate bound directly via the acetylenic bond. Based on the measurements shown in Fig. 31, we can now conclude with more confidence that the primary PTC is the energetically favored species shown in Fig. 32A because for the PTC in Fig. 32B we would expect efficient hyperpolarization at significantly lower fields of 3.5 ± 0.3 µT, which is not observed.

\textbf{4.3.2.5 Lifetime measurements of hyperpolarized states}

Finally, since the asymmetric molecule allows for easy read out of both singlet state and magnetization, we can measure their lifetimes \(T_S\) or \(T_1\). As displayed in Fig. 38,
$T_S$ was measured at 0.3 mT and 50 mT respectively, and was fitted with exponential decay constants of 117(7) s and 69(4) s. For comparison, we measured $T_1$ at the field where it has longer $T_S$ (0.3 mT) and find that magnetization decays much more rapidly with exponential decay constant $T_1$ of $12(5)$ s. The $T_1$ lifetime of the $^{13}$C$_2$ pair at 8.45T is measured as $8(0.4)$ s.

![Figure 38: $T_1$ and $T_S$ measurements of 1-phenyl-2-(4-pyridyl) acetylene. For all measurements, the sample was first hyperpolarized in the shield using 0.35 $\mu$T (polarize magnetization) / 6 $\mu$T (polarize singlet order) then positioned at 0.3 mT or 50 mT. After varying the delay time, the sample was transferred to the magnet quickly to measure the remaining signal. The data points were sampled randomly to eliminate the effect of the slow triple bond hydrogenation, and the lifetime constants were obtained using single exponential fit.](image)

4.3.2.6 Conclusion

In this research project, I demonstrated that, both magnetization and long-lived singlet order can be induced on $^{13}$C$_2$ using SABRE-SHEATH. Hyperpolarization lifetime is extended to ~2 minutes, or 10 times of its $T_1$. Furthermore, I described direct
hyperpolarization of long-lived singlet order by SABRE-SHEATH when the $J$-coupling in
the targeted spin pair is much larger than the $J_{HH}$ coupling between the hydrides. This
contrasts with the first demonstration of heteronuclear ($^{15}$N$_2$) long-lived states
hyperpolarized by SABRE, where $J_{NN}$ and $J_{HH}$ were comparable in size leading to a
resonance condition that is matched at a broad range of fields, raising the question if long-
lived states could be hyperpolarized when $J_{NN}$ or $J_{CC}$ are much larger. Here I have shown
that specific $\mu$T-fields work in that case. Hyperpolarization levels and enhancements
remained relatively low in this first demonstration. A likely culprit is the quadrupolar $^{14}$N
nuclei, as we are finding that quadrupoles act as highly efficient polarization sinks at $\mu$T
fields. Therefore, the hyperpolarization could likely be boosted by additional $^{15}$N labeling
of the substrates and other strategies detailed in the literature [57], [58]. Also, since no
pyridine was used in the carbon hyperpolarization experiments, the catalyst may be
compromised lacking this ligand and polarization levels could be lowered. By adding
pyridine to the solution and adjusting its concentration, higher polarization results could
be achieved. Finally, clear evidence has been assembled for at least two potential PTC
species that simultaneously exist in solution and I presented arguments that lead us to
believe that polarization transfer is primarily mediated indirectly via protons in the
substrates. Overall, the presented results illustrate an avenue towards simple and fast
hyperpolarization of long-lived $^{13}$C hyperpolarization with potential applications in
biomolecular MRI or the observation of slower processes by hyperpolarized NMR. The
presented advances can be translated to biomolecules already shown to be amenable to heteronuclear SABRE hyperpolarization including nicotinamide [59], \textit{in vivo} pH sensor imidazole [60], hypoxia sensor metronidazole [53] and others [48], [52]. While the current work was performed in methanol solutions, recent advances in heterogeneous [61], [62] and water-soluble [59], [60], [63]–[65] SABRE catalysis may lead to \textit{in vivo} translation of the presented approach for fast and continuous hyperpolarization of long-lived $^{13}$C molecular probes.

4.4 Discussion and conclusion

In conclusion, we developed SABRE-SHEATH as a general, effective, convenient and cost-efficient method which can hyperpolarize biologically relevant nuclei, such as $^{15}$N and $^{13}$C. We have shown that, SABRE-SHEATH can be used to directly polarize the long-lived singlet states of the heteronuclear spin pairs. We have furthermore shown that, the $J$-coupling of the target spin-pairs is proportional to the resonance conditions, thereby allowing to selectively hyperpolarize specific spin orders by matching field to the $J$-coupling magnitude.

Hyperpolarized singlets offer enhanced and prolonged NMR signals and easy access to persistent and strong NMR signals, which is a great advantage that other hyperpolarization techniques cannot provide. Though there are structural requirements of the target substrates, SABRE can polarize a broad range of molecules, including the ones that are involved in biology and biochemistry.
Using the SABRE-SHEATH technique, we can investigate complicated processes and very diluted samples [61] using NMR. This extends the reach of NMR to provide access to reaction monitoring with low concentration intermediates, such as biochemical processes. For example, since the $^{15}\text{N}_2$ group on diazirine has similar structure as the -CH- groups of the carbon backbones in biomolecules, it could be used as a label which enables monitoring the metabolism of tagged compound.

Another important type of reaction is the “click” reaction [66], [67], which is widely used in pharmaceutical and material industries for target molecules screening. Because of its high specificity, high yield and fast reaction rate, molecules that undergo this type of reaction could also be used as bio-tags or markers to detect whether a certain chemical process exists in vitro or in vivo. My research in the next chapter exactly shows how we could achieve this goal by combining SABRE-SHEATH with a click reaction. It will be shown that, the spin states polarized during SABRE-SHEATH could be preserved over the chemical reaction, thus leading to the creation of singlet enriched $^{15}\text{N}_2$, which is also known as “para-\text{N}_2”. This is an exciting application of SABRE-SHEATH because para-nitrogen is like para-hydrogen, which should have long-lived lifetime and could yield greatly amplified signal once its symmetry is broken and the signal is unlocked. Furthermore, it could be used as a perfect imaging agent for the lung since it composes 70% of the air. SABRE-SHEATH provides a practical and efficient way to generate hyperpolarized singlet gas and it will be introduced in next chapter.
5. SABRE-SHEATH for bioorthogonal chemistry and unearthing \textit{para}-^{15}\text{N}_2

As shown in previous chapters, SABRE-SHEATH provides a novel approach to rapidly and continuously hyperpolarize heteronuclear spins, such as \textsuperscript{15}N and \textsuperscript{13}C, which can be used to augment the information of \textsuperscript{1}H NMR. Furthermore, SABRE-SHEATH can directly hyperpolarize long-lived singlet states, which will allow to trace metabolism and biological functions of endogenous molecules and their derivatives of the living organisms for an extended time.

However, so far nearly all the demonstrated SABRE-SHEATH results, including the hyperpolarization experiments in aqueous solution, are concerned only with the hyperpolarizable target molecules themselves. Besides the developments of bio-compatible solvents and catalysts for SABRE-SHEATH, the question that whether SABRE-SHEATH could be integrated with chemical reactions, is critical to advance this novel technique towards \textit{ex vivo} or \textit{in vivo} applications. Using the context of bioorthogonal chemistry, we found that SABRE-SHEATH is well suitable for such type of reactions. Depending on the initial states of the polarized substrate, we can create either magnetization or singlet hyperpolarized nitrogen-15 gas. The latter is known as \textit{para}\textsuperscript{15}\text{N}_2, which is also a spin rotational isomer as \textit{para}-hydrogen introduced in chapter 2 and is likely to have very long-lived lifetime as well. In addition, since it is natural and harmless,
hyperpolarized $\text{para}^{-15}\text{N}_2$ could be a perfect agent for lung imaging using magnetic resonance.

5.1 Materials and methods

5.1.1 Bioorthogonal chemical reactions

As the term suggests, bioorthogonal chemical reactions are reactions that do not interfere with biological processes found in vivo [66], [67]. The individual components of such reactions react with each other rapidly and highly selectively, thus they will not affect other functionalities that are necessary to sustain life even under physiological conditions. Based on their properties, bioorthogonal chemistries are primarily and intensively used to probe diverse classes of biomolecules, such as glycans, lipids, nucleic acids and various metabolites, in living systems in real time [68]–[74]. In these applications, one component will be integrated on a biomolecule as the chemical reporter, while its reaction partner is covalently linked with a certain probe (such as a fluorophore or an affinity probe). As the biomolecule, together with its reporter, is introduced into the cell, this biomolecule will go through various biochemical transformations and will finally be secreted out of the cell, with the reporter attached to the newly synthesized biomolecule. At the presence of the partner, bioorthogonal chemical reactions between the reporter and its partner will occur thus we could specifically probe the newly synthesized biomolecules, which are of great significance to understand the metabolism.
and the network of biomolecules. Schemes of bioorthogonal reactions are shown in Fig. 39.

![Scheme of bioorthogonal chemistry](image)

**Figure 39:** [From Ref. 64] Scheme of bioorthogonal chemistry. The blue solid circle, acting as the chemical reporter, will go through bioorthogonal reaction with the orange crescent, acting as the complementary component. The bioorthogonal reaction will not interfere other biological processes in a cellular or complex environment. Once bioorthogonal reactants are “clicked” together, the target biomolecule will be specifically linked with the probe, shown as the orange star and together they could be directly visualized or analyzed.

### 5.1.2 Hyperpolarizable bioorthogonal tags

To date, a wide range of possible reactions has been discovered and developed as bioorthogonal reactions, such as aldehyde/ketone condensation [75], Staudinger ligation [76], and azide-alkyne cycloadditions [77]. Among all the possible reactions, we find that inverse electron demand Diels-Alder (IEDDA) reaction [78], which is a well-studied cycloaddition reaction using tetrazine and cyclooctyne (shown in Fig. 40) can be used after hyperpolarization. In this reaction $^{15}$N(4)-tetrazines can be polarized by SABRE-SHEATH while exhibiting high reactivity with strained cyclic unsaturated hydrocarbons, such as cyclooctyne. Thus, tetrazine has a dual role as the polarization agent and can at the same time serve as the tag for biomolecules. We note that attempts to polarize azides, which also constitute broadly used precursors for bioorthogonal reactions, remained unsuccessful.
Apart from the possibility to induce $^{15}$N magnetization by means of SABRE-SHEATH, it is reasonable to assume from structural considerations and the strong $^{15}$N-$^{15}$N $J$-couplings, that the long-lived singlet spin order of the $^{15}$N pairs of the tetrazine can also be polarized. This gives tetrazine a special advantage as bio-tag because it would still yield contrast even consecutive reactions with reaction partners are proceeded by a slow step (e.g., diffusion). Furthermore, tetrazines react selectively and rapidly with strained azadienophiles by IEDDA, one of the fastest bioorthogonal reactions reported and various tetrazine-tagged biomolecules have been successfully used in vivo and in vitro [79]–[81]. All these special properties make tetrazine an ideal experimental motif to test our proposal.

**Figure 40:** Scheme of integrating bioorthogonal reaction and SABRE-SHEATH hyperpolarization using all nitrogen-15 enriched tetrazine. The tetrazine precursor here has dual roles: the nitrogen’s will first be hyperpolarized by SABRE-SHEATH, followed by the reaction with cyclooctyne, the hyperpolarized signal remaining on the nitrogen’s in the product is also a probe to selectively highlight and localize the reaction partner which contains the target.

In our “proof-of-concept” research, instead of using a real specific target biomolecule, we used strained cyclooctyne bicyclo[6.1.0]non-4-yn-9-ylmethanol (target in
Fig. 40 = $^1$H) as the reaction partner of 3-phenyl-$^{15}$N$_4$-1,2,4,5-tetrazine (R = Ph). Structures of the reaction partners used are also shown in Fig. 41.

5.1.3 Reaction-promoted hyperpolarization setup

The hyperpolarization setup and procedure is the same as shown in Fig. However, the SABRE catalyst is hydrogenation catalyst, therefore the reactant (cyclooctyne) needs to be injected after polarization buildup. Therefore, we modified our conventional para-hydrogen bubbling setup and enabled direct injection of cyclooctyne into the solution of hyperpolarized tetrazine by adding a capillary tube into the pressurized NMR tube, the sample tube setup is shown in Fig. 41.

Figure 41: Modified para-hydrogen flow and cyclooctyne injection system. After $^{15}$N magnetization buildup, the valve is open to release the residual para-H$_2$
pressure and cyclooctyne is injected. After 3 s for reaction the sample is transferred to 8.45T for detection.

5.2 Experimental results

5.2.1 Hyperpolarization of $^{15}$N$_4$-1,2,4,5-tetrazine

Hyperpolarization of tetrazine was examined by standard SABRE-SHEATH procedure. The $J$-coupling network of tetrazine and the possible polarization transfer complex is shown in Fig 42. Note that since tetrazine is symmetric along the reflection line in the middle, with the strong $J_{NN}$ coupling between nitrogen’s along the same side of tetrazine, i.e., $J_{N1N2} = J_{N4N5} = -25$ Hz, and the weak couplings cross the sides of the molecule, i.e., $J_{N1N4} = J_{N2N5} \approx J_{N1N5} = J_{N2N4} \approx 3$ Hz, the hyperpolarization situation of tetrazine could be considered very similar to the case of $^{15}$N$_2$-diazirine, except there are now two same $^{15}$N$_2$ spin-pairs instead of one. This helps to significantly simplify the analysis and as expected, depending on the chosen magnetic field at which para-hydrogen gas is applied to the sample, two different hyperpolarized states of $^{15}$N$_4$-1,2,4,5-tetrazine were observed. At very low magnetic fields, where they satisfy the resonance conditions:

$$\nu_H - \nu_N = \pm J_{HH}$$

magnetizations of the $^{15}$N spin pairs of the tetrazine are hyperpolarized and display in-phase signal upon detection at 8.45 T. Using the gyromagnetic ratios and hydride $J_{HH}$ couplings, which are $\gamma_H = 42.577$ Hz/$\mu$T, $\gamma_N = -4.316$ Hz/$\mu$T and $J_{HH} \approx -10$ Hz, the expected magnetic field is around $\pm 0.21 \mu$T. In experiments, the optimal magnetic field (only positive field was used in this research) to hyperpolarize magnetization is around
0.3 μT, which is close to the theoretical calculation, and the enhancement is around 2700-fold (over thermal signal at 8.5 T, corresponding to 0.74% polarization).

Figure 42: The $J$-coupling network of tetrazine and one possible polarization transfer complex configuration.

To hyperpolarize the singlet state, the analysis is also like that of the diazirine case. Since $J_{NN}$ coupling (around -25 Hz) is comparable to the para-hydrogen derived hydride coupling $J_{HH}$ (around -10 Hz), and due to the large uncertainty of these values, the resonance condition:

$$J_{HH} = J_{NN}$$

is always approximately satisfied thus the singlet should be polarizable for tetrazine. Upon transfer to high field for signal detection, the presence of asymmetric substituents, phenyl and $^1$H, will render the $^{15}$N positions on the same side magnetically inequivalent. Thus, adiabatically transport of the singlet from low to high field will convert the spin order $I_1, I_2$ to $I_z - I_{2z}$, resulting in an anti-phase signals with the two chemically shifted resonances on acquisition. The experimental results match the expectations: anti-phase
alignment of the anti-Zeeman magnetization is observed over relatively broad range of magnetic fields ($\sim 2 \mu T < B < \sim 20 \text{ mT}$). Beyond 20 mT the signal begins to decrease because the chemical shift frequency difference between the nitrogen’s is becoming significantly larger than the $J$-coupling and their eigenstates are deviating away from the singlet-triplet states. The observed field dependency of hyperpolarized states selection is consistent with previous work about the hyperpolarization of $^{15}$N$_2$-diazirines and $^{13}$C$_2$-pyridyl acetylenes.

In our research, we used 0.3 mT to hyperpolarization the singlet spin order, where magnetization cannot be polarized and the magnetic field is small enough to keep singlet as eigenstate. The enhancement is up to 3000-fold over the thermal signal (at 8.45 T), which is about 0.89% polarization.

![Field dependence of magnetization and singlet](image)

**Figure 43: Hyperpolarization field dependency of $^{15}$N-tetrazine.**

The magnetization and singlet lifetimes of $^{15}$N$_4$-tetrazine are measured by positioning the sample at a certain magnetic field for various delays before detection after
the polarization buildup. At 0.3 mT, the magnetization has a $T_1$ lifetime of $1.4 \pm 0.1$ min, and at the same field, lifetime of the singlet state of the $^{15}$N spin pairs, $T_s$, is $2.7 \pm 0.3$ min, which is about 2 times longer than the magnetization. This is as expected because the singlet state is protected from symmetry and is immune to intramolecular dipole-dipole relaxation, thus it should have a longer lifetime.

Note that the strong $J$-coupling (14 Hz) between $^1$H (6) and $^{15}$N (1) and $^{15}$N (5) (see Fig. 42), lifetime of the nitrogen spin pairs could be shortened by the scalar relaxation of the second kind [82], which scales with $J$-coupling, and accounts for a slow relaxing spin being pulled to equilibrium with a thermal bath by a fast relaxing one. As mentioned in chapter 3, replacing this $^1$H (6) with deuterium will reduce the $J$-coupling by 6.5 times and it could affect the enhancement and lifetime [35], [83].

With this expectation, we measured the signal enhancements and lifetimes using the single deuterated isotope tetrazine, finding a 2900-fold enhancement, with $T_s$ calculated to be $2.1 \pm 0.7$ min at 0.3 mT. There is no significant change of enhancement or lifetime for the singlet observed upon deuteration. However, the magnetization state is poorly polarized and the lifetime cannot be determined. The reason may be that though the $J$-coupling is decreased, the quadrupolar deuterium near the nitrogen spin-pairs quenches hyperpolarization at μT fields [56]. Results of these two types of tetrazines and their lifetimes are shown in Fig. 44.
5.2.2 Bioorthogonal reactions of hyperpolarized $^{15}$N₄-tetrazine and cyclooctyne

With hyperpolarization of protonated and deuterated tetrazines established successfully, we examined whether the hyperpolarization could be retained in the reaction products of IEDDA reactions. In an initial study using unlabeled tetrazine and cyclooctyne, we confirmed that the reaction rate of the cycloaddition at room temperature is sufficiently rapid. The reaction is completed within seconds: once the cyclooctyne
solution is injected into the tetrazine solution, the pink color of tetrazine will disappear almost immediately. However, though the reaction is fast, diffusion of the reactants turns to be a problem. The biggest challenge is to make sure all the reactants are consumed in a short amount of time, otherwise we found the measurements to be inconsistent. To overcome this difficulty, the end of the injection capillary was modified by poking pinholes using a thin needle, which accelerates the distribution of the injected cyclooctyne. To ensure good mixing and distribution we found a 3-second shaking period for all the reactions to be sufficient, for all reactants to complete reaction.

There are two advantages using this particular cycloaddition reaction. First, the formation of a single pyridazine product allows for a straightforward analysis, avoiding potential complexity from multiple products which arise when other known azadienophile partners, such as *trans*-cyclooctene, are used [66], [84]. We obtained the thermal $^{15}$N reference spectra of both reactant tetrazine and the product after IEDDA reaction, from which we observe a clear distinction between the reactant and product by their $^{15}$N chemical shifts. Second, the reaction product cannot be hyperpolarized by SABRE-SHEATH. Under the same conditions (concentrations, temperature and para-hydrogen quality), it is confirmed that there is no hyperpolarized magnetization or singlet signal of the cycloaddition product can be obtained. The reason could be that the bulky stereo structure of the product prevents its contact with the catalyst. This provides
convincing evidence that all signals observed for the reaction products originate from hyperpolarized tetrazine.

We first examined the cycloaddition of the protonated tetrazine after hyperpolarizing magnetization. After addition of a solution of cyclooctyne to a sample of hyperpolarized tetrazine at 0.3 mT and subsequent transfer to high field, we observed sharp, in-phase peaks at 372 ppm that matched the position and pattern of the peaks observed in the thermal spectra of the product. An additional signal was detected at 310 ppm, which corresponds to hyperpolarized $^{15}\text{N}_2$ gas. This data reinforces that the IEDDA reaction of hyperpolarized $^{15}\text{N}_4$-tetrazine successfully generates hyperpolarized $^{15}\text{N}$-containing products, including both $^{15}\text{N}_2$-pyridazine and $^{15}\text{N}_2$ gas. Based on the spectrum of hyperpolarized products, we calculated an enhancement of 540-fold over their thermal spectra. The magnetization lifetime $T_1$ for $^{15}\text{N}_2$-pyridazine was determined to be 13±4 s, substantially shorter than that of tetrazine.

Next we investigated the reaction of tetrazine starting from hyperpolarizing scalar order (induced by bubbling at 0.3 mT). After the solution of cyclooctyne was injected to the solution of hyperpolarized tetrazine at the same field (0.3 mT) and transferred to high field for detection, we observed anti-phase peaks at 372 ppm, with 140-fold signal enhancement over the thermal spectra. The singlet lifetime $T_s$ for $^{15}\text{N}_2$-pyridazine was determined to be 13±2 s, very like its $T_1$ lifetime. This is in stark contrast to the significant
difference observed between $T_1$ and $T_s$ for the protonated precursor molecule (1,2,4,5-$^{15}$N(4) tetrazine).

Last, we investigated the effect of deuteration in the cycloaddition reaction. As mentioned before, because magnetization is inaccessible, only scalar order could be tested for the deuterated tetrazines. Therefore, we were restricted to measurements of singlet lifetime $T_s$ in the deuterated pyridazine product. Very similar to the observation in the reaction of protonated tetrazine, anti-phase peaks at 372 ppm were detected with a similar enhancement level of 180-fold. Encouragingly, though the deuterated tetrazine did not show longer singlet lifetime, a substantially longer $T_s$ of 24±6 seconds was obtained from the deuterated product. All the hyperpolarized reaction results are shown in Fig. 45.
Figure 45: IEDDA Reaction and Hyperpolarization Transfer. (A) Thermal spectra of tetrazine 1a, compared to thermal spectra of cycloaddition product 3a. Upon the cycloaddition reaction, a noticeable difference in chemical shift is observed on both nitrogen atoms (i.e., N1 and N2 in 1a vs. N1’ and N2’ in 3a). (B) Spectra of hyperpolarized 1a and the spectra obtained after the addition of 2. Hyperpolarized 3a and $^{15}$N$_2$ are observed. (C, E, G) Representative $T_1$ or $T_2$ decay measurements for product 3a magnetization, 3a singlet order, and 3b singlet order, respectively. Note the presence of observable $^{15}$N$_2$ in the post-addition spectra when hyperpolarizing magnetization (C), but absence of this peak in hyperpolarized singlet (E, G), which strongly suggests singlet $^{15}$N$_2$ has been generated in these experiments. (D, F, H) Lifetime measurements data, exponential fit of the data, and calculated $T_1$ or $T_2$ values.
for product 3a magnetization, 3a singlet, and 3b singlet respectively. In the hyperpolarization-cycloaddition experiments, para-hydrogen was bubbled into a solution of 15N4-1,2,4,5-tetrazine 1a or 1b (1.5 mM), pyridine (1.0 mM), and Ir(COD)(IMes)Cl (0.15 mM) in methanol-d4 (400 μL), then a solution of 2 (1.5 equiv) in methanol-d4 (200 μL) was added. The sample was held at 0.3 mT for a variable amount of time before transport to the magnet for detection.

5.2.3 Product hyperpolarization lifetime measurements at high field

To measure both the hyperpolarized magnetization and singlet lifetimes of the reaction product of the 1H isotopomer of the tetrazine at high magnetic field (8.45 T), a series of small tip angle pulses with a spacing of 1.6 s is applied to the sample. To that end, after injection of the cyclooctyne in the sample and mixing for 3 seconds, the tube is inserted into the magnet immediately. With a small tip angle of 15° or 11.25° only a part of the signal is used per transient and fast signal relaxation can be investigated. Although the signal is much weaker than in 90° acquisitions, hyperpolarization yields sufficient signal. Using this strategy, even the fast ortho-15N2 relaxation could be recorded and its T1 lifetime could be estimated.
Figure 46: Small tip angle spectra of the protonated tetrazine hyperpolarized cycloaddition product. (A) Magnetization of $^{15}$N-enriched tetrazine 1a is first hyperpolarized at 0.4 μT, then a solution of 2 in methanol-$d_4$ is injected. Then, the sample is manually transferred as quickly as possible into the magnet, using 15° tip angle to acquire 6 spectra consecutively, with a 1.6 s delay between each acquisition. The product shows in-phase signal at ~372 ppm, and at ~310 ppm, we can clearly see a singlet corresponding to hyperpolarized $^{15}$N$_2$ gas in the first 4 spectra. (B) Singlet spin order of $^{15}$N-enriched tetrazine 1a is first hyperpolarized at 0.3 mT, then a solution of 2 in methanol-$d_4$ is injected. Then, the sample is manually transferred as quickly as possible into the magnet, using 11.25° tip angle to acquire 6 spectra consecutively, with a 1.6 s delay between each acquisition. As expected, the product shows anti-phase signal at ~372 ppm, but at ~310 ppm there is no signal, indicating that para-$N_2$ is generated (thus yielding no $^{15}$N signal). (C, D, E) Relaxation constant measurements of the hyperpolarized product magnetization, hyperpolarized $^{15}$N$_2$ gas, and hyperpolarized product singlet. The product magnetization has $T_1$ lifetime of ~6.9 s, which is quite similar as $T_s$ (~7.4 s), and this is consistent with the 90° pulse acquisition measurements at low field.

As can be seen from Fig. 46, more than two magnetization hyperpolarized product spectra have signals at 310 ppm, and they are decreasing quickly. In addition, none of
singlet hyperpolarized product spectra has signal other than at 370 ppm, which comes from the pyridazine. This also confirms that the peak observed at 310 ppm in the pulse acquire detections belongs to the hyperpolarized ortho-$^{15}$N$_2$, and by adjusting the polarization magnetic field we could generate hyperpolarized \textit{para}-^{15}$N$_2$. Lifetimes of the hyperpolarized magnetization ($T_1$) and singlet ($T_s$) of the pyridazine product were measured with small flip angle method. We found that decay times are identical within the experimental error (see Fig. 46, $T_1 = T_s \approx 7$ s). That there is no significant difference between singlet and magnetization decay time constants is also observed at low magnetic field, where both characteristic time constants are around 13 seconds (see Fig. 45 d, f). For the other reaction product, hyperpolarized \textit{ortho}-^{15}$N$_2$, we found the $T_1$ lifetime to be around 3 seconds at 8.45 T (in methanol-$d_4$ solution). We note that although the pyridazine’s signal appears to be much larger than the $^{15}$N$_2$ gas signal (see Fig. 45 c, Fig. 46 a), if we trace back relaxation for about 4 seconds, that is the sample transport time into the magnet, we would find that the initial signal for both $^{15}$N species is approximately equal. This is expected due to the symmetry of the tetrazine precursor (1a), where no steric or electronic factors render the $^{15}$N spin-pairs on either side of the mirror plane inequivalent, and hence they should exhibit same polarization levels after SABRE as well as reactivity with the cyclooctyne. This trace back method verifies that signals at 310 ppm are from \textit{ortho}-^{15}$N$_2$. Also, Colell \textit{et. al.} has measured \textit{ortho}-^{15}$N$_2$ thermal signals and
calibrated the ppm scale to be IUPAC using a gas sample, confirming our conclusion of the generation of ortho-$^{15}$N$_2$ gas.

5.2.4 Reactions of hyperpolarized tetrazine and cyclooctyne in aqueous solution

One important aspect of the bioorthogonal reaction-promoted hyperpolarization tagging approach is its possibility under aqueous conditions, which is critical to develop the method towards ex vivo or in vivo biomedical applications. Applicability of SABRE-SHEATH hyperpolarization in water using a water-soluble iridium catalyst and elevated temperatures (60 – 70 °C) [85], [86] has been reported. We attempted to adopt the same strategy, that is, to use D$_2$O as a solvent at higher temperatures for tetrazine hyperpolarization. However, the substrate tetrazine is not stable at high temperature for an extended period of time and the activation time required for aqueous SABRE is much longer due to water’s high viscosity. Instead, we achieved our best results using a volume ratio 3:1 methanol-$d_4$/D$_2$O mixture and by elevating the temperature to about 50 °C using a water bath while bubbling para-hydrogen through the solution. As shown in Fig. 47, the protonated tetrazine precursor is hyperpolarized with an enhancement of around 900-fold ($p = 0.27\%$) at 0.3 mT (i.e., singlet state is chosen to be hyperpolarized), which is about 30% compared to that using neat methanol-$d_4$. After hyperpolarization of the tetrazine a solution of cyclooctyne in 3:1 methanol-$d_4$/D$_2$O was injected.
Figure 47: SABRE-SHEATH experiment using methanol-\textit{d}_4/D_2O (3:1) mixture as solvent. (A) The $^{15}$N-tetrazine precursor can be hyperpolarized using the same protocol as previously described, and the enhancement is around 900-fold. However, the signal after injection is quite small: only \textasciitilde 1% of the hyperpolarized signal is observed as shown in (B), and there is large amount of residual tetrazine left, which could be caused by the high viscosity of the solvent and the compound cannot completely react in a short time period of 3 seconds.

As expected, though we could observe the hyperpolarized product signal, the polarization level was much lower than previous experiments in methanol. This could be caused by several reasons. First, the viscosity of the water-containing solvent mixture is much higher. This means that \textit{para}-hydrogen diffusion may be inhibited. Second, diffusion and mixing of the injected compound and reaction with the precursor are impeded. And last, relaxation times may be shorter in a different solvent. As can be seen from Fig. 47, residual tetrazine peaks are still of significant signal intensity after injection of the cyclooctyne: due to high viscosity and surface tension of water and the internal diameter of the pressure NMR tubes (4 mm) the sample cannot be mixed easily by shaking. Nevertheless, we find the preliminary result to be encouraging. Although there
is a need for optimization of the experimental setup and catalyst to enable aqueous SABRE at lower temperature, our results show that the strategy works.

5.3 Discussion

The result presented in this chapter show that, hyperpolarization of both magnetization and singlet order accessible by SABRE-SHEATH can be retained over a bioorthogonal reaction, leading to a new reaction-promoted hyperpolarization tagging strategy and paving the way for more general SABRE-SHEATH applications.

It is intriguing that, although the singlet of tetrazine (compound 1a) has a significantly longer lifetime than its magnetization, the cycloaddition product does not. On the other hand, for the deuterated tetrazine (1b), where the singlet lifetime is almost identical to the protonated version (1a), the reaction product (3b) has substantially longer lifetime than the protonated product (3a). This could be explained by the difference between the precursor four-spin and product two-spin systems. While the matching conditions for magnetization as well as singlet are in full analogy to the diazirines described in the last section due to the identical mechanism involved in polarization buildup and similar magnitude of the $^{15}\text{N}-^{15}\text{N} J$-coupling, there is distinctive difference in spin topology between tetrazine precursor and pyridazine product. The cycloaddition reaction removes all symmetry elements present in the tetrazine (a mirror plane and a C$_2$ axis). In addition, the reflection symmetry of the molecule along the middle line containing the proton could make the singlets less affected by the intramolecular
coupling. Thus, whether the strong $^1$H coupling is present or not will not significantly alter the singlet relaxation and the two versions of tetrazine have similar singlet lifetimes. However, more detailed and sophisticated calculations may be needed to support these arguments. On the other hand, after the cycloaddition reaction, two $^{15}$N’s will be eliminated from tetrazine as nitrogen gas and there are only two chemically inequivalent $^{15}$N’s left in the product. For these two $^{15}$N spins, since they are asymmetric and not protected from a more compact and symmetric system, the presence of the strong $^1$H coupling from one side is now critical to interfere with the relaxation, which could lead to much shorter singlet lifetime and in the experiments, it was measured statistically the same as its magnetization lifetime; while using deuterium to decrease the external coupling by 6.5 times less, the two nitrogen spins in the pyridazine product are more isolated and their singlet lifetime was seen to increase again.

Another interesting and exciting discovery is the production of para-nitrogen ($p$-N$_2$), which is a by-product of the cycloaddition reaction and has been ignored so far. The absence of nitrogen gas signals in the hyperpolarization reaction data (Fig. 46) provides indirect evidence for the generation of para-$^{15}$N$_2$, which is an intriguing new hyperpolarized species and should have very similar spin properties of para-H$_2$. For the dinitrogen molecule, since its ground state electronic configuration is $1\sigma_g^2 1\sigma_u^2 1\pi_u^4 2\sigma_g^2$, the electronic wavefunction has $^1\Sigma_g^+$ symmetry [5], which is the same as the dihydrogen molecule as introduced in chapter 2. This wavefunction is symmetric under inversion and
reflection operations. Also like $^1\text{H}$, $^{15}\text{N}$ is fermion with spin number of $\frac{1}{2}$, the overall wavefunction should be antisymmetric under exchange of the two spins and finally, there are two spin rotational isomers of $^{15}\text{N}_2$ molecules: the ortho-nitrogen with odd rotational states ($J = 1, 3, 5, \ldots$) and symmetric nuclear spin states (triplet), and the para-nitrogen with even rotational states ($J = 0, 2, 4, \ldots$) and antisymmetric nuclear spin state (singlet). Similar to para-hydrogen, the nuclear spin state of para-nitrogen is symmetry protected and should have a long-lived lifetime relaxing to ortho-nitrogen. However, the rotational constant, $B$, for dihydrogen and dinitrogen is different. It has been shown that dihydrogen has a rotational constant $B_H \approx 87.6 \, \text{kJ}\text{T}$ (or 60.864 cm$^{-1}$), while for dinitrogen the number is $B_N \approx 2.7 \, \text{kJ}\text{T}$ (or 1.858 cm$^{-1}$) [87], [88]. The much smaller rotational constant of dinitrogen makes it impossible to prepare para-nitrogen using the same way as preparing para-hydrogen (Fig. 48): while para-hydrogen will be dramatically enriched as the temperature decreases below 200 K, for nitrogen this only happens below 10 K, by which the nitrogen is already frozen to solid (melting point of nitrogen is 63 K). Hereby, our research provides a practical way to prepare the pure state para-nitrogen, which is an interesting spin isomer possessing long-lived lifetime and could have huge potential for lung imaging, with the obstacle on how to break the symmetry of para-N$_2$ and unlock the signal.
5.4 Conclusion

In conclusion, I examined a novel magnetic resonance strategy by integrating bioorthogonal reactions and hyperpolarization. This strategy is demonstrated using all nitrogen-15 enriched tetrazine, which can be readily hyperpolarized using SABRE-SHEATH and undergoes rapid cycloaddition with cyclooctyne to provide hyperpolarized cycloaddition products as well as hyperpolarized $^{15}$N$_2$ gas. This research suggests great potential of $^{15}$N$_4$-1,2,4,5-tetrazine as a powerful molecular tag in NMR and MRI with dual roles in hyperpolarization and bioorthogonal ligation. Excitingly, the observations in the
current study support the production of hyperpolarized $^{15}\text{N}_2$ gas in both its ortho and para spin isomers. Future studies will be directed toward systematic optimizations on the $^{15}\text{N}$-tetrazine cycloaddition-based hyperpolarization tagging strategy and characterization and application of para-$^{15}\text{N}_2$ gas.
6. Advances of SABRE-SHEATH

It has been shown that SABRE-SHEATH is an exciting novel technique with high polarization efficiency, easy chemical components and straightforward procedure. Using the μ-metal to shield out the earth magnetic field, we can reach quite low magnetic field, which is adjustable by using a solenoid inside the shield, to match the resonance conditions for polarizing heteronucleus, such as $^{13}$C and $^{15}$N. Thus, this technique could be broadly used to polarize various nucleus and substrates. Furthermore, the preliminary tests show that SABRE-SHEATH could be integrated with chemical reactions, such as bioorthogonal reactions where the polarized substrate acts as a specific linkage and at the same time a polarization tag. However, several drawbacks associated with the operating procedure still exist and could be improved. In this chapter, I will first discuss some of the downsides of the current SABRE-SHEATH method, then I will demonstrate several major improvements that have been achieved in our group, which could have huge impact on future developments and applications of SABRE.

6.1 Limitations of SABRE-SHEATH

The first limitation exists in the current SABRE-SHAETH setup. Inside the shield under the resonance conditions, which are revealed by the spin system Hamiltonian, the polarization transfer should be a coherent process: the off-diagonal perturbation elements will drive the population to evolve in between the connected states back and forth sinusoidally. This process is similar as the one demonstrated by equation 3.4, for the SLIC
pulse sequence. However, situation is slightly different for the current SABRE-SHEATH setup, where a constant voltage is used to provide the matching magnetic field for the polarization transfer to occur, and it is left on for a sufficiently long time (~1 to 2 minutes) for the polarization to build up till equilibrium. Thus, instead of a coherent transfer, the current polarization is a long-time averaged result of both the coherent transition and the substrate exchange process, which would miss the maximum polarization transition time point and lead to lower polarization levels.

Another drawback of SABRE-SHEATH, which is more obvious, is the required sample transportation between polarization and detection. Though the transportation time could be as short as 7 or 8 seconds, it still largely restricts more general applications of SABRE-SHEATH. Till now, the hyperpolarization process must happen in the shield, where the magnetic field is on the scale of micro-Tesla. However, detection of the polarized signal occurs inside the NMR instrument, where the magnetic field usually ranges from 1 to 8.45 T or even higher. Thus, transportation of the polarized sample is inevitable and it has several evident disadvantages.

One is that, the transportation will certainly decrease the polarized signal, making some of the polarization “wasted” and depending on the magnetic field profile of the sample transportation path, polarization could be severely scrambled. One example is the pull-out operation of the sample from the solenoid. Though the magnetic field in the middle of the solenoid (inside the shield) is stable and well aligned, near the exit of the
solenoid, however, the field is quite miss-aligned and each time the sample is pulled out it will go through the miss-aligned field region. The complicated profile of the field scrambles the polarization and relaxes the signal, bringing unwanted effects to the detection results, which usually makes some of the original polarization patterns lost.

Secondly, the sample transportation makes it almost impossible to apply SABRE-SHEATH for 2D experiments for the polarized substrates, especially for the heteronucleus. Such experiments include correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) or heteronuclear multiple quantum coherence (HMQC) spectroscopies [89], which requires continuous polarization and signal detection. Note that for the para-hydrogen derived hydrides, it is possible to perform these 2D experiments [90] because once the hydrides-iridium complexes are formed, symmetry of para-hydrogen is always broken and large magnetization signal will be unlocked, no matter what the magnetic field is (similar as the PASADENA phenomenon); however, this is not the case for the target substrate because polarization transfer only happens at specific magnetic field.

Excitingly, the progresses made in our lab, which are also parts of my research, showed that it is possible to overcome these limitations mentioned above and we have already achieved promising results. It is proved that using a pulsed magnetic field, which can be provided by a wave function generator, the polarization could also be modulated and yield the highest polarization level when the matching magnetic field is on resonance
with the polarization transfer process. On the other hand, we established another SABRE modality, called “low irradiation generates high Tesla-SABRE” (LIGHT-SABRE), which can polarize both homo and heteronuclear spins directly and efficiently in the high magnetic field using the rotation frame, thus eliminating the requirement of sample transportation before detection and largely expanding the application scope of SABRE. This modality could be implemented both on our 1 T and 8.45 T NMR instruments, which provides great convenience and freedom to perform hyperpolarization experiments under wanted magnetic fields. I will discuss about these new advances in detail in the following sections.

6.2 Magnetic field modulated SABRE for higher polarization

The first advance I will demonstrate in this section is the modulated matching field SABRE, where a simple but elegant idea could help to boost the polarization level up to two or three times larger than the conventional SABRE-SHEATH method, which uses a constant matching field for polarization to transfer.

6.2.1 Theoretical basics

Till now, all the theoretical work is done under the coherent transfer scheme, i.e., the population will transfer sinusoidally between the connected states under the dominating Hamiltonian sub-matrices. Thus, the Hamiltonians or numerical simulations only capture the coherent part of the polarization transition patterns. Note that SABRE or SABRE-SHEATH are fundamentally reversible exchange processes, where the para-
hydrogen, iridium catalyst and the $^{15}$N substrates are undergoing rapid exchange in the methanol solution with an exponentially distributed lifetime whose average is around 40 ms, so what we acquired in the experiments are essentially the long time and large number of molecules averaged signal combining both the exchange and the polarization transition dynamics. By simply leaving the magnetic field on for around 2 minutes with the strength required by the resonance conditions, we are having no control on the detailed dynamics of the polarization transfer and expecting the $z$ polarization will gradually build up till it reaches the equilibrium maximum. This may work just as fine, however, by manipulating the matching field to make it on-and-off coordinately with the coherent polarization transfer, we should be able to achieve better polarization results.

To understand how this would work, let us take a step back to examine the Hamiltonian of the spin system again. Suppose we have para-hydrogen, iridium catalyst and $^{15}$N pyridine mixed well in the methanol solution, connected by the iridium metal core, the para-hydrogen derived hydride and two pyridine molecules form an AA’BB’ spin system, as shown in Fig. 49 with the corresponding $J$-coupling parameters.
The full Hamiltonian of this spin system is:

\[
H = \omega_H (I_{1z} + I_{2z}) + \omega_N (S_{1z} + S_{2z}) + 2\pi J_{HH} I_1 \cdot I_2 + 2\pi J_{NN} S_1 \cdot S_2 + 2\pi J'_{NH} (I_1 \cdot S_1 + I_2 \cdot S_2) + 2\pi J'_{NH} (I_1 \cdot S_2 + I_2 \cdot S_1)
\]

where \(\omega_H\) is the chemically shifted frequency of the hydride \(^1\text{H}\), and \(\omega_N\) is the frequency of the pyridine \(^{15}\text{N}\). Correspondingly, \(I_1\) and \(I_2\) stand for the two \(^1\text{H}\)'s, and \(S_1\) and \(S_2\) are for the two \(^{15}\text{N}\)'s. Again, since the hydrides are in pure singlet state, this Hamiltonian could be better analyzed using the singlet-triplet basis set. The three symmetric triplet states are:

\[
T_+ = \alpha\alpha \\
T_0 = (\alpha\beta + \beta\alpha)/\sqrt{2} \\
T_- = \beta\beta
\]

and the one anti-symmetric singlet state is:

\[
S_0 = (\alpha\beta - \beta\alpha)/\sqrt{2}
\]
Since the initial states are four equally populated states \( S_0^H S_0^N, S_0^H T_+^N, S_0^H T_0^N, \) and \( S_0^H T_-^N \), applying the singlet-triplet basis set to express the Hamiltonian of equation 6.1, we would find several important sub-matrices that connect those states to other states thus generating hyperpolarization. For example, starting with the \( S_0^H S_0^N \) state, one of the sub-matrices of the singlet-triplet adapted Hamiltonian is:

\[
H = \begin{pmatrix}
S_0^H S_0^N & T_0^H T_-^N \\
\end{pmatrix}
\]

\[\begin{align*}
H &= \begin{pmatrix}
S_0^H S_0^N & T_0^H T_-^N \\
\end{pmatrix} \\
&= -J_{HH} - J_{NN} - \frac{1}{2} \Delta J_{NH} \\
&= -\frac{1}{2} \Delta J_{NH} - \frac{1}{2} \Sigma J_{NH} + \Delta \nu_{HN}
\end{align*}
\]  

(6.2a)

where \( \Delta J_{NH} = J_{NH} - J'_{NH} \), the difference of the out-of-pair \( J \)-couplings, and \( \Sigma J_{NH} = J_{NH} + J'_{NH} \), the sum of these \( J \)-couplings. A factor of \( 2\pi \) is dropped in the sub-matrices to simplify the expression, so the unit of Hz is used and \( \Delta \nu_{HN} = \frac{\omega_H - \omega_N}{2\pi} \), which is the chemically shifted frequency difference between \( ^1H \) and \(^{15}N \). Equation 6.2a could be considered as a two-level perturbation system, and by equalizing the diagonal elements, i.e., to make the original energy difference of the two levels equals 0, the off-diagonal perturbation terms will be the most effective to induce population transition between the non-crossing energy levels. So, the \( T_- \) state of the nitrogen spin pair will be hyperpolarized and the resonance condition is:

\[
\Delta \nu_{HN} = -J_{HH} - J_{NN} + \frac{1}{2} \Sigma J_{NH}
\]  

(6.2b)
Similarly, for the anti-symmetric group, we could find that the sub-matrix for the two-level perturbation system annihilating the \( T^+ \) state for nitrogen, which is:

\[
\begin{bmatrix}
0 & 0 & 0 & 1 \\
1 & 0 & 0 & 2 \\
0 & 1 & 0 & 1 \\
0 & 0 & 1 & 0 \\
\end{bmatrix}
\]

\[
H = \begin{bmatrix}
S_0^H T_+^N & T_+^H S_0^N \\
-S_{HH} + S_{NN} & \frac{1}{2} \Delta J_{NH} \\
\end{bmatrix}
\]

and the resonance condition is:

\[
\Delta \nu_{HN} = -J_{HH} + J_{NN}
\]  

(6.3b)

As mentioned earlier in chapter 4, these two resonance conditions (equations 6.2b and 6.3b) will work synergistically to enhance the overall negative \(^{15}\text{N}\) magnetization. By switching the magnetic field to the other direction (i.e., reverse the poles connection with the voltage provider), we could switch the sign of \( \Delta \nu_{HN} \) thus satisfying the other complimentary set of resonance conditions and generating hyperpolarized positive \(^{15}\text{N}\) magnetization. The resonance conditions are:

\[
\Delta \nu_{HN} = J_{HH} + J_{NN} - \frac{1}{2} \Sigma J_{NH}
\]  

(6.4a)

and

\[
\Delta \nu_{HN} = J_{HH} - J_{NN}
\]  

(6.4b)

Equations 6.2b, 6.3b and 6.4 compose the full resonance conditions for SABRE-SHEATH to hyperpolarize bulk magnetization for \(^{15}\text{N}\) pyridine in different directions. To let the
chemical shift frequencies difference, meet on the order of the \( J \)-couplings, it would only be satisfied in the extremely low magnetic field, such as micro-Tesla fields.

On the other hand, based on equations 6.2a and 6.3a, when the resonance conditions are fulfilled, the off-diagonal perturbation items in these sub-Hamiltonians will drive the populations to transfer from the initial states to the target states. Considering the Bloch sphere as mentioned in chapter 3, the optimal polarization should be achieved at the time point \( \tau_{\text{evolv}} = 1/(2\Delta J \text{_{NH}}) \), which is mathematically equivalent to the time of a \( \pi \) pulse that inverses the z spin angular momentum. The effects of the off-diagonal perturbations are also shown in Fig. 50.

\[
\rho_{\text{source}} = \left| S_0^H S_0^N \right\rangle \left\langle S_0^H S_0^N \right| \\
\rho_{\text{target}} = \left| T_0^H T_0^N \right\rangle \left\langle T_0^H T_0^N \right|
\]

\[
\rho_{\text{source}} = \left| S_0^H T_0^N \right\rangle \left\langle S_0^H T_0^N \right| \\
\rho_{\text{target}} = \left| T_0^H S_0^N \right\rangle \left\langle T_0^H S_0^N \right|
\]

**Figure 50:** [From Ref. 47] Coherent polarization transfer dynamics demonstrated with Bloch sphere.

Thus, if the resonance condition is left on for longer time than \( \tau_{\text{evolv}} \), the perturbation Hamiltonian will keep transferring the population between the states so the optimal polarization level could be missed or the polarization could even be 0, though the latter case will hardly happen in the real SABRE-SHEATH situation because the magnetic
field is turned on for sufficiently long time, and the result at the end is a long-time, large number of molecules averaged polarization. But still, if we can control the on and off time of the correct magnetic field to make it match with the coherent transfer time, we could coherently pump the magnetization and achieve high polarization levels, though the result is still weighted by the exchange process.

A simple wave function generator that could generate various wave profiles would accomplish this task discussed above, and the device is shown in Fig. 51. One additional point is that, if we directly turn off the magnetic field during the coherent pumping, when pull the sample out from the shield the polarization would be scrambled due to the complicated magnetic field near the exit of the solenoid, which will severely interfere with the detection. To avoid this problem, instead of turning the field to 0, we used quite high voltage thus generating high magnetic field, which could also stop the coherent transfer at the end of pumping and store the polarized magnetization. By doing so, we could have aligned magnetic field near the exit of the solenoid and the polarized signal will be well preserved.
6.2.2 Simulation and experimental results

The numerical simulations are performed using the $J$-coupling parameters shown in Fig. 49, and two cases are considered. In the first case no exchange effect is considered and the result is only about the spin evolutions. In the second one, the chemical exchange effect is considered and the spin evolution result from case one is weighted based on the exponential distribution, using an averaged polarization transfer complex (PTC) lifetime of 40 ms. Theoretically, the resonance condition should be $\Delta \nu_{NH} = \pm (8 \text{ or } 20) \text{ Hz}$, and the optimal transition time would be $\tau_{\text{evolv}} = 1/(2\Delta J_{NH}) = 21 \text{ ms}$. 

Figure 51: Device to coherently pump the polarization and the resonance magnetic field profile.
From the simulations in Fig. 52 we can see that, using the pulsed magnetic field when placing the sample inside the shield does yield better polarization results, and it could be up to 3 times larger than the conventional SABRE-SHEATH method, where only the averaged signal is obtained. More excitingly, the experimental results further confirmed the theory and simulation, as shown below.
Figure 53: Experimental results of pulsed matching field of SABRE-SHEATH, and fitting with the analytical models with exchange. In this fitting, $\Delta \nu_{NH}$ of 24.7 and -26 Hz are used respectively for the red and blue curves. The other parameters are: $J_{NH} = -25$ Hz, $J_{HH} = -8$ Hz and complex lifetime $\tau_{life} = 39$ ms. Data were acquired by Shannon Eriksson.

It is exciting to see that, the experimental data shows great consistency with the theoretical work and our numerical simulations. For both the positive (red) and negative (blue) results, the first optimal polarization points appear at around 20 ms, which is a half cycle on the Bloch sphere evolution and is very close to the time predicted from the two-level Hamiltonians. As we increase the time of the matching field using the wave function generator, the evolution time goes longer, and the exchange and averaging effect begin to
become more dominating for the outcome. Finally, the oscillation of the polarization begins to converge to the constant field result, which is used as the normalization basis here. Depending on the polarization system composition and the temperature where the experiments were performed, the parameters deciding the polarization oscillation patterns could be different, and we may obtain various optimal polarization levels. But in general, the pulsed matching field method could give larger polarization than the original constant field method under the same conditions.

6.3 LIGHT-SABRE and its variants

The other huge advance we have achieved, is the LIGHT-SABRE polarization modality, which could hyperpolarize heteronucleus directly inside the NMR magnet. This method was first introduced by Theis and Warren et. al. in 2015[91], and recently has been improved with higher polarization levels by Lindale et. al., generating a variant called DARTH-SABRE (delayed adiabatic ramps transfer hyperpolarization-SABRE). Essentially, it is the radio frequency continuous wave (CW) irradiation in the NMR rotating frame that plays the critical role to generate hyperpolarization inside the NMR magnet. By setting the CW pulse whether off or on-resonance with the heteronuclei (such as $^{15}$N) of the polarization transfer complex, we can directly polarize z or x magnetization. Furthermore, given the complexity of the polarization transition and evolution processes because of the introduction of the CW irradiation and the rotating frame, parameters of
the CW pulse, such as the offset frequency $\nu_{\text{off}}$, the nutation frequency $\nu_n$ (representing its power) and the irradiation time $\tau_n$, can be optimized using numerical simulations.

6.3.1 Hyperpolarize z magnetization at high Tesla

For LIGHT-SABRE and its variants, since the sample is at high magnetic field, the chemical shift difference between $^1\text{H}$ and $^{15}\text{N}$ is much larger, thus it is now an AA’XX’ system, instead of AA’BB’. In addition, when the CW pulse is applied, the analysis should be done using the rotating frame Hamiltonian, which is similar as the lab frame shown by equation 6.1, but with the relative offset frequencies replacing the “true” frequencies in front of the $z$ spin angular momentum operators [1]. Since the CW pulse is on the transverse plane, for simplicity, it could be supposed to have phase 0 so be aligned with the $+x$ direction. With the offset frequency $\Omega_{\text{off}}$ (could be 0 if it is right on resonance with $^{15}\text{N}$, and here the capital omega is used for the rotating frame) and the nutation frequency $\omega_n$, the rotation frame Hamiltonian could be written as:

$$
H = \Omega_{\text{ff}} (I_{z1} + I_{z2}) + \Omega_{\text{off}} (S_{z1} + S_{z2}) + \omega_n (S_{1z} + S_{2z})
+ 2\pi J_{NN} I_1 \cdot I_2 + 2\pi J_{NN} S_1 \cdot S_2
+ 2\pi J_{NN} (I_1 \cdot S_1 + I_2 \cdot S_2) + 2\pi J_{NN} (I_1 \cdot S_2 + I_2 \cdot S_1)
$$

(6.5)

Using the singlet-triplet basis to transform the new Hamiltonian, we could have sub-matrices that connect states belonging to the same symmetry group. However, if we look at the sub-matrix that connects $S_0^+ S_0^-$ and $T_+^N T_-^N$, we would find that:
Again, a factor of $2\pi$ is dropped to make the expression cleaner. In equation 6.6, $\nu_{\text{off}}$ is the $^{15}\text{N}$ offset frequency of the irradiation in the rotating frame in unit of Hz, and $\nu_{\text{H}}$ is the $^1\text{H}$ offset frequency. As mentioned, at high magnetic field, $^1\text{H}$ will have a mega-Hz order frequency difference with $^{15}\text{N}$, so the energy levels of the two states, $S_0^H S_0^N$ and $T_+^H T_-^N$, are of huge difference and it is impossible for the diagonal terms to meet with each other. The off-diagonal terms thus will not be able to perturb the system and could even be dropped in the secular approximation.

Similar situations occur for the other symmetry groups, where one of the sub-Hamiltonians is:

$$H = \begin{pmatrix} S_0^H S_0^N & T_+^H T_-^N \end{pmatrix}$$

$$-J_{HH} - J_{NN} \quad -\frac{1}{2}\Delta J_{NH}$$

(6.6a)

The diagonal terms cannot be equalized and there is no polarization transfer under this Hamiltonian either. The same thing happens for the positive magnetization. To summarize, at high Tesla the original mechanisms that build polarization for the SABRE-SHEATH scheme do not work.
However, things are different if we focus on the states that are coupled with the \( T_0^H \) state, which does not have the \(^1H\) frequency involved thus the energy level matching becomes possible at high Tesla in the rotating frame. The detailed transition dynamics are revealed in the sub matrices of the full Hamiltonian of equation 6.5, which are:

\[
H = \begin{pmatrix}
|S_0^H T_0^N\rangle & |T_0^H T_0^N\rangle & |T_0^H T_0^N\rangle \\
-J_{HH} - J_{NN} & -\frac{1}{2\sqrt{2}} \Delta J_{NH} & \frac{1}{2\sqrt{2}} \Delta J_{NH} \\
\frac{1}{2\sqrt{2}} \Delta J_{NH} & -v_{off} & 0 \\
\frac{1}{2\sqrt{2}} \Delta J_{NH} & 0 & v_{off}
\end{pmatrix}
\]

and

\[
H = \begin{pmatrix}
|S_0^H T_+\rangle & |S_0^H T_-\rangle & |T_0^H S_0^N\rangle \\
-v_{off} & 0 & -\frac{1}{2\sqrt{2}} \Delta J_{NH} \\
0 & -v_{off} & \frac{1}{2\sqrt{2}} \Delta J_{NH} \\
-\frac{1}{2\sqrt{2}} \Delta J_{NH} & \frac{1}{2\sqrt{2}} \Delta J_{NH} & J_{HH} - J_{NN}
\end{pmatrix}
\]

Things are quite interesting at this point and we can draw several conclusions from these two complimentary sub-Hamiltonians. Firstly, it is seen that the offset frequency of the \(^{15}N\) channel irradiation now needs to be matched with the \( J \)-couplings to meet the resonance conditions, which are:

\[
v_{off} = \pm \left( J_{HH} \pm J_{NN} \right)
\]

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Under these resonance conditions, nitrogen negative/positive z magnetization will be created by equation 6.7a and at the same time, positive/negative magnetization is being annihilated from equation 6.7b, thus they together enhance the negative or positive magnetization.

Secondly, it is surprising to note that, though the CW irradiation is applied, the nutation frequency representing its power, \( \nu_n \), does not play any role here for the bulk polarization transfer. However, together with \( \nu_{\text{eff}} \), they form an effective Hamiltonian which could drive the polarized z magnetization to deviate from the z axis, leading to a lower polarized signal. Thus, the optimization for all the parameters of the CW pulse are more complicated and they may be revealed after numerical simulations. But still, equation 6.8 gives a good start.

Finally, the off-diagonal perturbation terms determine how efficient the polarization transfer would be under resonance conditions. For the cases studied here, it is still the out-of-pair \( J \)-coupling difference breaks the symmetry, which is the same as the standard SABRE-SHEATH scheme. However, the coefficients now contain imaginary parts, making the transfer cycle time not as straightforward to be determined. We could use analytical calculation to better understand the coherent transfer cycle. For example, if the resonance condition to polarize the negative magnetization is satisfied, the two-level sub-Hamiltonian is just the upper left two by two part of equation 6.7a, dropping the \( J_{\text{NN}} \) term (which is small) it is:
For this two-level perturbation, since the initial state is the singlet states, the starting density matrix is:

\[
\rho_0 = \begin{pmatrix}
|S_0^H S_0^N\rangle & |T_0^H T_-^N\rangle \\
|S_0^H S_0^N\rangle & 1 & 0 \\
|T_0^H T_-^N\rangle & 0 & 0
\end{pmatrix}
\] (6.9b)

The time dependent evolution of the populations of the states are:

\[
\rho_t = \exp(-iH_{2\times2}t)\rho_0\exp(iH_{2\times2}t)
\] (6.9c)

Plugging in the numbers and equalizing the diagonal terms of equation 6.9a, the density matrix after evolution is:

\[
\rho_t = \begin{pmatrix}
|S_0^H S_0^N\rangle & |T_0^H T_-^N\rangle \\
|S_0^H S_0^N\rangle & \cos^2(12t) & \frac{(1+i)\sin(24t)}{2\sqrt{2}} \\
|T_0^H T_-^N\rangle & \frac{(1-i)\sin(24t)}{2\sqrt{2}} & \sin^2(12t)
\end{pmatrix}
\] (6.9d)

Since a factor of $2\pi$ was dropped in the original Hamiltonian, considering in unit of Hz, the half cycle time to reach the highest population in the $T_0^H T_-^N$ state is $1/(2*24)$ second, which can be considered as $1/(2\Delta J_{NH})$ and is exactly the same as the original SABRE-SHEATH scheme. Thus, though the perturbation terms connecting the target
states are complex numbers, considering only the polarization transfer, they should not alter the polarization transfer time.

**6.3.2 Simulations for z magnetization polarization**

To verify the theory, numerical simulations were performed with MATLAB using the SPINACH package [40], [92]–[94]. As mentioned, because of the existence of the x irradiation operator, which do not commute with the offset frequency operator and will drive the polarized magnetization off the z axis, finding the optimized offset frequency $\nu_{\text{off}}$, irradiation power $\nu_n$ and irradiation duration $\tau_n$ is complicated. The numerical simulation gives the advantage to optimize the parameters without considering much about the details of the dynamics, and the results are shown below.

Simulation was first performed by sweeping the offset frequency from -50 to 50 Hz, while the irradiation power was kept fixed as 5, 15 or 25 Hz, and the irradiation duration was 40 ms. The duration was chosen the same as the averaged PTC lifetime as mentioned in last section. The $J$-coupling parameters of the spin system used for simulation are the same, which were shown in Fig. 49.
Figure 54: Three simulation results of sweeping the offset frequency, using various irradiation powers.

From Fig. 54 we can see that, for all cases when the irradiation is right on resonance with nitrogen-15 ($\nu_{\text{off}}$ equals 0), there is no polarization generated. This result is as expected: first, the resonance conditions are not satisfied when the irradiation is on resonance; second, this could also be understood as that, if the irradiation is right on resonance, there would be no perturbation on the z axis that could flip the spins thus no enhancement can be generated. This is similar as the original SABRE-SHEATH situation when the magnetic field provided by the solenoid exactly equals 0 and there is no polarization (shown in Fig.35), because all the spins are magnetically equivalent and there is nothing breaking the symmetry. Generally, with negative offset frequencies, the polarizations are mainly negative and with positive offset frequencies the polarizations are mainly positive. These results are consistent with the prediction that negative or positive magnetizations will be enhanced at those specific resonance conditions.

However, the optimal polarization levels are around $\pm 10$ Hz, which are slightly off from the resonance conditions indicated by equation 6.8, where it should be $\pm 8$ Hz. Furthermore, as the nutation frequency is increased, in the negative offset region there is
small amount of positive signal and vice versa in the positive offset region. The offset frequencies for optimal polarization levels are also dependent on the nutation frequency: as \( \nu_n \) changes, the optimal \( \nu_{\text{off}} \) will shift correspondingly.

To further interrogate the relationships between the parameters and optimize the \( z \) polarization for high Tesla SABRE, the parameters \( \nu_{\text{off}} \) and \( \nu_n \) are optimized simultaneously in a 2D calculation, with the irradiation duration fixed as 40 ms. The parameters \( \nu_n \) and \( \tau_n \) are simultaneously optimized as well, with the offset frequency fixed as -10 Hz. The results are shown below.

![Figure 55: Two-dimensional optimizations for \( z \)-magnetization polarization at high Tesla.](image)

(A) The optimal combinations of offset and nutation frequencies are \( \nu_{\text{off}} = \pm 10 \text{ Hz} \), \( \nu_n = 18 \text{ Hz} \) and the polarization level at these points are normalized to around 34%. (B) Using \( \nu_{\text{off}} = -10 \text{ Hz} \) but varying the nutation frequency and irradiation time, the maximum polarization appears around \( \nu_n = 18 \text{ Hz} \), which is consistent with (A), however, the optimal irradiation time is different from theoretical calculation.

The 2D simulation in Fig. 55 (A) is consistent with the three 1D offset frequency sweeping calculations in Fig. 54. The polarizations are symmetric about the zero-frequency line, where there is no magnetization hyperpolarized. If the nutation frequency
is too small (less than 5 Hz), there is basically no polarization generated. As the nutation frequency increases but stays below 30 Hz, the optimal offset frequency generally stays around ±10 Hz but is being slightly pushed towards 0. When the nutation frequency exceeds 30 Hz, larger offset frequency is required to generate polarization, but the transition will be much less efficient. Also, as the offset frequency increases, the polarization level will decrease because the resonance conditions are getting harder to be satisfied. In Fig. 55 (B) the first optimal point appears when the nutation frequency is 18 Hz and the duration is 40 ms. The optimal duration is about 2 times longer than the coherent transfer time indicated in the Hamiltonians, where it should be $1/(2\Delta J_{NH})$ to act as a $\pi$-pulse that connects between the singlet and triplet states. This difference could be caused by the evolution of the generated $z$ magnetization around the irradiation Hamiltonian. If the nutation frequency increases, the other optimal $z$ magnetization will appear with even longer duration time.

6.3.3 Hyperpolarize x magnetization at high Tesla

Besides hyperpolarizing the $z$ magnetization inside the magnet, using the same scheme, i.e., bubbling para-hydrogen inside the magnet and applying a CW irradiation with certain offset and nutation frequencies, we can also polarize the $x$ magnetization and detect without sample transportation. This is the original scheme when LIGHT-SHABRE was first introduced and till now LIGHT-SABRE includes several variants, including the
one that polarizes the $z$ magnetization discussed in the last section, and other optimized ones.

Mechanisms for polarization $x$ magnetization could be better understood by referring to the SLIC pulse sequence as discussed in chapter 3. Instead of using the triplet-singlet basis set along the $z$ axis, another $x$ axis adopted triplet-singlet set is used. Using this basis set, the process to hyperpolarize the $x$ magnetization inside the magnet for the $AA'XX'$ spin pairs is quite similar as the SLIC pulse sequence, except the transfer direction is reversed: the handle is the same to accomplish the transitions, which is again the $\Delta J_{\text{NH}}$ term that breaks the symmetry; but here we are creating more $x$ axis triplet, by consuming the pure singlet state of the $para$-hydrogen derived hydride.

The Hamiltonian is the same as equation 6.5, so to polarize $x$ magnetization the dynamics would be similar as polarizing $z$ magnetization. The key difference lies in which basis is chosen used. As mentioned in chapter 3, the three $x$ triplet states, which are combinations of the $z$-axis adopted triplet states, are:

$$X_+ = \left( T_+ + \sqrt{2} T_0 + T_- \right) / 2$$

$$X_0 = \left( T_+ - T_- \right) / \sqrt{2}$$

$$X_- = \left( T_+ - \sqrt{2} T_0 + T_- \right) / 2$$

The singlet state, however, remains the same since it is a spin-0 state and has no spin angular momentum:

$$S_0 = (\alpha \beta - \beta \alpha) / \sqrt{2}$$
Using this basis set, the adapted Hamiltonian would again have the corresponding sub parts that transfer population between the targeted states under possible resonance conditions at high Tesla. For states connected with the $S_0^H S_0^N$ state, the sub Hamiltonian is:

$$H = \begin{pmatrix}
|S_0^H S_0^N\rangle & |T_0^H X_-^N\rangle & |T_0^H X_+^N\rangle \\
-(J_{HH} + J_{NN}) & -\frac{1}{2\sqrt{2}} \Delta J_{NH} & \frac{1}{2\sqrt{2}} \Delta J_{NH} \\
\frac{1}{2\sqrt{2}} \Delta J_{NH} & -\nu_n & 0 \\
\frac{1}{2\sqrt{2}} \Delta J_{NH} & 0 & \nu_n \\
\end{pmatrix}$$

(6.10a)

And the sub Hamiltonian matrix for the other symmetry group is:

$$H = \begin{pmatrix}
|T_0^H X_-^N\rangle & |T_0^H X_+^N\rangle & |S_0^H S_0^N\rangle \\
\nu_n & 0 & -\frac{1}{2\sqrt{2}} \Delta J_{NH} \\
0 & -\nu_n & \frac{1}{2\sqrt{2}} \Delta J_{NH} \\
\frac{1}{2\sqrt{2}} \Delta J_{NH} & \frac{1}{2\sqrt{2}} \Delta J_{NH} & J_{HH} - J_{NN} \\
\end{pmatrix}$$

(6.10b)

From equations 6.10 we can see that these two sub Hamiltonian matrices are nearly the same as those in equations 6.7. Besides some alterations of the signs, the most critical difference is that, instead of the offset frequency, it is the nutation frequency of the CW pulse now needs to be adjusted to satisfy the resonance conditions, which are:

$$\nu_n = \pm (J_{HH} \pm J_{NN})$$

(6.11)
Since $\nu_n$ is the power of the irradiation, it can only be positive, and because $J_{1H}$ is negative (-8 Hz), only the positive x magnetization could be polarized. So the off-diagonal perturbation will create the positive x magnetization from the $S_0$ singlet and at the same time annihilate the negative x magnetization to the central $X_0$ triplet state, thus synergistically enhancing the positive x magnetization.

6.3.4 Simulations for x magnetization polarization

As is seen from equations 6.10, the offset frequency, $\nu_{\text{off}}$, is not directly playing any role here for the x polarization transfer and we can set the irradiation exactly on resonant with $^{15}$N (i.e., to let $\nu_{\text{off}}$ equal 0). Similar as the z magnetization polarization case, introduction of the CW pulse and rotating frame makes the dynamics more complicated. To search for the optimal conditions to yield better polarization results, again the two-dimensional numerical calculations could be used. By varying the irradiation offset and nutation frequencies at the same time, or the irradiation duration and nutation frequency at the same time, we can have a direct view of polarizations under different conditions.
Figure 56: Two-dimensional optimizations for x-magnetization polarization at high Tesla. (A) The highest x magnetization polarization is achieved using the irradiation with power of ~16 Hz and duration of ~33 ms. While the duration time is nearly the same as predicted by the Hamiltonian, the nutation frequency is off by larger amount, which could be due to the complexity caused by introduction of the CW pulse on x axis.

From the two-dimensional simulations we can clearly see that, unlike the z magnetization, the best x polarization is achieved at 0 offset, i.e., the irradiation is right on resonance with the $^{15}$N of the complex, and the nutation frequency is around 16 Hz, which is two times larger than the value firstly predicted by the rotating frame Hamiltonian. Though at some off-resonant positions there are polarizations appearing, the results are much weaker. In Fig. 56 (B), the optimal polarization appears at around 33 ms, which is also different from the predicted 21 ms. These differences may be originated from that, while the theoretical values are obtained using the rotating frame Hamiltonian, the results shown in the simulations are from the static field frame calculations.

On the other hand, when applying the x magnetization polarization scheme, one additional critical difference from the z polarization scheme exists. Since the x
magnetization is polarized, upon dissociation of the hydride complex, the polarized x magnetization of the $^{15}$N on the substrates will quickly lose phase, destroying the signal and there would be little left for detection. To avoid this problem, a $90^\circ$ y phase pulse could be inserted after the x phase CW irradiation, converting the x magnetization to the z direction, where the signal could be stored even the complex disassociates. The detailed pulse sequence is shown in Fig. 57.

Figure 57: Pulse sequence to polarize and store the x-magnetization for detection. As labelled in the figure, pulse (1) is the soft CW irradiation that is set on-resonance with the nitrogen-15 Larmor frequency, creating the hyperpolarized x magnetization. After the x hyperpolarization is created, pulse (2), which is also a soft pulse (but “harder” than the CW pulse), selectively flip the x magnetization to the z direction for storage. The combination of pulse (1) and (2) are performed for n cycles till the hyperpolarization reaches to the equilibrium. Finally, a broad band hard $90^\circ$ pulse is applied to read out the polarized signal.

Finally, as can be seen from the simulations, the idea of the modulated matching field is already indicated for both the z and x polarizations directly inside the magnet, and this idea could be readily applied to reach a better polarization result. That is, instead of using an arbitrarily long CW pulse, we use the optimal duration time to pump the polarization and stop the polarization transfer by turning off the CW pulse. Furthermore,
the amplitude shape of the CW pulse could also be modified for better polarizations, such as using an adiabatically decreasing pulse instead of using a constant nutation frequency [95]. One straightforward and similar method that can be used for the pulsed-SABRE is that, we can always follow the maximum (or minimum) polarization points in Fig. 52 thus generating the optimal evolution magnetic field profile to obtain the optimal polarization during the whole process.

6.4 Conclusion

In conclusion, it is demonstrated that the recent advances of the SABRE based method, including the pulsed-SABRE, LIGHT-SABRE and its variants, work well to overcome the drawbacks that the original SABRE-SHEATH method has. With these advances, we can achieve better polarization and generate hyperpolarization directly inside the high Tesla magnet, which bring great advantages and much broader and more general applications for the SABRE based heteronuclear hyperpolarization methods. Finally, different choices of the reference frames between the theory and simulations may lead to different results, but in principle, the numerical simulations reveal the optimal combinations of the CW pulse parameters to hyperpolarize the z or x magnetization directly inside the magnet, and these parameters could be further tested by experimental results.
7. Future directions

With the discovery and advances of SABRE-SHEATH and LIGHT-SABRE, now it is possible that more interesting and exciting NMR and MRI based experiments could be performed. In this chapter, I will briefly discuss the new possibilities, for the more general applications using SABRE.

7.1 Design new generation catalysts

To improve the efficiency of SABRE based hyperpolarization and to polarize a broader range of molecules with different motifs (still primarily polarize nitrogen), one key aspect is to design new generations of catalysts. It is evident that the polarization level depends on the magnetic field strength, as well as the catalyst structure, which determines the lifetime of the polarization transfer complex (PTC). The optimal PTC lifetime would be around the polarization transfer time, which is $1/(2\Delta J_{NH})$ so that the complex will dissociate when the maximum polarization transfer is achieved in one cycle.

As mentioned in previous chapters, currently for the IrCl(COD)(IMes) catalyst that was designed by Duckett et. al., the residence lifetime of pyridine or acetonitrile is around 40 ms, which is about 2 times longer than the optimal polarization transfer lifetime. Though it works well to polarize the nitrogen because the out-of-pair $J$-coupling is strong, advances could be made by designing new catalysts that would have similar residence lifetimes as the polarization transfer time.
Furthermore, designing the catalyst with ligands that will not exchange into solution can also help to achieve other goals. By fixing the ligand to the iridium atom (Fig. 58), we may reduce the steric congestion caused by the carbene, which could better coordinate the \textit{para}-hydrogen with the metal core thus improving the hyperpolarization efficiency. In addition, using the bi-dentate [96] or tri-dentate ligand catalyst [97], we could control the number of substrate molecules that are attached to the iridium core. Combining with the pulsed-SABRE method, we may be able to further explore the detailed chemical exchange dynamics for a certain substrate and catalyst.

![Figure 58: Three generations of SABRE catalysts.](image)

\textbf{7.2 Apply SABRE for 2D NMR spectroscopy}

As mentioned in last chapter, the development and advances of LIGHT-SABRE and its variant make it possible to hyperpolarize the substrates directly inside the magnet
at high field, such as 1T or even higher fields with no limitation. This approach allows for 2D NMR experiments, such as COSY and HSQC. By combining hyperpolarization with 2D NMR spectroscopy, we will not only have much higher signal to noise ratio (SNR) of the 2D spectra, but will also greatly shorten the experiments’ time, especially for detection of heteronuclei (carbon-13 and nitrogen-15). The heteronuclear $T_1$ relaxation time is usually much longer than proton and between each transient of the 2D acquisition, at least one period of $T_1$ time is required for the magnetization of the heteronucleus to recover thus the pulse sequence of the next transient could be performed. Often, the waiting time between transients could be up to 5 minutes and the number of transients could be around hundreds if we want to have a 2D spectrum of good resolution, thus leading to a tremendous amount of total experimental time. However, because of the different mechanism of the polarization creation of LIGHT-SABRE, the polarization buildup will usually finish in one minute, which will dramatically decrease the time for experiments. Additionally, considering the high expense of the $^{13}$C or $^{15}$N enriched compounds, using LIGHT-SABRE will also save lots of budget by performing experiments with small amounts of substrates but high signal. In general, by applying LIGHT-SABRE, we could have much more time and cost efficient 2D NMR results with well enhanced signal.

To incorporate the LIGHT-SABRE polarization pulse with a 2D pulse sequence is straightforward: as shown in Fig. 59, what is required is to put the continuous wave irradiation pulse in front of each transient of the 2D pulse sequence. Since the
magnetization is prepared for 2D applications, the z magnetization polarization is better used rather than the x because of its simpler pulse sequence without the extra 90° pulse for storage. The idea could be illustrated using the example of heteronuclear multiple-quantum correlation (HMQC) spectrum.

Figure 59: Scheme of incorporating the LIGHT-SABRE hyperpolarization pulse with HMQC pulse sequence and the sketch of the HMQC spectrum.

In Fig. 59, the 15N and 1H channels are used to show that we can use the polarized 15N signal to perform the HMQC experiments, which yields a 2D spectrum showing the correlations between the nitrogen and its bound protons. For the pulse sequence shown in Fig. 59, part (1) is the continuous wave irradiation with the optimal parameters (offset and nutation frequencies, and pulse duration, which can be optimized using simulation and experiments) that generates the best polarization. Since the z magnetization is polarized, after (1) the density operator can be expressed as $\varepsilon I_z$, where $\varepsilon$ is the enhancement level and $I_z$ is the z angular momentum operator. After pulse (2), the density operator is:

$$\rho_{(2)} = -\varepsilon I_y$$
Following the $J$-coupling evolution of $\tau$, at time point (3) before the 90°x pulse, it is:

$$\rho_{(3a)} = -\varepsilon I_N^y \cos(\pi J_{NH}\tau) + \varepsilon I_N^z I_H^x \sin(\pi J_{NH}\tau)$$

If $\tau$ is chosen to be $1/(2J_{NH})$, the first term of $\rho_{(3a)}$ will vanish and only the second term is left. Then the 90°x pulse on $^1H$ channel will transfer it to:

$$\rho_{(3b)} = -\varepsilon I_N^y I_H^x$$

which is a double quantum coherence of nitrogen and proton, and is also the reason of how the pulse sequence is named: the double quantum (multiple-quantum) coherence is used to probe the correlation of heteronuclei.

During the $t_1$ evolution, since it is merely a spin echo on the nitrogen channel, the offset evolution of nitrogen will be refocused. In addition, since the double quantum coherence will not evolve under the $J$-coupling Hamiltonian (they commute with each other), the only factor we need to consider is the offset evolution on proton channel for a total time of $t_1$, so the density operator is:

$$\rho_{(4a)} = -\varepsilon I_N^y I_H^x \cos(\Omega_{H}t_1) + \varepsilon I_N^z I_H^x \sin(\Omega_{H}t_1)$$

Again, the 90°x pulse of the proton channel converts it to:

$$\rho_{(4b)} = -\varepsilon I_N^y I_H^x \cos(\Omega_{H}t_1) + \varepsilon I_N^z I_H^x \sin(\Omega_{H}t_1)$$

which makes the first term an observable antiphase signal while the second term remains unobservable because it is a double quantum coherence and can be dropped. The final offset evolution turns the antiphase signal in-phase and ready for acquisition, which is:
\[ \rho_{s2} = -\varepsilon I^N_y \cos(\Omega_H t_1) \]

This is nitrogen signal with the proton characteristic offset frequency cosine modulation, by performing 2D Fourier transform we will have a two-dimensional spectrum, with the direct acquisition dimension showing the characteristic offset frequency of nitrogen as well as the $J$-coupling splitting, and the other indirect acquisition dimension showing the characteristic offset frequency of protons that are correlated with the nitrogen. By having the $t_1$ in an arrayed fashion with small steps, we can have a correlation map between the nitrogen and its correlated protons of a certain $J$-coupling. On the other hand, by choosing different $\tau$ values, we can detect the correlations between nitrogen and protons of different $J$-couplings.

It is obvious that, during the HMQC process, the enhancement level, $\varepsilon$, follows along and the final 2D spectrum will also have huge SNR due to the hyperpolarization. Thus, to incorporate LIGHT-SABRE hyperpolarization with 2D NMR experiments is practical and it provides a novel way to probe the correlations between heteronuclei, especially for molecules with very limited amounts. Besides, another huge advantage of this method is that, large amount of averages of the experiment is also permitted. That is, if one scan of the 2D experiment does not give satisfactory signal, we can simply perform multiple averages using the hyperpolarized signal to improve SNR.

One immediate application that could have significant meaning using this LIGHT-SABRE 2D NMR scheme is to probe the correlation between the nitrogen and protons of
nucleotide nitrogenous bases (nucleobases), for example, guanine (G). This is particularly interesting because it was found that, the nucleobases could undergo rapid exchange with their tautomeric or anionic structures [98], [99], thus forming Watson-Crick-like mismatches, which can evade the Watson-Crick fidelity checkpoints of the polymerase and could count for an important mechanism for spontaneous mutations and DNA replication and translation errors (Fig. 60). However, the tautomeric or anionic structures of the nucleobases were not directly observed because of their low abundance and short lifetimes. Instead, the existence of these structures was inferred by fitting the data acquired from a NMR method using the relaxation dispersion model [100]–[105]. Now using the hyperpolarization method inside the magnet, we may be able to directly observe the tautomeric species by elevating their NMR signals by orders of magnitude and interrogating their specific correlations with the nitrogen.
Figure 60: [From Ref. 97] Watson-Crick (WC) GC pair and Watson-Crick-like GT miss-pairs. (A) The Watson-Crick GC pair model. Since the protons attached on the nitrogens of guanine and thymine are spatially exclusive with each other, only the wobble (WB) form of mismatch could form if there are no tautomeric structures. The one-bond nitrogen-proton coupling (circled in dash orange oval) is around -70 Hz. (B) Possible tautomeric structures of guanine and thymine. With the enol or anionic structures, guanine and thymine could form WC-like pairs that could evade from the fidelity checkpoints and induce mutations. The three-bond nitrogen-proton coupling (marked as red) is around -5 Hz.

7.3 Apply SABRE for biomedical imaging

Clearly, it is more challenging to apply the SABRE based hyperpolarization methods for biomedical imaging, both in vitro or in vivo. Though water soluble catalyst has been designed and it shows good heteronuclear polarization efficiency, the condition for this catalyst to work is quite restricted: the activation of the pre-catalyst takes very long time (up to hours) and it must be at high temperature (up to 50 °C). These restrictions make it nearly impossible for real time biomedical imaging. However, by working with
organic chemist we may be able to design catalysts that could dissolve in water and be activated under normal conditions.

On the other hand, if the hyperpolarized substrates are to be injected in \textit{vitro} or \textit{in vivo}, the iridium metal catalyst must be easily and quickly separated from the solution before injection because of safety reasons, and it could cause fast relaxation of the polarized signal as well. This may be achieved by using the chelating agent which could combine with iridium and then be filtered out together. With the problems of water-solubility and catalyst-removal solved, we will be a huge step closer to biomedical imaging or metabolism monitoring using enhanced signal from SABRE.
8. Conclusion

In conclusion, this dissertation has reviewed the fundamental concepts of the singlet-triplet states and rotational spin isomers. Based on these concepts, it reviewed and discussed how we could prolong the signal lifetime by orders through accessing to the singlet state using both physical and chemical methods. The singlet state could be accessed by magnetic field cycling, RF pulse manipulation and chemistry interconversion. Specifically, for hydrogen its singlet state can be highly enriched using cooling in the presence of paramagnetic catalyst, thus generating \textit{para}-hydrogen.

This dissertation also shows the close relationship between rotational spin isomer and hyperpolarization and how we could achieve hyperpolarization using \textit{para}-hydrogen. Following SABRE, which specifically polarize proton as it was first discovered, we greatly expand this method to polarize heteronuclei, such as $^{13}$C and $^{15}$N, making the SABRE based polarization method more general. We explored the detailed polarization transfer dynamics from the fundamental quantum mechanical aspect and showed that using SABRE-SHEATH we can directly polarize the long-lived singlet state, which permits us to have the long-lived and boosted NMR signal at the same time.

Furthermore, combining the novel polarization method with bioorthogonal reactions reveals novel applications to track biomarkers. We also first generated \textit{para}-$^{15}$N$_2$ enriched nitrogen gas, whose production was never reported before. The \textit{para}-nitrogen
would be a very interesting pure-state gas to study its quantum mechanical properties and maybe developed as a new MR imaging agent.

Both signal prolonging and boosting are of essential significance to the further development and application of NMR and MRI. With the discovery and advances we have achieved for this novel polarization technique which is easy to install and cheap to use, the new era of magnetic resonance is emerging. We may imagine that, in the future beside the NMR and MRI instruments there would be such SABRE based hyperpolarization devices that can help generate both prolonged and highly boosted signals for multiple nuclear species, making the detections faster, more accurate and more dynamic.
References


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Biography

Zijian Zhou was born in February 1988, Dezhou, China. He received the Bachelor degree of Science (July 2010) with distinction from the department of life science at Shandong University in Jinan, China. Inspired by his interest in engineering, he earned the Master degree of Science (May 2013) from the department of biomedical engineering at Duke University, then he continued the Ph.D. study with Prof. Warren and Theis focused on magnetic resonance in the department of chemistry at Duke. During his Ph.D. career, he received multiple scholarships and awards, including the Kathleen Zielek Fellowship, two ENC (Experimental NMR Conference) Student Travel Awards and the Travel Award from department of chemistry. Below are the publications he had during his Ph.D. career.

Publications:


