



# **Zero- and Ultralow-Field NMR for Chemical and Biochemical Analysis**

by

Seyma Alcicek

supervised by Prof. Szymon Pustelny

A dissertation submitted to the in partial fulfilment of the  
requirements for the degree of

Doctor of Philosophy

in

Biophysics

in the

Faculty of Physics, Astronomy and Applied Computer Science

of the

Jagiellonian University in Krakow

June 2022

Copyright 2022

by

Seyma Alcicek

# Abstract

Zero- and ultralow-field (ZULF) nuclear magnetic resonance (NMR) is a novel, low-cost, and portable NMR incarnation, where the phenomenon is studied in the absence or with very weak external magnetic fields. In contrast to conventional NMR, in which spin interactions with external fields dominate, ZULF NMR spectroscopy is performed under dominant spin-spin interactions. Direct observation of intramolecular spin interactions, which contains valuable information about molecular structures, enables high-precision (bio-)chemical analysis.

This dissertation presents ZULF NMR studies, revealing the potential of the technique in many areas of modern science and technology (chemistry and life sciences, but also agricultural and pharmaceutical industry). The new capabilities of ZULF NMR are demonstrated through the development of the method and its combination with different experimental techniques.

The first original part of the dissertation is dedicated to the comprehensive investigation of the group of phosphorus-containing organic compounds using zero-field NMR. Due to the 100% natural abundance of the spin-1/2 phosphorous isotope ( $^{31}\text{P}$ ), the organophosphorus compounds provide strong ZULF NMR signals of even thermally-polarized samples. This allows for high-precision chemical analysis of the compounds without the need for their expensive isotope labelling. The research is extended to key metabolites in protein metabolism. Specifically, urea and amino acids are investigated and the role of the solvent, by demonstrating via the chemical-exchange process, is studied.

As the next step, different versions of the parahydrogen-induced polarization (PHIP) techniques are employed to overcome the inherent low-sensitivity problem of NMR. For example, non-hydrogenative PHIP is harnessed for sensitive zero-field detection of naturally abundant  $^{15}\text{N}$ -pyridine derivatives. These molecules are important for drug development because of their antimicrobial, antiviral, or anticancer properties. Also, the first-ever

## ABSTRACT

---

zero-field spectrum of a SABRE hyperpolarized biomolecule, nicotinamide, is reported. Additionally, the dissertation presents the studies of newly emerged proton-exchange-based polarization technique (PHIP-X) for sensitive detection of such biomolecules as urea, glycine, ammonium, and glucose. Specifically, the first studies on the implementation of the PHIP-X technique into ZULF NMR spectroscopy are presented.

The final part of the dissertation is dedicated to ZULF NMR relaxometry, which is introduced to study a molecular environment. This goal is realized via monitoring the relaxation properties of observed ZULF NMR signals. While the strong influence of paramagnetic oxygen on heteronuclear long-lived singlet state is demonstrated, the first-ever human blood ultra-low-field NMR relaxometry study is presented as a proof of concept.

The dissertation presents broad, self-contained, interesting, and often pioneering research in the emerging field of ZULF NMR and forms solid grounds for future studies in the field.

*To love of my life and my sonshine*

# Table of Contents

|                                                                  |             |
|------------------------------------------------------------------|-------------|
| <b>Abstract</b>                                                  | <b>iv</b>   |
| <b>List of Tables</b>                                            | <b>vii</b>  |
| <b>List of Figures</b>                                           | <b>viii</b> |
| <b>1 Introduction</b>                                            | <b>1</b>    |
| <b>2 Theoretical Background</b>                                  | <b>3</b>    |
| 2.1 Nuclear Magnetic Resonance Spectroscopy . . . . .            | 3           |
| 2.1.1 Nuclear Spin and Magnetism . . . . .                       | 3           |
| 2.1.2 Nuclear Zeeman Effect . . . . .                            | 5           |
| 2.1.3 Precession and Larmor Frequency . . . . .                  | 7           |
| 2.1.4 Longitudinal and Transverse Relaxation . . . . .           | 8           |
| 2.1.5 NMR Signal . . . . .                                       | 10          |
| 2.1.6 Nuclear Spin Interactions . . . . .                        | 12          |
| 2.1.6.1 Chemical Shift . . . . .                                 | 12          |
| 2.1.6.2 Indirect Dipole-Dipole Coupling: $J$ -Coupling . . . . . | 13          |
| 2.1.6.3 Dipolar Coupling . . . . .                               | 14          |
| 2.1.6.4 Quadrupolar Coupling . . . . .                           | 14          |
| 2.1.6.5 Spin-Rotation Coupling . . . . .                         | 14          |
| 2.1.7 Relaxation Mechanisms . . . . .                            | 15          |
| 2.1.8 Product Operator Formalism . . . . .                       | 18          |
| 2.1.9 INEPT Pulse Sequence . . . . .                             | 21          |
| 2.2 Exotic NMR: Zero- and Ultralow-Field Spectroscopy . . . . .  | 22          |

## TABLE OF CONTENTS

---

|          |                                                                                                                 |           |
|----------|-----------------------------------------------------------------------------------------------------------------|-----------|
| 2.2.1    | Introduction of Zero-Field Nuclear Magnetic Resonance . . . . .                                                 | 23        |
| 2.2.2    | Generating Zero-Field NMR Signal by Thermal Prepolarization . . . . .                                           | 24        |
| 2.2.2.1  | Sudden Transfer to Zero Field . . . . .                                                                         | 25        |
| 2.2.2.2  | Adiabatic Transfer to Zero Field . . . . .                                                                      | 29        |
| 2.2.3    | Interpretation of Zero Field $J$ -Spectroscopy . . . . .                                                        | 30        |
| 2.2.4    | Ultralow-Field NMR . . . . .                                                                                    | 36        |
| 2.2.5    | Detection of Zero and Ultralow Field NMR:<br>Optical Magnetometry . . . . .                                     | 38        |
| 2.3      | Parahydrogen-Based Hyperpolarization . . . . .                                                                  | 41        |
| 2.3.1    | Parahydrogen . . . . .                                                                                          | 41        |
| 2.3.2    | Hydrogenative Parahydrogen-Induced Polarization . . . . .                                                       | 43        |
| 2.3.3    | Non-hydrogenative Parahydrogen Induced Polarization: Signal Am-<br>plification by Reversible Exchange . . . . . | 47        |
| <b>3</b> | <b>Zero-field NMR of Organophosphorus Compounds</b>                                                             | <b>51</b> |
| 3.1      | Motivation and Overview . . . . .                                                                               | 51        |
| 3.2      | Material and Method . . . . .                                                                                   | 52        |
| 3.2.1    | Sample Preparation . . . . .                                                                                    | 52        |
| 3.2.2    | Experimental Setup . . . . .                                                                                    | 54        |
| 3.2.3    | Data Processing and Simulation . . . . .                                                                        | 55        |
| 3.3      | Results and Discussion . . . . .                                                                                | 56        |
| 3.3.1    | Simple $XA_n$ Spin Systems . . . . .                                                                            | 56        |
| 3.3.2    | $(XA_n)B_m$ Spin Systems . . . . .                                                                              | 58        |
| 3.3.3    | Generating Zero-field NMR Signal in $^1\text{H}$ - $^{31}\text{P}$ Spin Systems . . . . .                       | 63        |
| 3.3.4    | Limitations and Outlook . . . . .                                                                               | 64        |
| 3.4      | Conclusion . . . . .                                                                                            | 66        |
| <b>4</b> | <b>Zero-field NMR of Biomolecules:<br/>Spin Topology Modification Under Chemical Exchange</b>                   | <b>68</b> |
| 4.1      | Motivation and Overview . . . . .                                                                               | 68        |

## TABLE OF CONTENTS

---

|          |                                                                                                                                 |           |
|----------|---------------------------------------------------------------------------------------------------------------------------------|-----------|
| 4.2      | Material and Methods . . . . .                                                                                                  | 70        |
| 4.2.1    | Sample Preparation . . . . .                                                                                                    | 70        |
| 4.2.2    | Zero-field NMR Experiments . . . . .                                                                                            | 70        |
| 4.2.3    | Data Processing and Simulations . . . . .                                                                                       | 71        |
| 4.3      | Results and Discussion . . . . .                                                                                                | 71        |
| 4.3.1    | Zero-field $J$ -spectra of Urea in Protic and Aprotic Solvents . . . . .                                                        | 71        |
| 4.3.2    | Effects of pH-dependent Chemical Exchange on Zero-field Spectra . . . . .                                                       | 72        |
| 4.3.3    | Zero-field $J$ -spectra of Deuterium Isotopologues of Urea . . . . .                                                            | 77        |
| 4.3.4    | Zero-field $J$ -spectra of Amino acids in Aqueous Solutions . . . . .                                                           | 79        |
| 4.4      | Conclusion . . . . .                                                                                                            | 83        |
| <b>5</b> | <b>Zero-field NMR of Natural Abundance <math>^{15}\text{N}</math>-Pyridine Derivatives with In Situ SABRE Hyperpolarization</b> | <b>85</b> |
| 5.1      | Motivation and Overview . . . . .                                                                                               | 85        |
| 5.2      | Material and Methods . . . . .                                                                                                  | 86        |
| 5.2.1    | Parahydrogen Generator . . . . .                                                                                                | 86        |
| 5.2.2    | Bubbling System . . . . .                                                                                                       | 87        |
| 5.2.3    | Sample Preparation . . . . .                                                                                                    | 89        |
| 5.2.4    | Experimental Setup . . . . .                                                                                                    | 89        |
| 5.3      | Results and Discussion . . . . .                                                                                                | 90        |
| 5.4      | Conclusion . . . . .                                                                                                            | 95        |
| <b>6</b> | <b>Proton-Exchange-Based Hyperpolarization for Biomolecules</b>                                                                 | <b>97</b> |
| 6.1      | Motivation and Overview . . . . .                                                                                               | 97        |
| 6.2      | PHIP-X in Conventional NMR . . . . .                                                                                            | 98        |
| 6.2.1    | Material and Methods . . . . .                                                                                                  | 98        |
| 6.2.2    | Results and Discussion . . . . .                                                                                                | 100       |
| 6.3      | PHIP-X in ZULF NMR . . . . .                                                                                                    | 108       |
| 6.3.1    | Material and Methods . . . . .                                                                                                  | 108       |
| 6.3.2    | Results and Discussion . . . . .                                                                                                | 109       |

## TABLE OF CONTENTS

---

|          |                                                                          |            |
|----------|--------------------------------------------------------------------------|------------|
| 6.4      | Conclusion . . . . .                                                     | 114        |
| <b>7</b> | <b>Zero- and Ultralow-Field NMR Relaxometry</b>                          | <b>116</b> |
| 7.1      | Motivation and Overview . . . . .                                        | 116        |
| 7.2      | Material and Methods . . . . .                                           | 117        |
| 7.2.1    | Sample Preparation . . . . .                                             | 117        |
| 7.2.2    | Experimental Technique . . . . .                                         | 118        |
| 7.3      | Results and Discussion . . . . .                                         | 120        |
| 7.3.1    | Effect of Oxygen on Zero-field NMR . . . . .                             | 120        |
| 7.3.2    | Effect of Paramagnetic $\text{CuSO}_4$ on Ultralow Field NMR . . . . .   | 123        |
| 7.3.3    | Ultralow-Field Relaxometry of Whole Blood and Plasma . . . . .           | 126        |
| 7.4      | Conclusion . . . . .                                                     | 128        |
| <b>8</b> | <b>Summary and Outlook</b>                                               | <b>130</b> |
|          | <b>Appendix A</b>                                                        | <b>134</b> |
| A.1      | $J$ -Coupling Constants . . . . .                                        | 134        |
| A.2      | Zero-field NMR Spectral Analysis via First-order Perturbation Theory . . | 136        |
| A.3      | Calculation of Optimal Delays for the INEPT Sequence . . . . .           | 137        |
| A.4      | Conventional NMR Spectral Analysis for Kinetic Study of PHIP reaction    | 137        |
| A.5      | Calibration of the Magnetic Field Using Gaussmeter . . . . .             | 139        |
|          | <b>Bibliography</b>                                                      | <b>139</b> |

# List of Tables

|     |                                                                                                                                                                                                                                                                                                                                |     |
|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 2.1 | Frequencies of allowed transitions between levels characterized with the quantum numbers $F$ and $F_T$ , corresponding to the dominant spin subsystem XA and total spin of the whole (XA)B <sub>3</sub> system, obtained using first-order perturbation theory. . . . .                                                        | 35  |
| 3.1 | Frequencies ( $\nu$ ) of the allowed transitions between the energy levels characterized with the quantum numbers $F$ and $F_T$ , corresponding to the dominant spin subsystem XA <sub>6</sub> , and the total spin $F_T$ of the whole (XA <sub>6</sub> )B system, obtained using the first-order perturbation theory. . . . . | 61  |
| 3.2 | Frequencies $\nu$ of the allowed transitions between the energy levels characterized with the quantum numbers $F$ and $F_T$ , corresponding to the dominant spin subsystem XA, and the total spin $F_T$ of the whole (XA)B <sub>6</sub> system, obtained using the first-order perturbation theory. . . . .                    | 62  |
| 4.1 | Transition frequencies ( $\nu$ ) between the levels characterized with the quantum numbers $F$ and $F_T$ , obtained using first-order perturbation theory, in the (XA)B spin subsystem. . . . .                                                                                                                                | 79  |
| A.1 | The $J$ -coupling constants of neat <sup>15</sup> N-pyridine and <sup>15</sup> N-pyridine in methanol solution. . . . .                                                                                                                                                                                                        | 134 |
| A.2 | The $J$ -coupling constants of <sup>15</sup> N-nicotinamide and <sup>15</sup> N-3,5 dichloropyridine in methanol. . . . .                                                                                                                                                                                                      | 135 |
| A.3 | The $J$ -coupling constants of <sup>15</sup> N-3-methoxypyridine and <sup>15</sup> N-4-methoxypyridine in methanol. . . . .                                                                                                                                                                                                    | 135 |
| A.4 | The $J$ -coupling constants of <sup>15</sup> N-3-methylpyridine and <sup>15</sup> N-4-methylpyridine in methanol. . . . .                                                                                                                                                                                                      | 136 |
| A.5 | The observable transitions for <sup>13</sup> C-methanol in ultralow field. . . . .                                                                                                                                                                                                                                             | 136 |
| A.6 | Spin-echo delays used for the INEPT pulse sequence in the detection of each molecule of interest. . . . .                                                                                                                                                                                                                      | 137 |

# List of Figures

|      |                                                                                                                                                                                                                                                                                                                                            |    |
|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 2.1  | Zeeman splitting of the energy levels for a nuclear spin-1/2. . . . .                                                                                                                                                                                                                                                                      | 6  |
| 2.2  | Larmor precession in $B_0$ magnetic field. . . . .                                                                                                                                                                                                                                                                                         | 7  |
| 2.3  | Longitudinal magnetization after application of instant magnetic field. . .                                                                                                                                                                                                                                                                | 8  |
| 2.4  | Demonstration of a basic NMR experiment. . . . .                                                                                                                                                                                                                                                                                           | 9  |
| 2.5  | Components of the complex FID signal and corresponding real and imaginary parts of the NMR spectrum. . . . .                                                                                                                                                                                                                               | 11 |
| 2.6  | Illustration of $^1\text{H}$ and $^{13}\text{C}$ NMR spectra of a $^{13}\text{C}$ -methyl group. . . . .                                                                                                                                                                                                                                   | 14 |
| 2.7  | Autocorrelation $G(\tau)$ and the spectral density $J(\omega)$ functions of fast and slow fluctuating local fields $B_{\text{loc}}$ . . . . .                                                                                                                                                                                              | 16 |
| 2.8  | Dependence of the relaxation times $T_1$ and $T_2$ on the correlation time. . .                                                                                                                                                                                                                                                            | 17 |
| 2.9  | Basic and refocused INEPT pulse sequences. . . . .                                                                                                                                                                                                                                                                                         | 21 |
| 2.10 | Energy levels of the eigenstates for a coupled spin-1/2 pair under the high and zero field. . . . .                                                                                                                                                                                                                                        | 24 |
| 2.11 | Magnetic field observed in ZULF NMR employing prepolarization and sudden transfer approach without and with an additional transverse DC magnetic pulse. . . . .                                                                                                                                                                            | 26 |
| 2.12 | Amplitude of the zero-field NMR signal of the $^{13}\text{C}$ - $^1\text{H}$ nuclear system versus the $^1\text{H}$ rotation angle $\theta_H$ induced by the field applied in the $x$ -direction. Experimental J-spectra of $^{13}\text{C}$ -formic acid after the sudden transition to zero-field with/without optimum $x$ pulse. . . . . | 27 |
| 2.13 | Behavior of the $^{13}\text{C}$ - $^1\text{H}$ system after applying a transverse pulse. . . . .                                                                                                                                                                                                                                           | 28 |
| 2.14 | Energy levels and observable $\Delta m_F=0$ transitions for the $\text{XA}_3$ spin system in zero-field NMR. Zero field spectra of $^{13}\text{C}$ -methanol, 2- $^{13}\text{C}$ -acetonitrile, and the mixture of these compounds. . . . .                                                                                                | 32 |

## LIST OF FIGURES

---

|      |                                                                                                                                                                                                                                                                                                     |    |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 2.15 | Energy-level diagram of the $XA$ and $(XA)B_3$ spin systems along with the ZULF NMR observable transitions indicated by dashed arrows. Experimental zero-field spectrum of $[2-^{13}C]$ -L-alanine with an inset showing the molecular structure of the compound. . . . .                           | 34 |
| 2.16 | Simulated and experimentally measured zero-field spectra of $^{15}N$ -pyridine. . . . .                                                                                                                                                                                                             | 36 |
| 2.17 | Schematic energy-level diagram of the $XA_3$ spin system at the ultralow field and experimental ultralow-field spectrum of $^{13}C$ -methanol. . . . .                                                                                                                                              | 37 |
| 2.18 | Schematic representation of four possible states of the hydrogen molecule and the equilibrium populations of the hydrogen isomers as a function of temperature. . . . .                                                                                                                             | 42 |
| 2.19 | Demonstration of parahydrogen-based hyperpolarization techniques. . . . .                                                                                                                                                                                                                           | 44 |
| 2.20 | Population models used to explain PHIP experiments. . . . .                                                                                                                                                                                                                                         | 45 |
| 2.21 | Pairwise addition of parahydrogen to dimethyl acetylenedicarboxylate to form dimethyl maleate. Zero-field spectra of hyperpolarized dimethyl maleate via repeated PHIP. The experimental average and simulated spectra of hyperpolarized dimethyl maleate with $^{13}C$ in the vinyl group. . . . . | 46 |
| 2.22 | Schematic of SABRE reaction. Experimental and simulated zero-field spectra of hyperpolarized $^{15}N$ -labelled pyridine by SABRE. Relative amplitude of the peaks in zero-field spectra of hyperpolarized $^{15}N$ -pyridine versus transient number. . . . .                                      | 49 |
| 3.1  | Photographs of the sample-preparation setup. . . . .                                                                                                                                                                                                                                                | 53 |
| 3.2  | Schematics of the experimental setup. . . . .                                                                                                                                                                                                                                                       | 55 |
| 3.3  | Structural formulas of trimethyl phosphate and trimethyl phosphite with heteronuclear $J$ -coupling. Energy-level diagram along with the observable transitions of the $XA_9$ spin system. Experimental and simulated $J$ -spectra of trimethyl phosphate and trimethyl phosphite. . . . .          | 57 |
| 3.4  | Energy-level diagrams of the $XA_6$ to $(XA_6)B$ spin system and related observable transitions. Experimental and simulated dichlorvos spectra along with transition predicted by the first-order perturbation theory. . . . .                                                                      | 59 |
| 3.5  | Energy-level diagram of the $XA$ and $(XA)B_6$ spin systems along with transitions observable at the zero field. Experimental and simulated spectra of dimethyl phosphite along with transition predicted by the first-order perturbation theory. . . . .                                           | 60 |

## LIST OF FIGURES

---

|     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |    |
|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| 3.6 | Structural formula of dimethyl methylphosphonate with the heteronuclear $J$ -couplings and experimental $J$ -spectrum of dimethyl methylphosphonate.                                                                                                                                                                                                                                                                                                                                                      | 63 |
| 3.7 | Amplitude of the zero-field NMR signal of the $^1\text{H}$ - $^{31}\text{P}$ nuclear system versus the $^1\text{H}$ rotation angle $\theta_H$ induced by the field applied along the $y$ -axis. Simulated $J$ -spectra of the $^1\text{H}$ - $^{31}\text{P}$ spin system, following sudden transition to zero-field with/without the optimum $y$ -pulse. Experimental $J$ -spectra of trimethyl phosphate obtained after the sudden transition to zero-field with/without optimum the $y$ -pulse. . . . . | 65 |
| 4.1 | Simulated and experimental zero-field spectra of $^{15}\text{N}_2$ -urea and $^{13}\text{C}$ , $^{15}\text{N}_2$ -urea in aprotic (DMSO) and protic ( $\text{H}_2\text{O}$ ) solvents. . . . .                                                                                                                                                                                                                                                                                                            | 73 |
| 4.2 | Experimental (top) and simulated (bottom) zero-field $J$ -spectra of $^{15}\text{N}_2$ -urea and $^{13}\text{C}$ , $^{15}\text{N}_2$ -urea in aqueous solutions at various pH values. . . .                                                                                                                                                                                                                                                                                                               | 75 |
| 4.3 | Zero-field $J$ -spectra of $^{15}\text{N}_2$ -urea solutions measured for guiding fields of 0.01 mT and 0.1 mT. . . . .                                                                                                                                                                                                                                                                                                                                                                                   | 76 |
| 4.4 | Simulated zero-field $J$ -spectra of deuterated $^{15}\text{N}_2$ -urea isotopologues with corresponding structural formulas. . . . .                                                                                                                                                                                                                                                                                                                                                                     | 78 |
| 4.5 | Experimental and simulated zero-field $J$ -spectra of $^{15}\text{N}_2$ -urea in aqueous solutions with various $^1\text{H}/\text{D}$ ratios. . . . .                                                                                                                                                                                                                                                                                                                                                     | 78 |
| 4.6 | Energy-level structures of the (XA)B, and $\text{XA}_2$ spin subsystems. Experimental spectra of $^{15}\text{N}_2$ -urea in the mixture of $\text{D}_2\text{O}/\text{H}_2\text{O}$ . . . . .                                                                                                                                                                                                                                                                                                              | 80 |
| 4.7 | Simulated and experimental zero-field NMR spectra of $[2\text{-}^{13}\text{C}]$ -glycine, $[2\text{-}^{13}\text{C}]$ -alanine, and $[2,3\text{-}^{13}\text{C}_2]$ -L-alanine. . . . .                                                                                                                                                                                                                                                                                                                     | 82 |
| 5.1 | Photograph of the home-built parahydrogen generator. . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                              | 87 |
| 5.2 | Schematics and photograph of the bubbling system. . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                 | 88 |
| 5.3 | Photograph from the outside and schematics of the inside of the zero-field NMR setup for SABRE measurements, using the commercial optically-pumped magnetometers. . . . .                                                                                                                                                                                                                                                                                                                                 | 91 |
| 5.4 | Experimental and simulated zero-field spectra of natural abundance $^{15}\text{N}$ -pyridine and its derivatives. . . . .                                                                                                                                                                                                                                                                                                                                                                                 | 93 |

## LIST OF FIGURES

---

|     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |     |
|-----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 6.1 | Scheme of two-steps hydrogenation of propargyl alcohol. Kinetic of propargyl alcohol hydrogenation reaction versus hydrogen bubbling time for the various concentration of Rh(I)-catalyst. The relative intensity of signal arising from hyperpolarized allyl alcohol and propanol via ALTADENA-PHIP for various parahydrogen bubbling times. . . . .                                                                                                                                                                                                                                                                                                                                           | 101 |
| 6.2 | Schematic representation of the PHIP-X process and basic and refocused $^1\text{H}$ - $^{15}\text{N}$ INEPT NMR spectra of [ $^{13}\text{C}$ , $^{15}\text{N}_2$ ]-urea and [ $^{15}\text{N}_2$ ]-urea hyperpolarized via PHIP-X. . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 102 |
| 6.3 | Effect of parahydrogen bubbling time on peak intensity in the $^1\text{H}$ - $^{15}\text{N}$ INEPT NMR spectra of hyperpolarized [ $^{13}\text{C}$ , $^{15}\text{N}_2$ ]-urea. Averaged $^1\text{H}$ - $^{15}\text{N}$ refocused INEPT NMR spectra of hyperpolarized [ $^{13}\text{C}$ , $^{15}\text{N}_2$ ]-urea for each bubbling time interval. The magnetic field in which the parahydrogen bubbling was conducted versus signal enhancement in the $^{15}\text{N}$ -NMR spectrum of hyperpolarized [ $^{13}\text{C}$ , $^{15}\text{N}_2$ ]-urea. The image of the adjustable magnet consisting of transversely oriented Halbach arrays that was used for adjusting bubbling field. . . . . | 104 |
| 6.4 | Experimental setup used for the ALTADENA-PHIP and PHIP-X experiments. Intensity of hyperpolarized [ $^{13}\text{C}$ , $^{15}\text{N}_2$ ]-urea versus the position of NMR tube inside the bore of spectrometer during parahydrogenation. . .                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 105 |
| 6.5 | Refocused $^1\text{H}$ - $^{15}\text{N}$ INEPT NMR spectra of hyperpolarized [ $^{15}\text{N}$ ]-glycine, [ $^{15}\text{N}$ ]-ammonium, and [ $^{15}\text{N}$ ]-benzamide by PHIP-X. Refocused $^1\text{H}$ - $^{13}\text{C}$ INEPT NMR spectrum of D-[1- $^{13}\text{C}$ ]-glucose hyperpolarized via PHIP-X . .                                                                                                                                                                                                                                                                                                                                                                               | 107 |
| 6.6 | Experimental sequence for the zero-field NMR experiment using PHIP. Scheme of parahydrogenation reaction of propargyl alcohol to form allyl alcohol. Experimental and simulated zero-field spectra of hyperpolarized allyl alcohol. . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 111 |
| 6.7 | Experimental sequence for the ULF NMR experiment using PHIP and PHIP-X. Ultralow-field NMR spectrum of hyperpolarized allyl alcohol. .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 113 |
| 7.1 | Experimental sequence for zero- and ultralow-field NMR relaxometry. . .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 119 |
| 7.2 | Zero-field NMR resonance measured in the $J$ -spectra of the trimethyl phosphate samples prepared with a different number of the freeze-pump-thaw cycles. . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 120 |
| 7.3 | Relaxation times $T_1$ , $T_f$ , $T_s$ and $T_2$ of $^{13}\text{C}$ -formic acid versus the number of the freeze-pump-thaw cycles performed during sample preparation. . .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 121 |

## LIST OF FIGURES

---

|     |                                                                                                                                                                                                                                                                                          |     |
|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 7.4 | Ultralow-field experimental spectra versus the storage time. The variation in $T_1$ relaxation time constant of water protons at various ULFs with respect to the different $\text{CuSO}_4$ concentration (mM) in a solution prepared with/without 2 cycles of freeze-pump-thaw. . . . . | 124 |
| 7.5 | A serial experimental ultralow-field spectra versus various $\text{CuSO}_4$ concentration. $T_2$ relaxation time constants of water protons versus different $\text{CuSO}_4$ concentration in solution prepared with/without 2 cycles of freeze-pump-thaw. . . . .                       | 125 |
| 7.6 | $^1\text{H}$ ULF NMR signal of human whole-blood and blood plasma. . . . .                                                                                                                                                                                                               | 126 |
| 7.7 | $T_1$ and $T_2$ relaxation magnetic-field profiles of human whole-blood and blood-plasma measured at ultralow fields. . . . .                                                                                                                                                            | 127 |
| A.1 | Peak assignments on $^1\text{H}$ NMR spectrum of the sample consisting of propargyl alcohol and Rh-catalyst in acetone. . . . .                                                                                                                                                          | 138 |
| A.2 | Photograph of a gaussmeter used for calibration of the benchtop NMR fringe magnetic field to which an NMR sample was subjected. . . . .                                                                                                                                                  | 139 |

# Acknowledgments

I am grateful to many people who supported me throughout my PhD journey. This work would not have been possible without their help and kindness.

Foremost, I am particularly indebted to my PhD supervisor, Prof. Szymon Pustelny, for giving me the opportunity to be in this wonderful scientific environment of his group as a Marie Skłodowska-Curie Early-Stage Researcher. I would like to express my sincere thanks to him for providing endless support and guidance while giving me space to develop in line with my scientific curiosity and interest. Working with such a brilliant scientist has been a wonderful privilege for me.

At the beginning of the project, I had a pleasure to visit Prof. Malcolm Levitt's group at the University of Southampton. I am incredibly grateful to him for his kind invitation and inspiring lectures, which allowed me to learn the principles of NMR from the marvelous expert. Special thanks go to his group for a great working environment during my visit.

During the last period of my PhD, I had a chance to visit Dr. Danila Barskiy's group at the Johannes Gutenberg University in Mainz. While the research presented in Chapter 6 is the result of this visit, he also contributed to the study presented in Chapters 4 and 7, where I greatly benefited from fruitful discussions with him. I would like to thank his team, Erik Van Dyke and Jingyan Xu, who constructed the setup for the high-field NMR study presented in Chapter 6.

My special thanks to Prof. Szymon Pustelny's group with many intelligent young researchers at the Jagiellonian University in Kraków. I would like to express my special thanks to Piotr Put who built two ZULF NMR setups, which were used for all ZULF NMR studies presented in this dissertation. Most of the works presented in this dissertation are products of our ambition, hard work, and countless hours of discussion with him (often ending with a crazy idea). I would like to thank Łukasz Bodek for help constructing the parahydrogen generator, bubbling system, and vapor condenser. Also, I would

---

like to thank Oksana Bondar and Adam Kubrak for their contribution to the studies demonstrated in Chapters 5 and 7 performed in the last period of my PhD. I would like to thank Vladimir Kontul scientifically for developing digital signal processing software for ZULF NMR data and contributing to the construction of the optical-magnetometer setup. Moreover, my special thanks to him and my dear Aleksandra Sierant, for being my close friends, and making every work day joyful and meaningful. I feel incredibly fortunate to meet such great people in Kraków! I always miss our coffee breaks!

I would like to thank my parents, Serpil and Atila Sezer, who always think that I do a great job and are proud of me!

Finally, I am deeply grateful to my love Fatih, for standing beside me throughout the long and winding road toward this dissertation. I would not have made it without his understanding and unconditional support. Besides our life-long collaboration, being parents of our son, we had a chance to collaborate on a scientific base providing the results presented in Chapter 7. Last, but not least, I would like to thank my son, Umut, for his understanding, while I was spending the weekends and evenings on this dissertation instead of playing with him.

# Chapter 1

## Introduction

Zero- and ultralow-field (ZULF) nuclear magnetic resonance (NMR) is a novel, portable, and cost-effective spectroscopic technique that enables high-precision chemical analysis through direct observation of intramolecular spin interactions in the absence of an external magnetic field. In such a regime, the Zeeman interaction vanishes, and indirect spin-spin interactions ( $J$ -coupling), which exist between spins of nuclei in molecules, become dominant when other interactions averaged out to zero in isotropic liquids.  $J$ -coupling is mediated by the molecule's electrons over distances of several chemical bonds, hence providing information about the molecular structure and the strength and orientation of its chemical bonds [1]. This enables the identification, characterization, and quantification of molecular structures by providing the high-resolution measurement of spin-spin couplings with remarkably narrow resonance linewidths as a result of the high absolute field homogeneity and long spin-coherence times [2, 3]. Furthermore, the greater skin depth at low frequencies enables us to measure samples, and monitor chemical and biochemical reactions inside even metal containers [4]. ZULF NMR signals are often detected by optically-pumped magnetometers, which have several advantages such as high sensitivity, low-price, small footprint, and non-cryogenic operation [5]. Nowadays, such sensors are also commercially available, which facilitates their application by non-experts [6, 7].

This dissertation explores the applicability of several ZULF NMR spectroscopy techniques in many areas, especially for chemical and biochemical analysis. Chapter 2 provides essential background on conventional NMR which might be crucial in grasping the basic principles of ZULF NMR spectroscopy. Further, parahydrogen-based polarization techniques are introduced briefly for conventional NMR as well as ZULF NMR spectroscopy. Chapter 3 discusses the high-selective detection of organophosphorus com-

pounds by observing  $^1\text{H}$ - $^{31}\text{P}$  spin systems at zero-field regime. Chapter 4 concerns the influence of the chemical-exchange process on spin systems under ZULF conditions, providing many examples of measurements of key biomolecules (e.g., urea and amino acids). Chapter 5 presents zero-field NMR of numerous natural-abundance  $^{15}\text{N}$ -pyridine derivatives with the implementation of *in situ* SABRE hyperpolarization. Chapter 6 discusses the hyperpolarization of a wide range of molecules through polarization transfer by the proton-exchange process, so-called PHIP-X, and this new technique is introduced for ZULF NMR studies. Chapter 7 demonstrates the ZULF NMR relaxometry for chemical and biological samples, including human whole-blood and plasma. Finally, Chapter 8 summarizes the overall presented studies and mentions potential future directions for ZULF NMR.

# Chapter 2

## Theoretical Background

*"I was taught that  
the way of progress  
is neither swift nor easy."  
-Marie Skłodowska Curie*

### 2.1 Nuclear Magnetic Resonance Spectroscopy

This section provides an essential background of conventional NMR to facilitate understanding of the work, particularly the work performed using high-field NMR presented in Chapter 6. It is also a starting point for understanding of ZULF NMR and PHIP techniques discussed in the following chapters. A detailed discussion on conventional NMR can be found in textbooks such as Refs. [8–10].

#### 2.1.1 Nuclear Spin and Magnetism

An atom is composed of a nucleus surrounded by electrons. The nucleus is made up of protons and neutrons that are also known as nucleons. An electron has a negative elementary charge  $-e$ . At the same time, a proton has a total charge of  $+e$ , while a neutron is electrically neutral. Since an electron is 1836 times lighter than a proton or a neutron, the center of mass of an atom lies within the nucleus and hence, in an atom, the electron moves with respect to the nucleus.

Along with mass and electric charge, a specific property of elementary particles is their spin. A nuclear spin is explained as an angular momentum that arises from an intrinsic “rotation” (spin) of nucleons and their arrangement within the nucleus (nuclear

shell model). Despite this composite nature, the resultant angular momentum of a nucleus is called a nuclear spin. The magnitude of the nuclear spin  $\mathbf{I}$  is quantized and given by:

$$|\mathbf{I}| = [I(I + 1)]^{1/2}\hbar, \quad (2.1)$$

where  $\hbar$  is the reduced Planck constant and  $I$  is the spin quantum number, which takes a value of an integer or a half-integer. Projection of the spin on the quantization axis is given by the magnetic quantum number  $m_I$  that takes one of the  $2I + 1$  integer values between  $-I$  and  $I$  separated by a unity,  $m_I = -I, -I + 1, \dots, I$ . In the absence of external magnetic and electric fields, energies associated with different spin orientation are identical and the corresponding energy levels are degenerate.

Nucleons are spin-1/2 particles. A net spin of a nucleus  $I_{\text{ALL}}$  is determined by a combination of spins of all nucleons and takes the values according to the rule:

$$I_{\text{ALL}} = |I_1 - I_2 - \dots - I_n|, |I_1 - I_2 - \dots - I_n| + 1, \dots, I_1 + I_2 + \dots + I_n, \quad (2.2)$$

where  $I_n$  is the spin quantum number of the  $n^{\text{th}}$  particle. An energy level of each possible nuclear spin state is different. The spin quantum number of the nuclear state with the lowest energy is called the ground-state nuclear spin. There are some guiding rules for determining the ground-state spin of an atomic nucleus:

1. If the number of neutrons and the number of protons are both even, the ground-state nuclear spin is 0. Examples of such nuclei are:  $^{12}\text{C}$ ,  $^{16}\text{O}$ .
2. If the number of neutrons and the number of protons are both odd, the ground-state nuclear spin is an integer value higher than zero. Examples are:  $^2\text{H}$  ( $I = 1$ ),  $^{14}\text{N}$  ( $I = 1$ ).
3. If the number of nucleons is odd, then the ground-state nuclear spin is a half-integer. Examples are:  $^1\text{H}$  ( $I = 1/2$ ),  $^{13}\text{C}$  ( $I = 1/2$ ),  $^{15}\text{N}$  ( $I = 1/2$ ),  $^{31}\text{P}$  ( $I = 1/2$ ),  $^{35}\text{Cl}$  ( $I = 3/2$ ).

In conventional NMR, the ground-state nuclear spin is often simply referred to as the

nuclear spin.

Nuclei with spin-1/2 have spherical distribution of electric charge, thus the electric and magnetic fields originating from the nucleus surrounding such nuclei are homogeneous in all directions. This is a great property that facilitates the understanding of their behavior during NMR experiments. On the other hand, for nuclei with spins larger than 1/2, the charge distribution is not spherically symmetric, which results in nuclear quadrupole and (potentially) higher moments. In principle, this can complicate the nuclear spin dynamics in NMR.

Another property of subatomic particles is their magnetic moments. Electrons and nuclei have a permanent magnetism linked to their spin. Thus, the nuclear magnetic moment  $\boldsymbol{\mu}$  is proportional to the nuclear spin angular momentum  $\mathbf{I}$  by:

$$\boldsymbol{\mu} = \gamma \mathbf{I}, \quad (2.3)$$

where  $\gamma$  is a gyromagnetic ratio, a unique proportionality constant for each nucleus. In a very simplistic picture, one can assume that nuclei with a non-zero magnetic moment interact with the magnetic field as if they were small bar magnets. The potential energy of this interaction  $E_{\text{mag}}$  is determined by the magnetic field  $\mathbf{B}$  and the magnetic moment  $\boldsymbol{\mu}$ :

$$E_{\text{mag}} = -\boldsymbol{\mu} \cdot \mathbf{B}. \quad (2.4)$$

This relation shows that the stronger the field, the larger the energy of the interaction.

### 2.1.2 Nuclear Zeeman Effect

When an external magnetic field is applied, the  $(2I + 1)$ -fold degeneracy of the nuclear-spin state is alleviated and the magnetic sublevels start to have different energies. The Zeeman energy-level shift of the  $m_I$  sublevel, induced by the magnetic field of magnitude  $B_0$  applied the quantization ( $z$ -) axis is given by:

$$E_{m_I} = -\gamma \hbar m_I B_0. \quad (2.5)$$

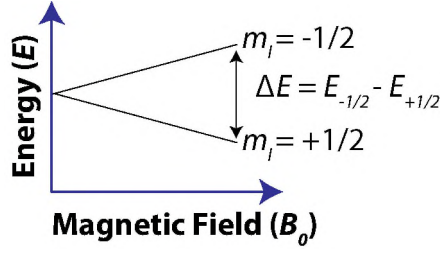


Figure 2.1: Zeeman splitting of the energy levels for a nuclear spin-1/2 with  $\gamma > 0$  as a function of the magnitude  $B_0$  of the  $z$ -oriented magnetic field.

Figure 2.1 shows an energy-level dependence of the  $m_I$  sublevels versus the magnitude of the  $z$ -oriented magnetic field  $B_0$  of a generic spin-1/2 particle.

The energy difference between two magnetic sublevels of the spin-1/2 particle subjected to the magnetic field of magnitude  $B_0$  is given by:

$$\Delta E = E_{m_{-1/2}} - E_{m_{+1/2}} = \gamma \hbar B_0. \quad (2.6)$$

Although the spin-1/2 particle can be in the  $m_{-1/2}$  or  $m_{+1/2}$  states, in thermal equilibrium, the lower energy state  $m_{+1/2}$  is more occupied. This is explained by the Boltzmann distribution, which is a probability measure that defines the distribution of spin populations between states as a function of energy and temperature. The population ratio between two energy sublevels, that is, the probability ratio of finding particles in either of the two states separated by the energy difference  $\Delta E$ , can be calculated using the Boltzmann equation:

$$\frac{P_{m_{-1/2}}}{P_{m_{+1/2}}} = \exp\left(-\frac{|\Delta E|}{k_B T}\right) = \exp\left(-\frac{\gamma \hbar B_0}{k_B T}\right), \quad (2.7)$$

where  $P_i$  is the population in  $i^{\text{th}}$  state,  $T$  is the temperature, and  $k_B$  is the Boltzmann constant. The nuclear polarization of an ensemble of spin-1/2 nuclei is given by:

$$p = \frac{P_{m_{+1/2}} - P_{m_{-1/2}}}{P_{m_{+1/2}} + P_{m_{-1/2}}}. \quad (2.8)$$

Equations (2.7) and (2.8) allow one to calculate the polarization of an NMR sample. It shows that even in several tesla magnetic fields, nuclear spin polarization is on the

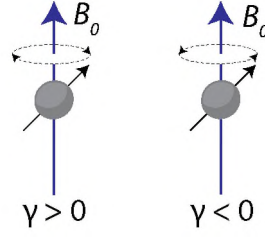


Figure 2.2: Larmor precession of two nuclei characterized by an opposite sign gyromagnetic ratios in a given magnetic field. For positive  $\gamma$ , the direction of rotation is clockwise, whereas for negative  $\gamma$ , the direction is counterclockwise.

order of  $10^{-5}$ . As a result, the total nuclear magnetization of an ensemble is very small. This results in low sensitivity of NMR as an analytical tool.

### 2.1.3 Precession and Larmor Frequency

In the absence of an external magnetic field, the spatial orientation of the magnetic moments is isotropic, meaning that all possible directions are equally represented. However, once the spins are placed in the field, the nuclear magnetic moment is either in the same direction as the spin polarization (for nuclei with  $\gamma > 0$ ), or in the opposite direction of the spin polarization (for nuclei with  $\gamma < 0$ ). The spin polarization begins to move around the field maintaining a constant angle between the spin magnetic moment and the external magnetic field, this movement is known as spin precession. The reason for such behavior is that spins have angular momentum, as well as a magnetic moment under a magnetic field. The frequency of spin precession  $\omega_0$  is called the Larmor frequency and is equal to the frequency of transition between two successive magnetic sublevels of  $\Delta m_I = 1$ . The frequency can be calculated using Eq. (2.6) and the well-known relationship between the energy and frequency,  $E = \hbar\omega$ :

$$\omega_0 = -\gamma B_0. \quad (2.9)$$

For a given magnetic field  $B_0$ , the direction of Larmor precession depends upon the sign of the gyromagnetic ratio. As shown in Fig. 2.2, the magnetic moment precesses clockwise for positive gyromagnetic ratios ( $\gamma > 0$ ) and counter-clockwise for negative gyromagnetic ratios ( $\gamma < 0$ ).

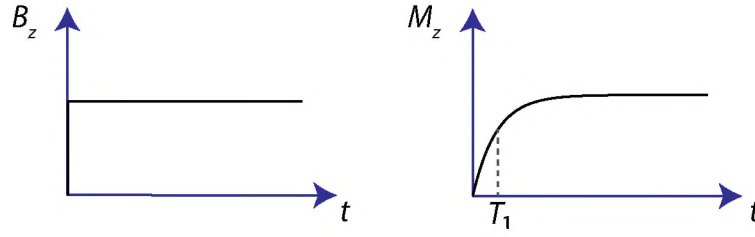


Figure 2.3: Longitudinal magnetization (right) after application of an instant static magnetic field along the  $z$ -axis (left). The  $T_1$  time constant corresponding to the shown longitudinal relaxation process is indicated with a dashed line.

### 2.1.4 Longitudinal and Transverse Relaxation

The net magnetic moment oriented along the external magnetic field (conventionally denoted in high-field NMR as the  $z$ -axis) is called longitudinal magnetization. When the field is applied, the longitudinal magnetization grows over time (Fig. 2.3), while the spin states establish the Boltzmann-equilibrium distribution:

$$M_z(t) = M_z(eq)(1 - e^{-t/T_1}), \quad (2.10)$$

where  $M_z(eq)$  is the equilibrium magnetization and  $T_1$  is the constant called the longitudinal relaxation time or the spin-lattice relaxation time. Relaxation occurs by the interaction of nuclear spin with magnetic fields produced by fluctuating magnetic dipoles because of random molecular motions, and this process lies between microseconds to minutes (for detailed discussion see Sec. 2.1.7).

As described above, spins precess at the Larmor frequency around the main field while reaching thermal equilibrium. In such a situation, there is no net magnetization perpendicular to the main field because the magnetization distribution in thermal equilibrium is symmetric around the field. In conventional high-field NMR experiments, in order to convert (rotate) the longitudinal magnetization into the transverse magnetization, an oscillating magnetic-field pulse, the so-called radio-frequency (rf) pulse, is applied. If longitudinal magnetization is along the  $z$ -axis and is rotated by  $\pi/2$  about the  $x$ -axis, then the result is the transverse magnetization along the  $-y$  axis (Fig. 2.4). In this case, it might be considered as the pulse duration is considerably shorter than the precession

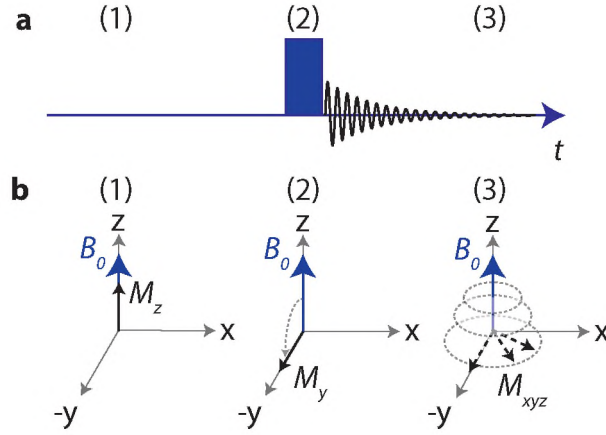


Figure 2.4: Schematics of basic NMR experiment. (a) Simple pulse sequence along with the NMR signal and (b) nuclear magnetization during the NMR experiment at three stages of a NMR experiment: (1) The longitudinal magnetization  $M_z$  at thermal equilibrium under the  $B_0$  magnetic field applied along the  $z$ -axis; (2) Rotation of magnetization due to application of the  $x$ -oriented  $\pi/2$  rf pulse, tilting the magnetization toward the  $-y$  axis (generation of the transverse magnetization,  $M_y$ ); (3) Evolution of transverse magnetization (Larmor precession) around the  $z$ -axis and relaxation toward the longitudinal magnetization. Note that for clarity no precession is shown during stage 2 (assumption of pulse duration much shorter than the precession period).

period, thus no spin precession occurs during the pulse.

If the system is exposed to an rf pulse of the same frequency as the Larmor frequency of the nuclei, the rf field is resonant with the precession of the nuclear spins. Thus, the nuclei gain energy, which gives rise to a transition between nuclear spin states in the system (e.g., the transition between the  $m_{+1/2}$  and  $m_{-1/2}$  states in the spin-1/2 nuclei). However, this resonance does not occur if the rf pulse does not have the appropriate frequency and duration for this transition. Therefore, the optimal pulse for a specific nucleus depends on the energy difference between states, thus the gyromagnetic ratio of this nucleus and the applied magnetic field, as shown in Eq. (2.6). For instance, the rf pulse tuned to the Larmor frequency of  $^1\text{H}$  (on-resonant pulse for  $^1\text{H}$  spins) does not have same effect on  $^{15}\text{N}$  spins (off-resonant pulse for  $^{15}\text{N}$  spins).

When the pulse is turned off, the transverse-magnetization component  $M_{xy}(0)$  precesses around the main static field at the Larmor frequency. Simultaneously, the transverse magnetization decreases exponentially (relaxes), whereas the longitudinal magneti-

ization recovers. Transverse magnetization at  $t$  time  $M_{xy}(t)$  is given by:

$$M_{xy}(t) = M_{xy}(0)e^{-t/T_2}, \quad (2.11)$$

where  $T_2$  is a constant called the transverse relaxation time or the spin-spin relaxation time.

The decay of the transverse magnetization can be written separately for the  $x$ - and  $y$ -axes:

$$\begin{aligned} M_x(t) &= M_x(0) \sin(\omega_0 t) e^{-t/T_2}, \\ M_y(t) &= -M_y(0) \cos(\omega_0 t) e^{-t/T_2}, \end{aligned} \quad (2.12)$$

where  $M_x(0)$  and  $M_y(0)$  are the corresponding initial (immediately after the pulse) components of the magnetization along  $x$  and  $y$ .

### 2.1.5 NMR Signal

After the rf pulse, precessing and decaying nuclear transverse magnetization is detected by inductive coils surrounding the spin ensemble (NMR sample). According to the Faraday induction law that is employed in the pickup-detection scheme, the change of magnetic flux through an enclosed loop of a conductor induces an electric current in the loop. Thereby, the NMR signal is associated with an oscillating electric current, the so-called free induction decay (FID).

In conventional NMR, the so-called quadrature detector may be used. The scheme involves the simultaneous acquisition of an NMR signal with the coils along the  $x$ - and  $y$ -axes, which allows to determine complex FID  $s(t)$ , which is given by:

$$\begin{aligned} s(t) &= s_x(t) + i s_y(t) \\ &= s(0)[\cos(\omega_0 t) + i \sin(\omega_0 t)]e^{-t/T_2} \\ &= s(0)e^{-i\omega_0 t} e^{-t/T_2}, \end{aligned} \quad (2.13)$$

where  $s_x(t)$ , and  $s_y(t)$  are  $x$ - and  $y$ -components of the complex NMR signal. The components depend on time-dependent magnetization components  $M_x(t)$  and  $M_y(t)$ .

Since the interpretation of time-domain FID is challenging, Fourier transform is used to convert the NMR signal from the time to the frequency domain. Presenting the NMR signal as its spectrum is useful for discerning information. Application of the (one-sided) Fourier transform to the NMR signal given by Eq. (2.13) yields the so-called complex Lorentzian:

$$\begin{aligned}
 s(\omega) &= \mathcal{F}\{s(t)\} \\
 &= \int_0^\infty s(t)e^{-i\omega t} dt \\
 &= \int_0^\infty s(0)e^{-i\omega_0 t} e^{(-t/T_2)} e^{-i\omega t} dt \\
 &= \frac{s(0)T_2}{1 + [T_2(\omega_0 - \omega)]^2} - i \frac{s(0)T_2^2(\omega_0 - \omega)}{1 + [T_2(\omega_0 - \omega)]^2}.
 \end{aligned} \tag{2.14}$$

As shown in Eq. (2.14), the complex Lorentzian consists of real and imaginary parts, which are called the absorption and dispersion Lorentzian, respectively. The real part of NMR spectrum consists of resonance at the Larmor frequency  $\omega_0$  with a full width at half maximum (FWHM) given by  $1/(\pi T_2)$ , as illustrated in Fig. 2.5.

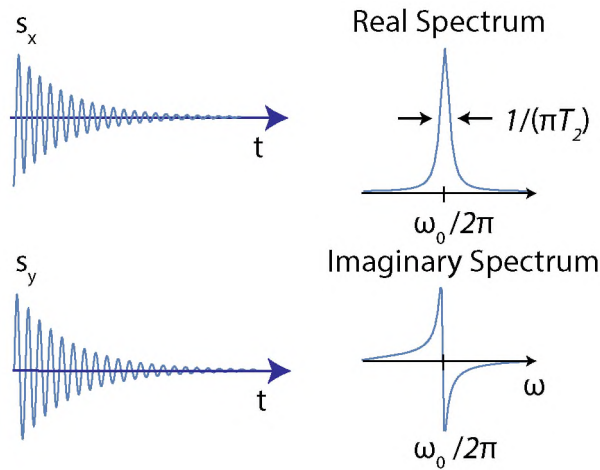


Figure 2.5:  $x$ - (top left) and  $y$ - (bottom left) components of the complex FID signal and corresponding real (top right) and imaginary (bottom right) parts of the NMR spectrum. The FID signal manifests in the spectrum as a resonance of the central frequency  $\omega_0/2\pi$  and FWHM  $1/(\pi T_2)$ .

### 2.1.6 Nuclear Spin Interactions

The nuclear spin interaction can be divided into external and internal interactions. To some extent, the external interactions, i.e., the interactions with static and oscillating magnetic fields, were discussed above. Here, we briefly review the internal spin interactions to better understand the original work presented in this dissertation.

#### 2.1.6.1 Chemical Shift

The indirect magnetic interaction between the external magnetic field and the nuclear spins that is associated with electrons of the investigated molecules is known as a chemical shift. Since electrons possess a magnetic moment (both at internal and external levels), in the presence of the external field they circulate about the direction of the applied magnetic field around the nucleus and produce a current. This circulating current generates a magnetic field opposed to the external field (Lenz law), called an induced field. The strength of the induced field  $\mathbf{B}_{\text{ind}}$  is proportional to the strength of the external field  $\mathbf{B}_0$  with the proportionality constant being called the chemical-shift tensor  $\sigma$  (in general, the directions of the external and induced field are not parallel):

$$\mathbf{B}_{\text{ind}} = \sigma \mathbf{B}_0. \quad (2.15)$$

The induced field  $\mathbf{B}_{\text{ind}}$  modifies the magnetic field to which nuclear spin  $j$  is subjected, resulting in the effective change of its Larmor frequency:

$$\omega_j = -\gamma_j B_0 (1 - \sigma_j). \quad (2.16)$$

It should be noted that the chemical-shift tensor is specific for each nuclear spin in a molecule due to differences in the electronic environment, unless the spins are chemically equivalent. In order to provide an external-field-independent measure for this parameter, a relative scale is introduced:

$$\delta_j = \frac{\omega_j - \omega_{\text{ref}}}{\omega_{\text{ref}}}, \quad (2.17)$$

where  $\delta_j$  is the chemical shift of the  $j^{\text{th}}$  nucleus and  $\omega_{\text{ref}}$  is the reference Larmor frequency of this type of nucleus in a reference molecule of tetramethylsilane (the chemical shift of the reference compound is assumed to be zero). Because chemical shifts are small numbers, they are usually expressed in parts per million (ppm).

### 2.1.6.2 Indirect Dipole-Dipole Coupling: $J$ -Coupling

The indirect dipole-dipole interactions of nuclear spins, the so-called  $J$ -coupling, are mediated through the electrons of the molecule over the distances of several chemical bonds. Unlike a chemical shift,  $J$ -coupling is magnetic-field independent.

The mechanism of  $J$ -coupling involves the magnetic interaction of the electron with two nuclei (the Fermi contact interaction) and the mutual interaction of the electrons which obeys the Pauli exclusion principle. Because of the participation of the electrons,  $J$ -coupling is orientation-dependent, therefore it survives averaging over molecular rotation in isotropic liquids.

If two nuclear spins with the same sign of gyromagnetic ratios are connected by one bond, the  $J$ -coupling constant takes a positive value and vice versa. On the other hand, the sign of longer-range  $J$ -coupling constants depends on molecular structural parameters, such as bond angles and torsional angles.

The  $J$ -coupling interaction contributes to the energy of a spin system. When the  $J$ -coupling constant is positive and the spins are parallel, the energy of the spin system increases and vice versa. This induced energy difference by the  $J$ -coupling interaction results in small frequency changes; as a consequence, specific coupling patterns arise in the NMR spectra, which may be used for determination of the molecular structures.

Since this dissertation is mainly based on heteronuclear  $J$ -coupling interactions, as a simple example,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of  $^{13}\text{C}$ -methyl group are demonstrated in Fig. 2.6. Due to heteronuclear  $J$ -coupling interaction between  $^{13}\text{C}$  and  $^1\text{H}$  in this group, the  $^1\text{H}$  resonance peak observed in the NMR spectrum is split into a doublet, while the  $^{13}\text{C}$  resonance peak is split into a quartet. In the following section, ZULF NMR spectra of  $^{13}\text{C}$ -methyl are also demonstrated to better understand the difference from conventional

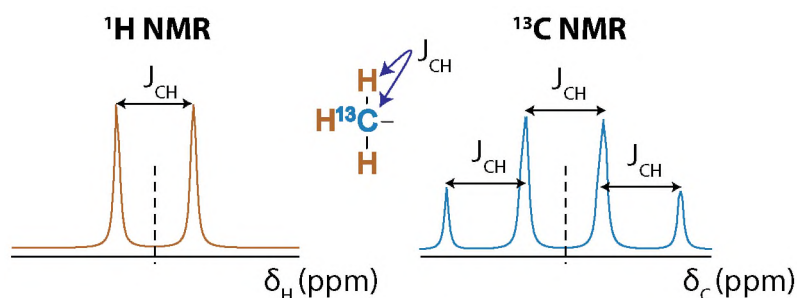


Figure 2.6: Illustration of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of a  $^{13}\text{C}$ -methyl group, originating from the heteronuclear  $J$ -coupling interaction of the  $J_{\text{CH}}$  constant between  $^{13}\text{C}$  and  $^1\text{H}$ .

NMR.

### 2.1.6.3 Dipolar Coupling

The interaction of nuclear spins with each other through space is called direct dipole-dipole coupling. This coupling is averaged to zero in isotropic liquids by rapid molecular tumbling in solution. On the other hand, in the case of large molecules, this motion is not fast enough for a complete averaging out of dipole-dipole interaction and the remaining part contributes to relaxation especially for spins-1/2. This subject is discussed in a following section from the point of view of a relaxation mechanism.

### 2.1.6.4 Quadrupolar Coupling

As mentioned above, nuclei with spin larger than 1/2 possess nuclear quadrupole moment due to the non-spherical charge distribution. The electric quadrupole moment of the nucleus interacts strongly with the electric-field gradients created by a surrounding-electron cloud. The quadrupolar interaction is averaged by rapid isotropic molecular rotation in liquids and contributes only indirectly to relaxation processes, leading to short coherence times and hence broad resonance lines as discussed in the following section.

### 2.1.6.5 Spin-Rotation Coupling

Since a molecule comprises positive and negative electric charges (nucleus and electrons), its motion causes the charge circulation, resulting in an electric current. Thus, the molecular rotation produces local magnetic fields. The interaction of the nuclear spins with

this field is known as a spin-rotation coupling. The only importance of this interaction for the work presented in this dissertation could be its contribution to relaxation, however, this contribution is negligible except for small molecules in gases or non-viscous liquids.

### 2.1.7 Relaxation Mechanisms

In the previous section, the processes of transverse and longitudinal relaxations are briefly introduced. Here, the mechanism of the relaxation process is discussed in more detail, since understanding of its key concepts is needed for the discussion presented in Chapter 7.

Since the molecule randomly tumbles and reorients over time, the magnitude and direction of local magnetic fields around a nucleus are subjected to fluctuation. There are several sources causing such fluctuation including dipole-dipole interaction, chemical-shift anisotropy, etc. In turn, the field fluctuations cause relaxation of spin polarization.

In general, the fluctuations of different interactions are correlated with each other. The simplest approach to understanding these correlations requires the assumption that each nucleus is exposed to an independent, time-varying random field  $B_{\text{loc}}(t)$ , which averages out to zero over long times:

$$\langle B_{\text{loc}}(t) \rangle_t = 0. \quad (2.18)$$

Although the field averages out, the magnitude of this fluctuating field is not zero, as it is equal to the square of the field:

$$\langle B_{\text{loc}}^2(t) \rangle_t \neq 0. \quad (2.19)$$

To define how fast the field fluctuates, the autocorrelation function  $G(\tau)$  for a time interval  $\tau$  is introduced:

$$G(\tau) = \langle B_{\text{loc}}(t)B_{\text{loc}}(t + \tau) \rangle_t \neq 0. \quad (2.20)$$

As shown, slow field fluctuations cause slow decay of the autocorrelation function  $G(\tau)$ ,

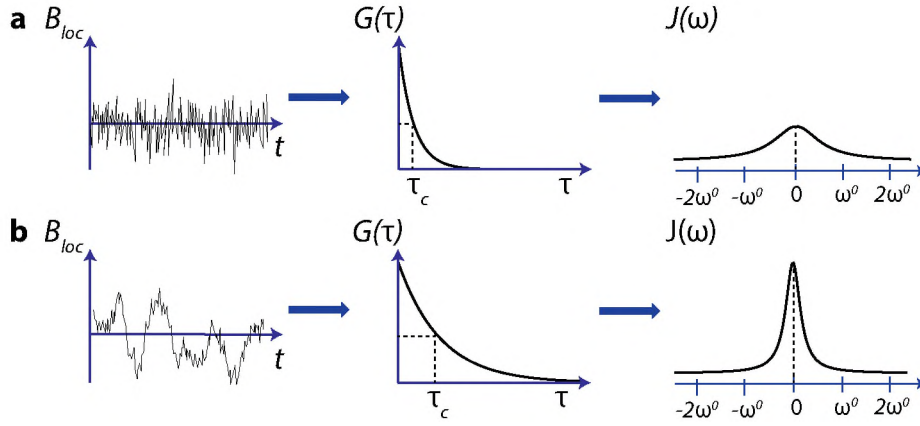


Figure 2.7: Autocorrelation  $G(\tau)$  and the spectral density  $J(\omega)$  functions of fast (a) and slowly (b) fluctuating local fields  $B_{loc}$ .

and fast fluctuations result in a quick decay of the autocorrelation function  $G(\tau)$ . This function is independent of time  $t$  and is equal to the magnitude of the fluctuating field at  $\tau = 0$ :

$$G(0) = \langle B_{loc}(t)^2 \rangle_t. \quad (2.21)$$

In general, the autocorrelation function is assumed as a simple exponential function [11]:

$$G(\tau) = \langle B_{loc}(t)^2 \rangle e^{-|\tau|/\tau_c}, \quad (2.22)$$

where  $\tau_c$  is a measure of the rate of fluctuations and is known as the correlation time. The correlation time is the average time that the molecule rotates by one radian. In general, the correlation time increases with molecular size and viscosity and decreases with temperature.

Since the autocorrelation function is defined in the time domain, the Fourier transform can be applied to obtain a measure of the amount of motion present at different frequencies. It is called a spectral-density function  $J(\omega)$ , which is given by:

$$J(\omega) = \mathcal{F}\{G(\tau)\} = 2 \int_0^\infty G(\tau) e^{-i\omega\tau} d\tau. \quad (2.23)$$

The real part of the spectral-density function is

$$\text{Re}\{J(\omega)\} = 2 \langle B_{loc}(t)^2 \rangle \frac{\tau_c}{1 + \omega^2 \tau_c^2} = G(0) \frac{2\tau_c}{1 + \omega^2 \tau_c^2}. \quad (2.24)$$

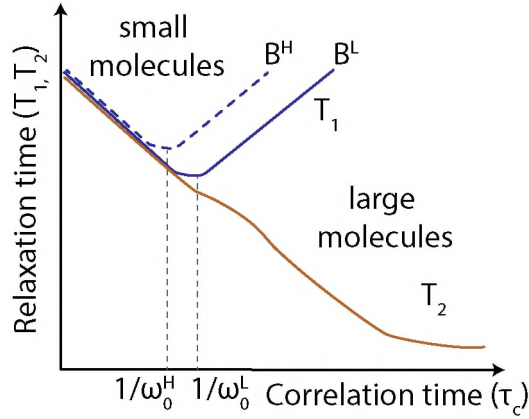


Figure 2.8: Dependence of the relaxation times  $T_1$  and  $T_2$  on the correlation time  $\tau_c$ .  $B^H$  and  $B^L$  indicate higher and lower magnetic fields applied during the spin evolution and  $\omega_0^H$  and  $\omega_0^L$  indicate the corresponding the Larmor frequencies. Minimum value of the relaxation time  $T_1$  is equal to the inverse of the frequency. The correlation time basically increases by a molecular size. Adapted from Refs. [12, 13].

As illustrated in Fig. 2.7, the fast fluctuation of the local field results in short  $\tau_c$  and broad spectral-density function (the case of small molecules in non-viscous solutions), while the consequence of slow fluctuations is long  $\tau_c$  and narrow spectral-density function (the case of large molecules).

Based on the assumption introduced in Ref. [12], relaxation originates from only the intramolecular dipole-dipole interaction, which is the main source of polarization relaxation for spin-1/2 nuclei. The simplest model for the dependence of relaxation times  $T_1$  and  $T_2$  on the correlation time  $\tau_c$  is given by [8, 12]:

$$\begin{aligned} \frac{1}{T_1} &= \frac{3b}{10}[J(\omega_0) + 4J(2\omega_0)], \\ \frac{1}{T_2} &= \frac{3b}{20}[3J(\omega_0) + 5J(\omega_0) + 2J(2\omega_0)], \end{aligned} \quad (2.25)$$

where  $b$  is the dipole–dipole coupling constant. When the correlation time is very short, the  $T_1$  and  $T_2$  relaxation times are equal. This condition is known as the extreme narrowing limit ( $\omega_0\tau_c \ll 1$ ). If the correlation time increases,  $T_2$  continuously decreases, while the value of  $T_1$  first decreases, until  $T_1$  reaches its minimum [12], and then it starts to increase again. The minimum value of the relaxation time  $T_1$  depends on the magnetic field applied during relaxation, since it is equal to the inverse of the Larmor frequency ( $1/\omega_0$ ) in that field (Fig. 2.8).

There are several sources of relaxation in liquids. As mentioned above, for relaxation of spin-1/2 nuclei, the most significant interaction is the direct dipole-dipole coupling. This is because of an orientation dependence of the interaction. Another considerable source is the chemical-shift anisotropy related to the variation of magnetic shielding with regards of the orientation of molecules relative to the external field. In the case of spin larger than 1/2, relaxation is dominated by the quadrupolar interaction. Also, a large magnetic moment, arising from unpaired electrons in paramagnetic species (e.g., oxygen or some transition-metal compounds), affects the local field of a nuclear spin, this process also introduces relaxation.

### 2.1.8 Product Operator Formalism

To explain basic NMR experiments, the semi-classical vector model was used in the previous section. However, this approach is not universal and cannot be applied to modern multiple-pulse experiments. To predict the outcome of such experiments, the product-operator formalism is often used [14]. Specifically, this approach enables the analysis of temporal evolution of magnetization under the action of pulses oriented in different directions.

The product-operator formalism uses two important quantum-mechanical concepts - wave functions and operators. A wave function describes a quantum state of a system (e.g., a nuclear spin) and operators represent such “observable quantities” of the system as position, momentum, and energy. Combination of these concepts enables the evaluation of physical quantities describing the system.

In the context of NMR, nuclear spins are represented by the Cartesian-base operators ( $\hat{I}_x$ ,  $\hat{I}_y$ ,  $\hat{I}_z$ ). The eigenstates of the spin operators are indicated by two quantum numbers,  $I$  and  $m_I$ , which are described in the previous section. For instance, the eigenequation of  $\hat{I}_z$  is:

$$\hat{I}_z|I, m_I\rangle = m_I|I, m_I\rangle, \quad (2.26)$$

where  $|I, m_I\rangle$  is the eigenstate of the system and  $m_I$  is the eigenvalue of the  $\hat{I}_z$  operator (the Zeeman eigenstate).

For a nuclear spin-1/2 ( $I = 1/2$ ) there are two Zeeman eigenstates of the  $\hat{I}_z$  operator, denoted by  $|\frac{1}{2}, m_I\rangle$ , with two eigenvalues  $-\frac{1}{2}$  or  $\frac{1}{2}$ . For these Zeeman eigenstates, the following notation is often used below:

$$\begin{aligned} |\alpha\rangle &= |\frac{1}{2}, +\frac{1}{2}\rangle, \\ |\beta\rangle &= |\frac{1}{2}, -\frac{1}{2}\rangle. \end{aligned} \quad (2.27)$$

The superposition state  $|\psi\rangle$  of the Zeeman eigenstates can be written as:

$$|\psi\rangle = c_\alpha |\alpha\rangle + c_\beta |\beta\rangle = \begin{pmatrix} c_\alpha \\ c_\beta \end{pmatrix}, \quad (2.28)$$

where  $c_\alpha$  and  $c_\beta$  are the superposition coefficients. These coefficients are normalized as:

$$|c_\alpha|^2 + |c_\beta|^2 = 1. \quad (2.29)$$

The spin density operator  $\hat{\rho}$  that describes the state of the entire spin ensemble can be defined as:

$$\hat{\rho} = |\psi\rangle\langle\psi| = \begin{pmatrix} |\alpha\rangle & |\beta\rangle \\ \langle\alpha| & \begin{pmatrix} p_{\alpha\alpha} & p_{\beta\alpha} \end{pmatrix} \\ \langle\beta| & \begin{pmatrix} p_{\alpha\beta} & p_{\beta\beta} \end{pmatrix} \end{pmatrix}, \quad (2.30)$$

where  $p_{\alpha\alpha}$  and  $p_{\beta\beta}$  are the diagonal elements of the matrix called populations, which are the probabilities of occupation of the  $|\alpha\rangle$  and  $|\beta\rangle$  level, respectively. The off-diagonal elements,  $p_{\alpha\beta}$  and  $p_{\beta\alpha}$ , refer to the coherences between the  $|\alpha\rangle$  and  $|\beta\rangle$  states. The coherence order between two states in a magnetic field is defined as the difference in their eigenvalues  $m_I$ . Specifically, a single-quantum coherence is the coherence between the sublevels of  $\Delta m_I = \pm 1$ . It should be noted that these coherences give rise to a signal in conventional NMR.

The energy of a system is given by the Hamiltonian operator  $\hat{\mathcal{H}}$ . The interaction of a

spin-1/2 with an external magnetic field along the  $z$ -axis is given by:

$$\hat{\mathcal{H}}_Z = \omega_I \hat{I}_z, \quad (2.31)$$

where  $\omega_I$  is the Larmor frequency of the spin and  $\hat{I}_z$  is the spin operator defined in Eq. (2.26). A defined density operator  $\hat{\rho}(0)$ , corresponding to the initial state of the system, evolves under the Hamiltonian  $\hat{\mathcal{H}}_Z$  according to:

$$\hat{\rho}(t) = e^{-i\hat{\mathcal{H}}_Z t} \hat{\rho}(0) e^{i\hat{\mathcal{H}}_Z t}. \quad (2.32)$$

For instance, if  $\hat{\rho}(0) = \hat{I}_z$ , which is the case of thermal equilibrium in the  $z$ -oriented field, there is no evolution of the state, as  $[\hat{\mathcal{H}}_Z, \hat{\rho}(0)] = 0$ . However, if  $\hat{\rho}(0)$  is equal to  $\hat{I}_x$ , it does not commute with the Hamiltonian, hence, the system evolves as follows:

$$\hat{\rho}(t) = e^{-i\omega_I \hat{I}_z t} \hat{I}_x e^{i\omega_I \hat{I}_z t} = \cos(\omega_I t) \hat{I}_x + \sin(\omega_I t) \hat{I}_y, \quad (2.33)$$

which is the case occurs in basic NMR experiments mentioned above.

For a system with two coupled spins-1/2, given by operators  $\hat{I}$  and  $\hat{S}$ , under the action of the external field along the  $z$ -axis, the Hamiltonian can be given as a sum of the Zeeman Hamiltonian  $\hat{\mathcal{H}}_Z$  and the  $J$ -coupling Hamiltonian  $\hat{\mathcal{H}}_J$ :

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_Z + \hat{\mathcal{H}}_J, \quad (2.34)$$

where the terms are given by:

$$\begin{aligned} \hat{\mathcal{H}}_Z &= \omega_I \hat{I}_z + \omega_S \hat{S}_z, \\ \hat{\mathcal{H}}_J &= J_{IS} \hat{\mathbf{I}} \cdot \hat{\mathbf{S}}, \end{aligned} \quad (2.35)$$

where  $J_{IS}$  is the  $J$ -coupling constant between the  $I$  and  $S$  spins of the  $\omega_I$  and  $\omega_S$  are the corresponding Larmor frequencies of the spins. The Hamiltonian is more complicated for coupled multi-spin systems, which are the main interest in this dissertation. However, the absence of the Zeeman interaction in zero field enables us to observe the terms solely

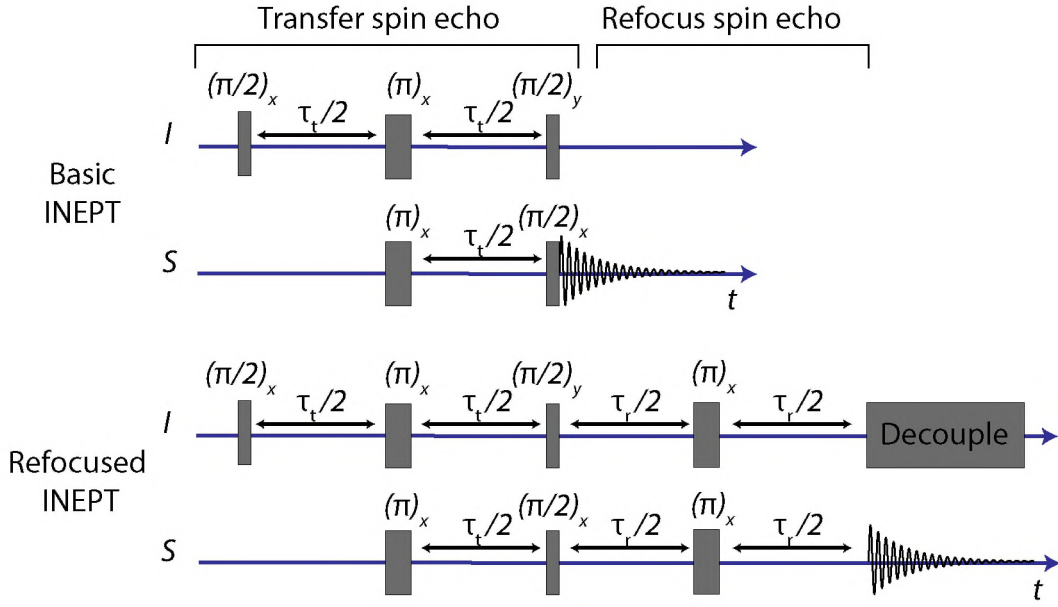


Figure 2.9: Basic and refocused INEPT pulse sequences. The transfer and refocus spin-echo time intervals are denoted as  $\tau_t/2$  and  $\tau_r/2$  respectively. In the case of the coupled two-spin system, the spin-echo time intervals are equal to  $1/(4|J_{IS}|)$ , where  $J_{IS}$  is the  $J$ -coupling constant between the  $I$  and  $S$  spins.

originating from the  $J$ -coupling interactions. This specific case is discussed in further sections using the expressions introduced briefly here.

### 2.1.9 INEPT Pulse Sequence

Many NMR-active ( $I > 0$ ) nuclei present in biologically important molecules suffer from poor inherent sensitivity in the NMR spectroscopy. This originates from a low natural isotopic abundance of the nuclei and low gyromagnetic ratio, which corresponds to low thermal polarization and hence low magnetization, but also low Larmor frequency. In turn, this significantly reduces the signal-to-noise ratio of NMR signals of the nuclei. The prime examples of such nuclei are  $^{13}\text{C}$  and  $^{15}\text{N}$ . In order to enhance the signal of these nuclei, insensitive nuclei enhanced by polarization transfer (INEPT) pulse sequence is often used [15]. With this pulse sequence, the polarization from the nuclei with high  $\gamma$  is transferred to the nuclei with low  $\gamma$  through the  $J$ -couplings between the spins.

In Fig. 2.9, the basic INEPT pulse sequence is demonstrated for a two-spin system, where the nucleus  $I$  has a higher gyromagnetic ratio than the nucleus  $S$  and they are coupled with the coupling constant  $J_{IS}$ . In the method, the polarization transfer from

the spin  $I$  to the spin  $S$  occurs as follows: Initially, a transverse polarization of the  $I$  spin  $I_y$  is created on the spin  $I$  by the application of the  $\pi/2$ -pulse applied along the  $x$ -axis. To facilitate the understanding of spin-echo period, one might consider first the application of  $\pi$  pulse along  $x$ -axis which generates  $I_yS_z$ , and then  $t = \tau_t = 1/(2|J_{IS}|)$  delay (combination of two  $\tau_t/2$  periods). During  $\tau_t$  delay,  $I_yS_z$  term evolves into an anti-phase magnetization  $I_xS_z$  due to the  $J$ -coupling. Finally, the  $\pi/2$ -pulse is applied along the  $y$ -axis for the  $I$  spin and simultaneously the  $\pi/2$  around the  $x$  axis is applied for the spin  $S$ . As a result of these pulses, the anti-phase polarization  $I_zS_y$  is generated resulting in anti-phase peaks in the spectrum with  $J$ -splitting. In the case of application of an additional decoupling field,  $J$ -splitting disappears, hence anti-phase peaks merge and cancel out each other. In order to yield single peaks in the spectrum using a decoupling field, the enhanced anti-phase terms can be refocused resulting in an in-phase term ( $S_y$ ) which corresponds to in-phase peaks. This pulse sequence consisting of transfer and refocus spin-echo is known as refocused INEPT (Fig. 2.9). Further discussion regarding polarization transfer can be found in Ref. [8].

## 2.2 Exotic NMR: Zero- and Ultralow-Field Spectroscopy

In contrast to conventional NMR, ZULF NMR is performed in the absence of a magnetic field. In such a regime, the Zeeman interaction vanishes, and spin-spin interactions that exist between the nuclei in a molecule become dominant (assuming non-existence of quadrupolar and dipole-dipole couplings). The spin-spin interactions are mediated by the electrons of the molecules over the distances of several chemical bonds, providing information about molecular structure and the strength and orientation of chemical bonds [1]. This opens means for identification, characterization, and quantification of molecular structures by high-resolution measurements provided by remarkably narrow ZULF NMR resonance linewidths. The linewidths result from high absolute magnetic-field homogeneity and long spin-coherence times [2, 3]. Furthermore, a greater skin depth at low frequencies enables measurements of samples and monitoring chemical and bio-

chemical reactions inside metal containers [4]. In order to detect ZULF NMR signals, atomic magnetometers are often used. Their main advantages are low price, small size, and non-cryogenic operation, leading to technical simplicity and near-zero maintenance [5]. This is further reinforced by the ever-growing commercial availability of such sensors.

In the following section, the basic principles of ZULF NMR spectroscopy are discussed. The subject is covered in the context of the dissertation. Further discussion of ZULF NMR can be found in Refs. [1, 5, 16].

### 2.2.1 Introduction of Zero-Field Nuclear Magnetic Resonance

Operation at ZULF requires attenuation of external magnetic fields (e.g., the Earth's magnetic field). Specifically, zero-field conditions are achieved when the Larmor precession of a nuclear spin  $\mathbf{I}$ , in the residual field of the magnitude  $B_0$ , is smaller than the spin relaxation rate  $1/T_2$ :

$$|\gamma_I B_0| < \frac{1}{T_2}. \quad (2.36)$$

As a result, contributions of the external-field-dependent spin interactions, such as the Zeeman effect or chemical shift, are negligible. Moreover, in isotropic liquids consisting of spin-1/2 at zero field, all spin-dependent interactions are averaged out to zero except for the  $J$ -coupling.

Let us now consider a coupled spin system with two spin-1/2 nuclei  $I$  and  $S$  at zero field. The total nuclear spin Hamiltonian  $\hat{\mathcal{H}}$  is equal to the  $J$ -coupling Hamiltonian  $\hat{\mathcal{H}}_J$ :

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_J = J_{IS} \hat{\mathbf{I}} \cdot \hat{\mathbf{S}}, \quad (2.37)$$

where  $\hat{\mathbf{S}}$  and  $\hat{\mathbf{I}}$  are the corresponding spin operators and  $J_{IS}$  is the  $J$ -coupling constant between the nuclei.

In order to generate an observable zero-field signal, the nuclear spins need to be polarized. At zero field, sufficient polarization can be achieved conventionally with thermal prepolarization (prior to the measurements), using a permanent magnet that is placed outside of the detection region. Alternatively, instead of thermal prepolarization, hyper-

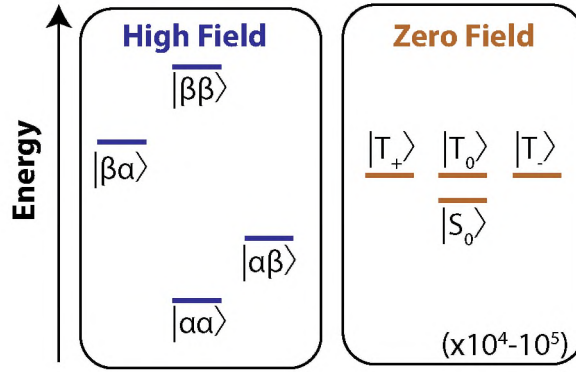


Figure 2.10: Energy levels of the eigenstates for a coupled spin-1/2 pair under the- high and zero-field conditions. Note that the two diagrams are not drawn to scale.

polarization techniques can be used. Both techniques are discussed below.

### 2.2.2 Generating Zero-Field NMR Signal by Thermal Prepolarization

As illustrated in Fig. 2.10, the eigenstates of the molecule, consisting of two coupled spin-1/2 at high and zero fields, are different. When the Zeeman interaction is dominant (high field), the system is described by the projection of the individual spins onto the quantization axis (often coinciding with the static magnetic field), forming the  $|\alpha\alpha\rangle$ ,  $|\alpha\beta\rangle$ ,  $|\beta\alpha\rangle$ ,  $|\beta\beta\rangle$  eigenstates. When the  $J$ -coupling dominates (zero field), the eigenstates of the system are those of the singlet (total spin zero) and triplet (total spin one) states,  $|S_0\rangle$ ,  $|T_0\rangle$ ,  $|T_+\rangle$ ,  $|T_-\rangle$ . The states correspond to the high-field states via:

$$\begin{aligned}
 |T_+\rangle &= |\alpha\alpha\rangle, \\
 |T_0\rangle &= \frac{1}{\sqrt{2}}(|\alpha\beta\rangle + |\beta\alpha\rangle), \\
 |T_-\rangle &= |\beta\beta\rangle, \\
 |S_0\rangle &= \frac{1}{\sqrt{2}}(|\alpha\beta\rangle - |\beta\alpha\rangle).
 \end{aligned}
 \tag{2.38}$$

### 2.2.2.1 Sudden Transfer to Zero Field

After prepolarization at high field that is applied along the quantization axis  $z$ , the initial density matrix  $\hat{\rho}_{\text{pol}}$  can be written as:

$$\hat{\rho}_{\text{pol}} \propto (\omega_I \hat{I}_z + \omega_S \hat{S}_z) = \frac{\omega_I + \omega_S}{2} (\hat{I}_z + \hat{S}_z) + \frac{\omega_I - \omega_S}{2} (\hat{I}_z - \hat{S}_z), \quad (2.39)$$

where  $\omega_I$  and  $\omega_S$  are the Larmor frequencies of the corresponding fields at the prepolarization field  $B_{\text{pol}}$ . If the guiding field is suddenly turned off, the high-field state is maintained during the transfer to zero field. As derived in Ref. [17], the high-field density matrix can be projected onto the zero-field basis, so that the prepolarization state [Eq. (2.39)] can be rewritten into the zero-field basis

$$\hat{\rho}_{\text{sudden}} \propto \frac{\omega_I + \omega_S}{2} (|T_+\rangle\langle T_+| - |T_-\rangle\langle T_-|) + \frac{\omega_I - \omega_S}{2} (|S_0\rangle\langle T_0| - |T_0\rangle\langle S_0|), \quad (2.40)$$

which contains coherences between the  $|S_0\rangle$  and  $|T_0\rangle$  states. Since this matrix does not commute with the  $J$ -coupling Hamiltonian (2.37), the state immediately starts to evolve, giving rise to an oscillating magnetic field. The field can be detected by a sensor with a sensitive axis along the  $z$ -direction (Fig. 2.11a). This time-dependent signal, related to the oscillating magnetization of the sample  $\langle M_z \rangle_{\text{sudden}}$ , can be derived using Eqs. (2.32) and (2.39):

$$\begin{aligned} \langle M_z \rangle_{\text{sudden}}(t) &\propto \text{Tr}\{(\gamma_I \hat{I}_z + \gamma_S \hat{S}_z) e^{-i\hat{\mathcal{H}}_J t} \hat{\rho}_{\text{sudden}} e^{i\hat{\mathcal{H}}_J t}\} \\ &\propto B_{\text{pol}}(\gamma_I - \gamma_S)^2 \cos(2\pi J_{IS} t), \end{aligned} \quad (2.41)$$

where in the second line we used Eq. (2.33).

As seen in Eq. (2.41), the net magnetization of the system oscillates along the  $z$ -axis (the direction of the initial polarization) at the coupling constant  $J_{IS}$  that is typically given in frequency units. The Fourier transform of this time-domain signal yields a  $J$ -spectrum with a single peak at the frequency  $J_{IS}$ . The amplitude of the oscillating magnetic field depends on the prepolarization field as well as the square of the gyromagnetic ratio difference of the coupled nuclei. Therefore, one of the main limitations of

zero-field NMR is that only heteronuclear systems are directly observable with the technique. Nonetheless, the homonuclear couplings can be observed through perturbation of the heteronuclear couplings.

It should be noted that the spin system also produces non-oscillating (static) magnetization along the  $z$ -axis (the zero-frequency peak). Since this peak is not used for chemical analysis, it is beyond the scope of this work and hence is not included in Eq. (2.41). Nonetheless, one can find discussion of the static magnetization in Ref. [18].

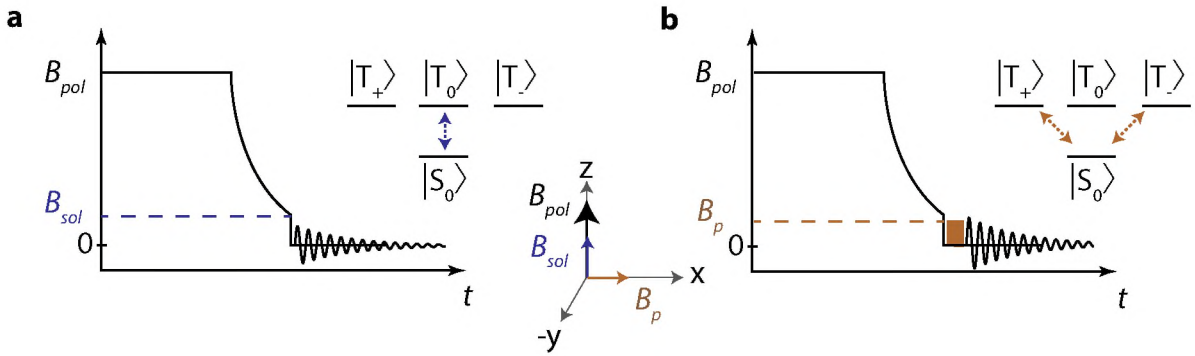


Figure 2.11: Time dependence of the static magnetic field during sudden transition experiments without (a) and with (b) an additional transverse DC magnetic pulse. The initial period of prepolarization in the strong field  $B_{pol}$  is followed by a non-adiabatic transport through the guiding field and eventually the sample reaches a magnetically-shielded region. The guiding field  $B_{sol}$  is then switched off suddenly and the signal is either observed immediately (a) or a sharp DC pulse is first applied in the  $x$ -direction, which allows to observed an enhanced signal (b). The energy levels and induced transitions are presented with the spin quantization axis aligned with  $B_{sol}$  (a) and  $B_p$  (b).

In an alternative approach, after switching off the guiding field, a sharp, transverse ( $x$ - or  $y$ -oriented) DC pulse, with duration  $\tau_p$  and amplitude  $B_p$ , is applied (Fig. 2.11b). Unlike conventional NMR, in this case, the DC magnetic field is preferred. This is so because the Larmor frequency is zero in the absence of an external magnetic field. For instance, in the coupled spin-1/2 spin system, the Hamiltonian for a DC  $x$ -pulse is given by:

$$\hat{\mathcal{H}}_p = \gamma_I B_p \hat{I}_x + \gamma_S B_p \hat{S}_x. \quad (2.42)$$

During the pulse,  $J$ -coupling evolution is neglected by assuming  $B_p \gamma_I, B_p \gamma_S \gg J_{IS}$ . In this case, the DC  $x$ -pulse rotates spin  $I$  and  $S$  by angles  $\theta_I = \gamma_I B_p \tau_p$  and  $\theta_S = \gamma_S B_p \tau_p$

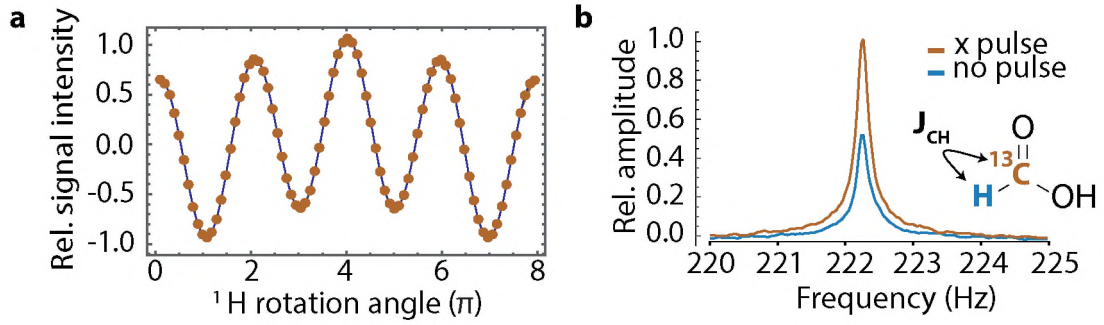


Figure 2.12: (a) Amplitude of the zero-field NMR signal of the  $^1\text{H}$ - $^{13}\text{C}$  nuclear system versus the  $^1\text{H}$  rotation angle  $\theta_{\text{H}}$  induced by the field applied in  $x$ . The dots correspond to numerical simulations (SpinDynamica), while the solid line is determined based on Eq. (2.44). The signal is normalized to the maximum amplitude. (b) Experimental  $J$ -spectra of  $^{13}\text{C}$ -labelled formic acid after the sudden transition to the zero field with/without the optimum  $x$ -pulse. The spectra are the result of four averages.

around the  $x$ -axis. Thus, after the pulse, the density matrix  $\hat{\rho}_{\text{pulse}}$  is:

$$\hat{\rho}_{\text{pulse}} \propto \frac{\omega_I + \omega_S}{2} \left( \hat{I}_z \cos \theta_I + \hat{S}_z \cos \theta_S \right) + \frac{\omega_I - \omega_S}{2} \left( \hat{I}_z \cos \theta_I - \hat{S}_z \cos \theta_S \right). \quad (2.43)$$

Through the analysis of Eqs. (2.39) and (2.40), it is clear that the term  $\hat{I}_z + \hat{S}_z$  is diagonal in the zero-field basis and hence it does not evolve over time. As this term remains stationary under  $\hat{\mathcal{H}}_J$ , it corresponds to decaying static magnetization as mentioned above for the previous case. However, the term  $\hat{I}_z - \hat{S}_z$  evolves due to the Hamiltonian, giving rise to a signal oscillating at  $J_{IS}$ .

It is noteworthy that, with optimum pulsing, the factor next to  $\hat{I}_z - \hat{S}_z$  can be maximized. This is achieved when two prefactors in terms in Eq. (2.43) are swapped. The dependence of coherence (off-diagonal terms which evolves) amplitude on the rotational angle of the transverse pulse can be derived as:

$$\hat{\rho}_{\text{coherence}} \propto \frac{\omega_I + \omega_S}{2} (\cos \theta_I - \cos \theta_S) + \frac{\omega_I - \omega_S}{2} (\cos \theta_I + \cos \theta_S). \quad (2.44)$$

If the gyromagnetic ratios of both spins have the same sign, an angle of the pulse, maximizing the coherence amplitude, satisfies one of the following conditions:  $\cos \theta_I = 1$  and  $\cos \theta_S = -1$  or  $\cos \theta_I = -1$  and  $\cos \theta_S = 1$ . For example, for a  $^1\text{H}$ - $^{13}\text{C}$  system, in which  $\gamma_{\text{C}}/\gamma_{\text{H}} \approx 1/4$ , these conditions are achieved when  $\theta_{\text{H}} \approx 4\pi$  and  $\theta_{\text{C}} \approx \pi$ .

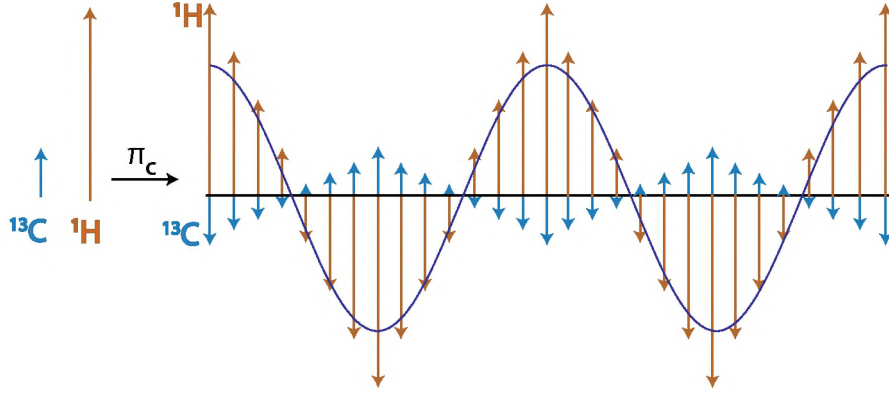


Figure 2.13: Behavior of the  $^1\text{H}$ - $^{13}\text{C}$  system after applying a transverse DC  $\pi$  pulse for  $^{13}\text{C}$  following thermal prepolarization and sudden field switch (see main text for experimental detail). The blue and orange vectors represent magnetization of  $^{13}\text{C}$  and  $^1\text{H}$  nuclei, respectively. The navy blue line shows the total magnetization over time which is an oscillation evolved from quantum beating under  $J$ -coupling in this situation.

To support the discussion, the zero-field NMR spectra of the  $^1\text{H}$ - $^{13}\text{C}$  system are simulated using the SpinDynamica package for Mathematica [19]. In Fig. 2.12(a), amplitudes of the signals simulated for different pulse lengths are shown as orange points. Simulations are performed using a finite-length pulse with an amplitude of  $50\mu\text{T}$ , which is realistic for an experimental implementation. The relationship between signal intensity and pulse angle is then calculated using Eq. (2.44) and shown in Fig. 2.12(a) as a blue solid line. As shown, both approaches give consistent results.

For educational purposes, the evaluation of the  $\hat{I}_z - \hat{S}_z$  term in the  $^1\text{H}$ - $^{13}\text{C}$  system, after application of the optimal pulse ( $4\pi$  rotation for  $^1\text{H}$  and  $\pi$  rotation for  $^{13}\text{C}$ ), is shown in Fig. 2.13 using the semi-classical vector model. In this particular case, after pulse rotation, the  $\hat{I}_z - \hat{S}_z$  term arises from the first term in the initial density matrix (2.40). Therefore, the amplitude of the corresponding coherence depends on the population difference between the  $|T_+\rangle$ ,  $|T_-\rangle$  states, because the transitions occur between these states and  $|S_0\rangle$  states [Fig. 2.11(b)].

After applying the optimal pulse, the detectable component of the magnetization  $M_z$  is proportional to [1, 20]:

$$\begin{aligned} \langle M_z \rangle_{\text{pulse}}(t) &\propto \text{Tr}\{(\gamma_I \hat{I}_z + \gamma_S \hat{S}_z) e^{-i\hat{\mathcal{H}}_J t} \hat{\rho}_{\text{pulse}} e^{i\hat{\mathcal{H}}_J t}\} \\ &\propto B_{\text{pol}}(\gamma_I + \gamma_S)(\gamma_I - \gamma_S) \cos(2\pi J_I s t). \end{aligned} \quad (2.45)$$

By comparing the  $z$ -magnetization amplitudes for sudden transfer without [Eq. (2.41)] and with [Eq. (2.45)] the transverse pulse, the signal enhancement due to the optimized DC transverse pulse can be calculated. For the  $^1\text{H}$ - $^{13}\text{C}$  system, the signal enhancement is:

$$\frac{(\gamma_{\text{H}} + \gamma_{\text{C}})}{(\gamma_{\text{H}} - \gamma_{\text{C}})} \approx 1.67. \quad (2.46)$$

As a supporting result, the experimental spectra of  $^{13}\text{C}$ -label formic acid are shown in Fig. 2.12(b). The spectra present two discussed cases measured with and without the transverse pulse applied after the sudden transition to the zero field.

It is worth mentioning that the application of the  $z$ -pulse after sudden transfer does not contribute to the signal, as the initial density matrix  $\hat{\rho}_{\text{pol}}$  commutes with the  $z$ -field Hamiltonian.

### 2.2.2.2 Adiabatic Transfer to Zero Field

If the guiding field is turned off slowly, population of the high-field eigenstates converts to the population of the zero-field eigenstates, and the density matrix remains diagonal in the eigenbasis of the Hamiltonian at each stage of the transfer. Thus, there are no coherences after this adiabatic transfer (as  $\hat{\rho}_{\text{pol}}$  commutes with  $\hat{\mathcal{H}}_J$ ). To generate the coherence, a DC pulse should be applied either along the transverse ( $x$ - or  $y$ -) direction or longitudinal ( $z$ -)axis.

Similarly to the sudden transfer, after application of the transverse pulse, the first term of the initial density matrix  $\hat{\rho}_{\text{pol}}$  evolves according to:

$$\hat{\rho}_{\text{transverse}} \propto \frac{\omega_I + \omega_S}{2} (\cos \theta_I - \cos \theta_S), \quad (2.47)$$

where the amplitude of the corresponding coherence depends on the population difference between the  $|T_+\rangle$  and  $|T_-\rangle$  states, the same as in the sudden-transfer case. On the other hand, the  $z$ -pulse causes the transition between  $|S_0\rangle$  and  $|T_0\rangle$  due to the second term of

the initial density matrix  $\hat{\rho}_{\text{pol}}$ , which is derived in Ref. [17] as:

$$\hat{\rho}_{\text{longitudinal}} \propto \frac{\omega_I - \omega_S}{2} (\sin \theta_I - \sin \theta_S). \quad (2.48)$$

It can be easily calculated from Eqs. (2.47) and (2.48) that the optimum angle to maximize the coherence amplitude is the  $\pi$  pulse for  $^{13}\text{C}$  ( $4\pi$  for  $^1\text{H}$ ) in transverse direction, and the  $\pi/6$  pulse for  $^{13}\text{C}$  ( $2\pi/3$  for  $^1\text{H}$ ) in the longitudinal direction. Furthermore, for a two-spin system, the ratio of amplitudes of signals following transverse and vertical pulses are given by

$$r_{tl} = \frac{\gamma_I + \gamma_S}{\gamma_I - \gamma_S}. \quad (2.49)$$

### 2.2.3 Interpretation of Zero Field $J$ -Spectroscopy

Several studies introduced methods for the interpretation of zero-field NMR spectra [3, 21, 22]. Here, these interpretation methods are discussed using representative zero-field NMR spectra.

**In a simple  $\text{XA}_n$  nuclear spin system**, composed of a heteronucleus X and a set of  $n$  magnetically-equivalent nuclei A, in absence of other interactions, the total nuclear spin Hamiltonian  $\hat{\mathcal{H}}$  is the heteronuclear  $J$ -coupling Hamiltonian:

$$\hat{\mathcal{H}} = \sum_i^n J_{\text{XA}} \hat{\mathbf{S}} \cdot \hat{\mathbf{I}}_{\text{A},i} = J_{\text{XA}} \hat{\mathbf{S}} \cdot \sum_i^n \hat{\mathbf{I}}_{\text{A},i} = J_{\text{XA}} \hat{\mathbf{S}} \cdot \hat{\mathbf{I}}_{\text{A}}, \quad (2.50)$$

where  $J_{\text{XA}}$  is the coupling constant and  $\hat{\mathbf{S}}$  and  $\hat{\mathbf{I}}_{\text{A},i}$  are the spin vector operators, corresponding to the spin X and the individual spins A, while  $\hat{\mathbf{I}}_{\text{A}}$  denotes the total spin of all A nuclei. Due to the dominant scalar interaction, the spins  $\mathbf{I}_{\text{A}}$  and  $\mathbf{S}$  couple into the total spin  $\mathbf{F}$ ,  $\mathbf{F} = \mathbf{I}_{\text{A}} + \mathbf{S}$ . The energy levels, mainly defined by three quantum numbers  $S$ ,  $I_{\text{A}}$ , and  $F$ , allow one to calculate the eigenenergies of the levels:

$$E^0 = \frac{J_{\text{XA}}}{2} [F(F+1) - I_{\text{A}}(I_{\text{A}}+1) - S(S+1)], \quad (2.51)$$

where  $F$  ranges from  $|I_{\text{A}} - S|$  to  $I_{\text{A}} + S$  by unity. Additionally, the magnetic quantum

number  $m_F$  can be defined for energy sublevels, which takes one of the  $2F + 1$  integer values between  $-F$  and  $F$  separated by a unity,  $m_F = -F, -F + 1, \dots, F$ . For the  $\text{XA}_n$  spin system, the observable transitions between the eigenstates of the energies given by Eq. (2.51) can be found by employing the selection rules [23]:

$$\begin{aligned}\Delta I_A &= 0, \\ \Delta F &= 0, \pm 1.\end{aligned}\tag{2.52}$$

It should be also mentioned that an additional selection rule for the quantum number  $m_F$  emerges. The rule depends on the quantization axis, which is chosen to explain energy levels and observable transitions (Fig. 2.11). If the pulse is applied along the  $z$ -axis, the selection rule is  $\Delta m_F = 0$ . On the other hand, when the pulse is applied along one of the transverse axes, the selection rule is  $\Delta m_F = \pm 1$  [22].

Using the selection rules and the energies of the levels introduced in Eq. (2.51), one can show that for a system of spin-1/2 nuclei ( $S = I_{A,i} = 1/2$ ) and an even number of A nuclei, the ZULF NMR spectrum consists of  $(n + 1)/2$  lines at frequencies from  $J_{\text{XA}}$  to  $(n + 1)J_{\text{XA}}/2$  with the  $J_{\text{XA}}$  intervals. Analogously, if  $n$  is even, the spectrum consists of  $n/2$  lines at frequency from  $J_{\text{XA}}/2$  to  $(n + 1)J_{\text{XA}}/2$  with the  $J_{\text{XA}}$  intervals [21].

To demonstrate the ability to chemically fingerprint using zero-field NMR spectroscopy, a mixture of  $^{13}\text{C}$ -methanol and 2- $^{13}\text{C}$ -acetonitrile, i.e., two compounds of the same  $\text{XA}_3$  spin topology, was first investigated. As explained above, the zero-field NMR spectrum of the  $\text{XA}_3$  spin system consists of two peaks at the frequencies  $J_{\text{XA}}$  and  $2J_{\text{XA}}$ , corresponding to the transitions between the  $F = 0$  and  $F = 1$  states and the  $F = 1$  and  $F = 2$  states [Fig. 2.14(a)]. In Fig. 2.14(b), the  $^{13}\text{C}$ -methanol spectrum, consisting of two peaks at  $J_{\text{CH}} \approx 141 \text{ Hz}$  and  $2J_{\text{CH}} \approx 282 \text{ Hz}$ , and 2- $^{13}\text{C}$ -acetonitrile spectrum, consisting of two peaks at  $J_{\text{CH}} \approx 136 \text{ Hz}$  and  $2J_{\text{CH}} \approx 272 \text{ Hz}$ , are shown. In the mixture, all four distinguishable zero-field peaks are observed in the spectrum [Fig. 2.14(b)]. It is worth noting that zero-field NMR spectrum is solely determined by the  $J$ -coupling interactions. Although in high-field NMR, these interactions are also present, they are observed indirectly as a splitting of the main peaks, arising from magnetic precession of spins (e.g.,  $^1\text{H}$

or  $^{13}\text{C}$  peaks).

The measurements shown in Fig. 2.14 were obtained using the thermal-prepolarization approach and the following sudden transition to the zero field. The details of the experimental method and data analysis are described in Chapter 3, where the subject is discussed in the context of organophosphorus compounds that are larger spin systems.

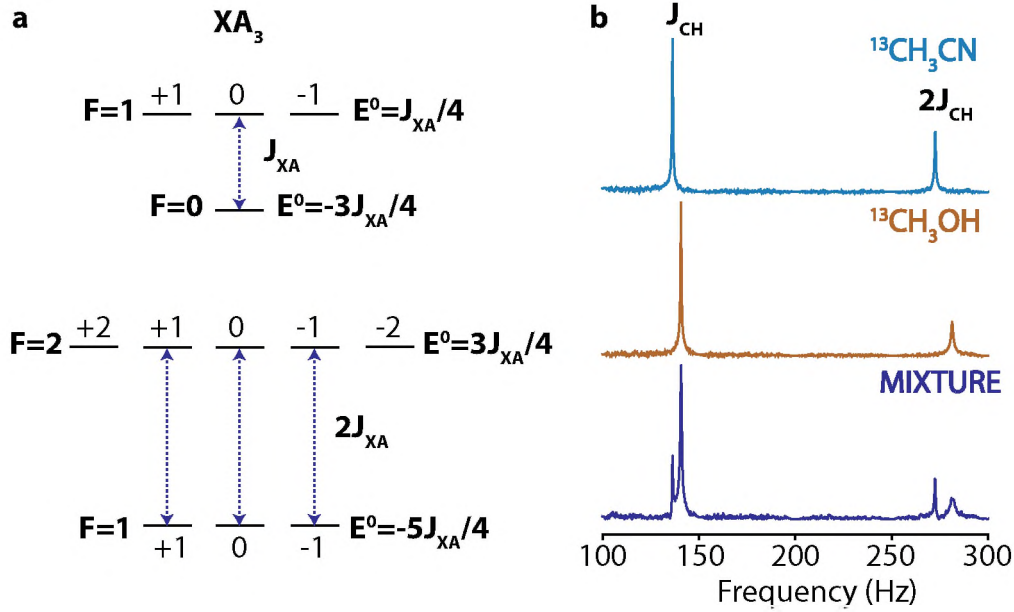


Figure 2.14: (a) Energy levels and observable  $\Delta m_F = 0$  transitions for  $\text{XA}_3$  spin system in zero-field NMR (see main text for details). The manifolds are grouped by the quantum number  $I_A = 1/2$  (top) and  $I_A = 3/2$  (bottom). Each state is labelled by its characteristic quantum numbers ( $F$  and  $m_F$ ). (b) Zero-field spectra of neat  $^{13}\text{C}$ -methanol (16 transients), neat 2- $^{13}\text{C}$ -acetonitrile (16 transients) and the mixture of these compounds (64 transients). The noise peaks at 50 Hz and its harmonics, stemming from a power line, are filtered out.

**More complex spin systems**,  $(\text{XA}_n)\text{B}_m$ , contain a heteronucleus X and two sets of homonuclei ( $n$  nuclei A and  $m$  nuclei B) with a dominant  $J_{\text{XA}}$ -coupling and weaker  $J_{\text{XB}}$ - and  $J_{\text{AB}}$ -couplings. The  $J$ -spectra of such spin systems can be explained using perturbation theory. The gist of this approach is that the weaker couplings perturb the system determined by the stronger coupling, which results in shifting or splitting of NMR lines observed in the spectrum [21]. To examine the  $(\text{XA}_n)\text{B}_m$  spin systems in more detail, we define additional spin operators. They are the total spin operator of all B nuclei  $\hat{\mathbf{I}}_B = \sum_i^n \hat{\mathbf{I}}_{B,i}$  and the total spin operator of the system  $\hat{\mathbf{F}}_T = \hat{\mathbf{I}}_B + \hat{\mathbf{F}}$ , with  $F_T$  being a total-spin quantum number ranging between  $|I_B - F|$  and  $I_B + F$  by a unity.

As shown in Refs. [21–23], using the operators and the corresponding quantum numbers, the energy shift  $E^1$  from an unperturbed energy of the level, calculated in the first-order perturbation theory, is:

$$E^1 = \frac{J_{\text{XB}}^1 + J_{\text{AB}}^1}{2} [F_{\text{T}}(F_{\text{T}} + 1) - F(F + 1) - I_{\text{B}}(I_{\text{B}} + 1)], \quad (2.53)$$

where  $J_{\text{XB}}^1$  and  $J_{\text{AB}}^1$  represent the scaled  $J$ -coupling constants that can be written as:

$$\begin{aligned} J_{\text{XB}}^1 &= \frac{J_{\text{XB}}}{2} \left[ 1 + \frac{S(S + 1) - I_{\text{A}}(I_{\text{A}} + 1)}{F(F + 1)} \right], \\ J_{\text{AB}}^1 &= \frac{J_{\text{AB}}}{2} \left[ 1 + \frac{I_{\text{A}}(I_{\text{A}} + 1) - S(S + 1)}{F(F + 1)} \right]. \end{aligned} \quad (2.54)$$

To find observable transitions between shifted energy levels, we employ the following selection rules [21]:

$$\begin{aligned} \Delta I_{\text{A}} &= \Delta I_{\text{B}} = 0, \\ \Delta F_{\text{T}} &= 0, \pm 1, \end{aligned} \quad (2.55)$$

which allows us to approximately determine the position of the split lines. To predict the frequency of the lines more precisely, higher-order terms must be included. This is especially needed in cases where closely spaced spectral lines correspond to transitions that are degenerate to the first order. Spectral lines with a distance of up to 0.1 Hz, degenerate within the first-order perturbation theory, can be distinguished by including second-order corrections or by exact numerical diagonalization [22]. However, even the first-order calculations are an approximate solution, they can provide an intuitive, qualitative interpretation of zero-field spectra.

As an example of the more complex system, the spectrum of [2- $^{13}\text{C}$ ]-L-alanine, being the  $\text{XAB}_3$  spin system, is shown in Fig. 2.15. [2- $^{13}\text{C}$ ]-L-alanine is composed of one  $^{13}\text{C}$  spin and a single  $^1\text{H}$  spin with one-bond coupling  $J_{\text{CH}}^1 = 146.1$  Hz and three equivalent  $^1\text{H}$  spins with two-bond couplings  $J_{\text{CH}}^2 = -4.5$  Hz. The homonuclear coupling constant between the inequivalent  $^1\text{H}$  spins  $J_{\text{HH}}^3$  is 7 Hz [24, 25]. The homo- and heteronuclear couplings

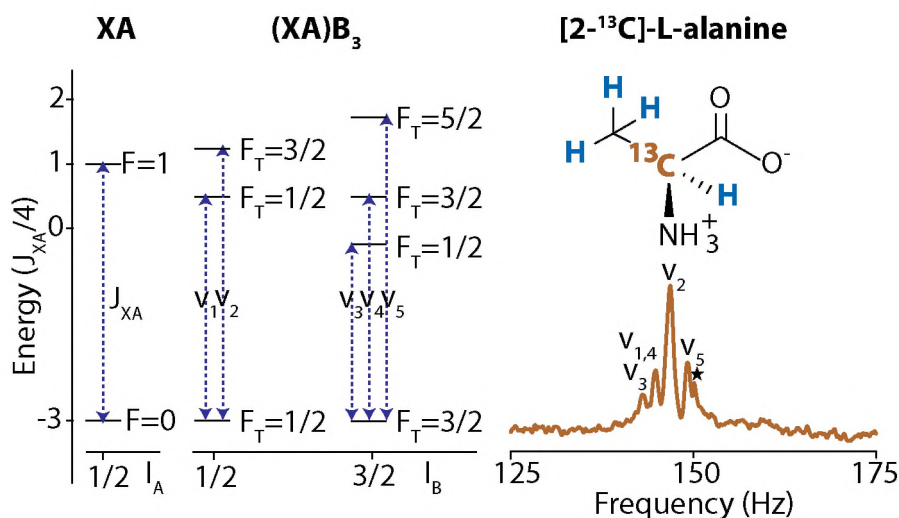


Figure 2.15: Energy-level diagram of the XA and (XA) $B_3$  spin systems (magnetic-sublevel structure is not shown) along with the ZULF NMR observable transitions indicated by dashed arrows (left). Experimental zero-field spectrum of [2- $^{13}\text{C}$ ]-L-alanine with an inset showing the molecular structure of the compound (right). The high-frequency transitions are denoted by  $v_{1-5}$ . The manifolds are grouped by the quantum numbers  $I_A$  and  $I_B$  and each manifold is labelled by its quantum number  $F$  or  $F_T$ . The peak observed in the spectrum at 150 Hz and marked by a black star is a third harmonic of 50 Hz power-line noise. The details of the experimental method and data analysis are described in Chapter 3.

of the groups with exchangeable protons of the  $-\text{OH}$  and  $-\text{NH}$  groups are averaged out under the rapid chemical exchange. This topic is discussed in further detail in Chapter 4.

The energy-level structure of the XA spin system is shown in Fig. 2.15. The levels are split and shifted due to weak  $J_{XB}$  and  $J_{AB}$  as shown in the diagram corresponding to the  $XAB_3$  spin system. The frequencies of the observable transitions are calculated using the first-order perturbation and are presented in Table 2.1. The high-frequency peaks, associated with  $\Delta F = 1$ , are labelled in the [2- $^{13}\text{C}$ ]-L-alanine spectra as  $v_{1-5}$ . Since low-frequency peaks, arising at frequencies lower than 4 Hz and associated with  $\Delta F = \pm 1$  transitions, are not evident in the experimental spectrum due to their spectral overlap (3 transitions within a 2 Hz window and larger linewidths of the peaks) and flicker ( $1/f$ ) noise, they are not presented in the figure. The flicker noise is usually observed at low frequencies and its amplitude strength is inversely proportional to the frequency.

Table 2.1: Frequencies  $\nu$  of allowed transitions between levels characterized with the quantum numbers  $F$  and  $F_T$ , corresponding to the dominant spin subsystem XA and total spin of the whole (XA) $B_3$  system, obtained using the first-order perturbation theory. The  $J$ -coupling constants for [2- $^{13}\text{C}$ ]-L-alanine, (XA) $B_3$ , are used for perturbative calculation (see main text).

| $F_T$             | $F$           | $\nu$                            | $\nu$ (Hz) |
|-------------------|---------------|----------------------------------|------------|
| $1/2 \mapsto 1/2$ | $0 \mapsto 1$ | $-1/2(J_{XB} + J_{AB}) + J_{XA}$ | 144.9      |
| $1/2 \mapsto 1/2$ | $0 \mapsto 1$ | $1/4(J_{XB} + J_{AB}) + J_{XA}$  | 146.7      |
| $1/2 \mapsto 3/2$ | $1 \mapsto 1$ | $3/4(J_{XB} + J_{AB})$           | 1.9        |
| $3/2 \mapsto 1/2$ | $0 \mapsto 1$ | $-5/4(J_{XB} + J_{AB}) + J_{XA}$ | 143.0      |
| $3/2 \mapsto 3/2$ | $0 \mapsto 1$ | $-1/2(J_{XB} + J_{AB}) + J_{XA}$ | 144.9      |
| $3/2 \mapsto 5/2$ | $0 \mapsto 1$ | $3/4(J_{XB} + J_{AB}) + J_{XA}$  | 148.0      |
| $1/2 \mapsto 3/2$ | $1 \mapsto 1$ | $3/4(J_{XB} + J_{AB})$           | 1.9        |
| $3/2 \mapsto 5/2$ | $1 \mapsto 1$ | $5/4(J_{XB} + J_{AB})$           | 3.1        |

**For even more complex spin systems than (XA $_n$ )B $_m$ ,** the first-order calculation might not be straightforward. In such a case, to be able to interpret  $J$ -spectra, simulations can be performed by numerical diagonalization of the density matrix, using the high-performance library for spin simulations [3, 26].  $^{15}\text{N}$ -pyridine with its XAA'BB'C topology can be an ideal example of such a complex spin system. This compound consists of one  $^{15}\text{N}$  spin and 5 magnetically inequivalent  $^1\text{H}$  spins. A and A' represent the chemically equivalent, however, magnetically inequivalent spins. In brief, the chemical equivalence is called for the same isotopic species when they precess at the same frequency (the same chemical shift). When the spins are chemically equivalent and have identical couplings to all other spins in the molecule, they are known as magnetically equivalent. Even if A and A' spins precess at the same frequency in the field, they display distinct coupling networks, as demonstrated in the Table A.1. The  $J$ -couplings between magnetically equivalent spins are unobservable.

In Fig. 2.16, the simulated and experimental zero-field spectra of  $^{15}\text{N}$ -pyridine are presented (details of the experimental method and data analysis are described in Chapter 3). Due to the long relaxation time of the pyridine molecule, narrow lines are observed in its  $J$ -spectrum. Despite the complex pattern of the spectrum, which is difficult to predict by basic calculations, numerical calculations allow determination of the pyridine unique spectrum, which agrees well with the experimental results. This paves the way for pattern

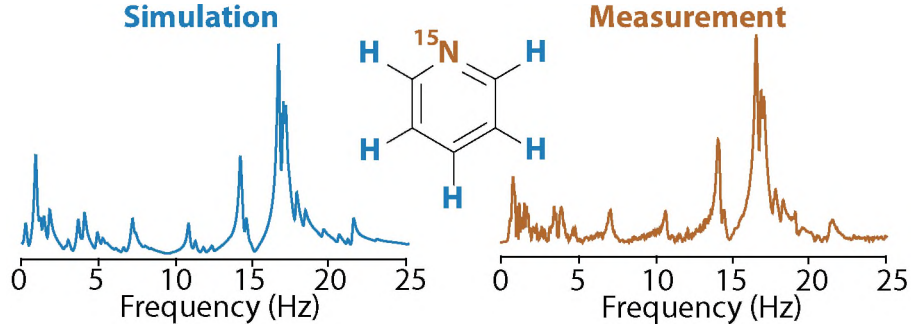


Figure 2.16: Simulated and experimentally measured zero-field spectra of  $^{15}\text{N}$ -pyridine. The  $J$ -coupling values used for the simulation are given in Table A.1.

recognition using zero-field NMR.

## 2.2.4 Ultralow-Field NMR

If the spins precession is larger than the transverse relaxation rate, i.e., when  $|\gamma B_0| > 1/T_2$ , but the Larmor precession difference is still smaller than the  $J$ -coupling  $J_{\text{XA}}$  between the nuclei, i.e.,  $|B(\gamma_I - \gamma_S)| < |J_{\text{XA}}|$ , we may consider the ultralow-field (ULF) range. Under the ULF regime, the total nuclear spin Hamiltonian  $\hat{\mathcal{H}}$  is equal to the sum of the  $J$ -coupling Hamiltonian  $\hat{\mathcal{H}}_J$  and the Zeeman Hamiltonian  $\hat{\mathcal{H}}_Z$ , which arises from the interaction of all nuclei with the external ultralow field  $\mathbf{B}$ :

$$\hat{\mathcal{H}} = \hat{\mathcal{H}}_J + \hat{\mathcal{H}}_Z = \hat{\mathcal{H}}_J - \sum_i (\gamma_i \hat{\mathbf{I}}_i \cdot \mathbf{B}), \quad (2.56)$$

where  $\hat{\mathbf{I}}_i$  and  $\gamma_i$  are the nuclear spin operator and the gyromagnetic ratio of the  $i^{\text{th}}$  nucleus, respectively.

To provide a comparison between zero, ultralow, and high-field regimes,  $^{13}\text{C}$ -methanol, being the  $\text{XA}_3$  spin system, is considered. In presence of an ultralow transverse ( $x$ - or  $y$ -oriented) magnetic field, the unperturbed (zero-order) energy-level structure of the  $\text{XA}_3$  system is modified due to the Zeeman interaction (Fig. 2.17). In this situation, the selection rules for observable transitions are determined as  $\Delta F = 0, \pm 1$ ,  $\Delta I_A = 0$ , and  $\Delta m_F = \pm 1$  [27] where the quantization axis is chosen along the applied small perturbative field direction, namely transverse axis.

As derived in Ref. [27] using first-order perturbation calculations, in the system with a

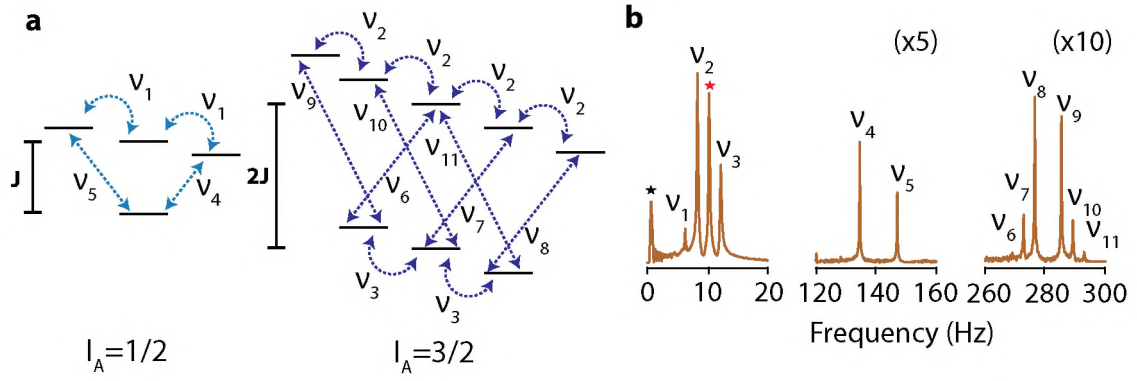


Figure 2.17: (a) Schematic energy-level diagram of the  $XA_3$  spin system at the ultralow field. In the presented case, the spin quantization axis is oriented along the magnetic field. The observable transitions are represented by  $\nu_{1-11}$ . The manifolds are grouped by the quantum number  $I_A$ . (b) Experimental ultralow-field spectrum of  $^{13}\text{C}$ -methanol. The black star denotes  $1/f$  noise. The peak denoted by red star arises from precession of uncoupled  $^1\text{H}$  spin of  $^{13}\text{C}$ -methanol.

total spin  $I_A = 1/2$ , which at zero field has a single line at a the frequency  $J_{\text{CH}}$ , additional line can arise at low frequency when there is non-zero magnetic field. The line appears at:

$$\nu_1 = \frac{\gamma_I + \gamma_S}{2} B_x, \quad (2.57)$$

and it is a result of a transition between successive magnetic sublevels of the  $F = 1$  state. For the total A-nuclei spin  $I_A = 3/2$ , the line at  $2J_{\text{CH}}$  is observed at zero field. However, due to the Zeeman interaction, the energies of magnetic sublevels change at non-zero field, both in the  $F = 1$  and  $F = 2$  levels. The energy shifts of the magnetic sublevels lead to the transition of the frequencies  $\nu_2$  and  $\nu_3$  in the  $F = 2$  and  $F = 1$  levels, respectively:

$$\begin{aligned} \nu_2 &= \frac{3\gamma_I + \gamma_S}{4} B_x, \\ \nu_3 &= \frac{5\gamma_I - \gamma_S}{4} B_x. \end{aligned} \quad (2.58)$$

The calculated frequency of observable transitions between the manifolds are summarized in Table A.5. The experimental ultralow-field spectrum of  $^{13}\text{C}$ -methanol is presented in Fig. 2.17 by defining all peaks with the corresponding transitions. The peak stemming from the precession of uncoupled  $^1\text{H}$  is discussed further in Chapters 6 and 7.

### 2.2.5 Detection of Zero and Ultralow Field NMR:

#### Optical Magnetometry

In conventional NMR, inductive detectors (pick-up coils) are used. The sensitivity of such sensors depends on an oscillating-field frequency and hence the static magnetic-field strength [28]. As the Larmor frequencies in high-field are on the order of tens or even hundreds of megahertz, pickup coils are well suited for the detection of NMR signals. In zero and ultralow fields, however, the dominant interaction comes from the scalar coupling, which ranges between a single and hundreds of hertz. For detection of such fields, conventionally used inductive sensors are not suitable.

Several methods were introduced to acquire ZULF NMR signals. Initially, zero-field experiments were performed by field cycling [29]. In this method, a sample is polarized in a high field and then transferred to the zero-field region for evolution. Finally, the sample is transferred back to the high-field region and measured with a high-field instrument. The field cycle approach is time consuming due to the need to detect the signal point by point by varying the evolution time in the zero field.

An example of a non-inductive detection method that was used for ZULF NMR is the superconducting quantum interference device (SQUID). Such sensors achieve a sensitivity of about  $10 \text{ fT}/\sqrt{\text{Hz}}$  [30], which makes them particularly attractive for the measurements. SQUIDs were used for both spectroscopy and imaging and their advantages, such as lower requirements for relative field homogeneity and the ability to measure in the presence of a metal object, were demonstrated [31, 32]. However, the necessity of a cryogenic cooling limits applicability/portability of the technique as well as increases its cost. Another non-inductive magnetic sensor is nitrogen-vacancy (NV) centers in diamond. The NV center have optically accessible electron spin states, which enables initialization and readout of the state. Moreover, the state is magnetically sensitive, thus can be used for magnetic-field detection. Unfortunately, the sensitivity of the method is still in the  $\text{nT}/\sqrt{\text{Hz}}$  range, i.e., it is significantly lower than those of SQUID (and atomic magnetometers discussed below and used in this dissertation) [33]. On the other hand, they are an attractive

method for nanoscale NMR because of their small size and ability to be positioned closer to the target sample [34].

In this dissertation,  $^{87}\text{Rb}$  atomic magnetometers are employed to detect ZULF NMR signals. The principle of this, used in this dissertation, magnetic-field sensors may be explained as follows: (1) Resonant, circularly polarized laser generates atomic (total-angular-momentum) polarization of alkali atomic vapor, creating their bulk magnetization along the optical pumping axis; (2) External magnetic field, originating from the NMR sample, causes the Larmor precession of the atomic polarization/magnetization; (3) Precession changes optical properties of the atomic vapor, which manifests by modification of properties of light. Monitoring these properties allows one to measure magnetic fields generated by NMR sample [35].

The main advantages of the atomic magnetometry is its low cost, as well as small size and non-cryogenic operation of the sensors. Combined with ever-growing commercial availability of the sensors, these are the attributes that make the magnetometers particularly attractive for ZULF NMR. These are also the main motivation for using the magnetometers for research presented within this dissertation.

The study presented in the dissertation can be divided into several groups with regard to the applied magnetometric scheme. Measurements of zero-field spectra of thermally-prepolarized samples are presented in Chapters 3 and 4. For the measurements, the home-built atomic magnetometer was used. On the other hand, for the ZULF NMR studies exploring parahydrogen-based hyperpolarization, i.e., the research presented in Chapters 5 and 6, a setup with commercially available magnetometers was used. The combination of commercial magnetometers with the piercing solenoid, enabling ultralow-field NMR, is described in Chapters 6 and 7. A comprehensive description of all these setups is provided later when related studies are discussed. Nonetheless, some general features of atomic magnetometry and its application in ZULF NMR are presented below.

The sensitivity of an atomic magnetometer is one of the main parameters determining the applicability of ZULF NMR in chemical and biochemical analysis. The fundamental sensitivity limit of atomic magnetometers arises from spin projection noise. This noise

can be recalculated in the limit on magnetic-field sensitivity, which is given by [35]:

$$\delta B = \frac{1}{\gamma_a \sqrt{n_a T_{2,a} V t_m}}, \quad (2.59)$$

where  $\gamma_a$  is the gyromagnetic ratio of an alkali atom,  $n_a$  is the density of alkali atoms (here,  $^{87}\text{Rb}$ ),  $T_{2,a}$  is the transverse spin relaxation time of alkali-atom polarization,  $V$  is the vapor-cell volume, and  $t_m$  is the measurement time. It is easy to deduce from Eq. (2.59) that to maximize the sensitivity of atomic magnetometers, one needs to increase the vapor density and relaxation time. A higher vapor density is achieved by increasing the temperature of a gas container (vapor cell), since it is a function of the parameter. Furthermore, the relaxation time is improved under low magnetic field with high alkali vapor density since the spin-exchange relaxation, being one of the main sources of relaxation in atomic vapors, vanishes when the spin-exchange collision rate is higher than the Larmor frequency of the atoms. This regime is known as a spin-exchange relaxation-free (SERF) [36]. The sensitivity of an atomic magnetometer operating in the SERF regime reaches  $< 1 \text{ fT}/\sqrt{\text{Hz}}$ . However, this sensitivity might not be adequate for measuring ZULF NMR signals from dilute samples or samples containing low-abundance isotopes, which are thermally polarized. To overcome this limitation, hyperpolarization techniques have been used and this subject is discussed in further section as well as in Chapters 5 and 6.

One of the drawbacks of an atomic magnetometer is its dead time. This is the time between the device being saturated or turned off and the time when it resumes its full measurement capabilities. It was discussed above that, for ZULF NMR measurements, a static field or magnetic-field pulses are applied to the samples. Often the same field also perturbs the atomic magnetometer. As the dynamic range of the magnetometer is typically below 10 nT, application of the field that is many orders of magnitude larger saturates the magnetometers. Thus, even after the fields have been turned off, the magnetometer resumes its full sensitivity within  $\approx 30\text{-}50 \text{ ms}$ . This constrains the ability to measure NMR samples with shorter relaxation times. Although the use of higher-power lasers can reduce the dead time to  $\approx 1 \text{ ms}$  [37], this approach was not explored for ZULF

NMR. It should be noted that the dead time in high-field NMR, arising from the use of the same coil to pulse and detect the signal, is on the order of 5-10  $\mu\text{s}$  [38]. Thus, it does not form a significant limitation for the measurements.

Another limitation of atomic magnetometers is their finite bandwidth. The bandwidth is determined by the transverse relaxation time of the alkali polarization (it reveals the opposite dependence on quantity as the sensitivity). The bandwidth of the magnetometer used in the work presented in Chapters 3 and 4 is about 400 Hz. Therefore, the sensitivity drops significantly above this frequency. However, despite this constraint, the ability of atomic magnetometers to detect compounds with high  $J$ -coupling constants, such as one-bond  $^1\text{H}$ - $^{31}\text{P}$ , is discussed further in Chapter 3.

## 2.3 Parahydrogen-Based Hyperpolarization

The main drawback of ZULF NMR, as well as conventional NMR, is low sensitivity, originating from the extremely low spin polarization of samples thermal polarized even in the strongest magnets. To overcome this limitation, such hyperpolarization methods as spin-exchange optical pumping, dissolution dynamic nuclear polarization (d-DNP), chemically-induced dynamic nuclear polarization, and parahydrogen-induced polarization (PHIP), were developed. Among them, parahydrogen-induced polarization methods are of particular interest since they offer fast hyperpolarization with inexpensive and simple equipment. To provide background for the studies presented in Chapters 5 and 6, the main principles of PHIP and related techniques are briefly discussed in this section. A detailed explanation of the spin dynamics and chemical kinetics behind parahydrogen-based hyperpolarization can be found in several comprehensive reviews (see, for example, Refs. [39–41]).

### 2.3.1 Parahydrogen

The hydrogen molecule consists of two coupled nuclear spin-1/2 particles. Due to the difference in nuclear-spin configurations, it exists as two nuclear spin isomers: parahy-

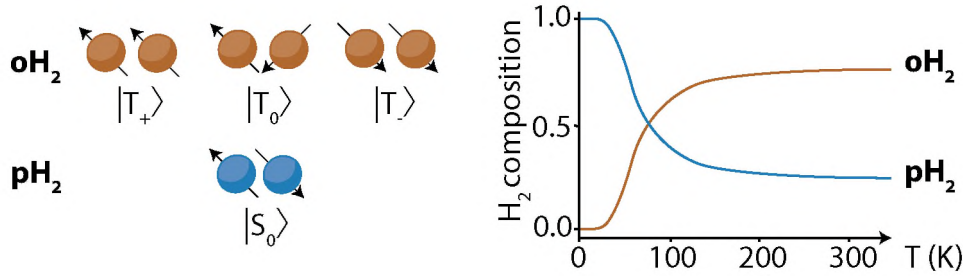


Figure 2.18: Left: Schematic representation of four possible states of the hydrogen molecule: grouped in parahydrogen ( $\text{pH}_2$ ) and orthohydrogen ( $\text{oH}_2$ ) states [42]. Right: The equilibrium populations of the hydrogen isomers as a function of temperature (Detailed calculation for isomer fraction can be found in the Ref. [39]).

drogen ( $\text{pH}_2$ ) and orthohydrogen ( $\text{oH}_2$ ). Parahydrogen is a singlet state, i.e., it has a total nuclear spin of  $I = 0$  and only one magnetic sublevel  $m_I = 0$ . Because of spin zero, parahydrogen is non-magnetic and hence NMR silent. Orthohydrogen has a total nuclear spin  $I = 1$ , therefore it has three magnetic sublevels ( $m_I = -1, 0, 1$ ) and is called a triplet. These four spin states of the hydrogen molecule can be written on a singlet-triplet basis as shown in Eq. (2.38). The hydrogen states are also artistically shown in Fig. 2.18.

The Pauli principle states that fermions, i.e., particles with half-integer spin (e.g.,  $^1\text{H}$ ) are described by the antisymmetric overall wave function [43]:

$$\psi(1, 2) = -\psi(2, 1). \quad (2.60)$$

The overall wave function of the hydrogen molecule can be written as the product of the following five wave functions:

$$\psi_{\text{tot}} = \psi_e^{\text{orb}} \psi_e^{\text{spin}} \psi_n^{\text{vib}} \psi_n^{\text{rot}} \psi_n^{\text{spin}}, \quad (2.61)$$

where  $\psi_e^{\text{orb}}$  is the wave function associated with the orbital motion of electrons,  $\psi_e^{\text{spin}}$  with the electron spin state,  $\psi_n^{\text{vib}}$  is the wave function associated with the vibrational motion of the nuclei,  $\psi_n^{\text{rot}}$  with the rotational motion of the nuclei, and  $\psi_n^{\text{spin}}$  with the wave function related to the nuclear spin state. Since the first three wave functions ( $\psi_e^{\text{orb}}, \psi_e^{\text{spin}}, \psi_n^{\text{vib}}$ ) are all symmetric, the product of the rotational and nuclear spin wave functions ( $\psi_n^{\text{rot}} \psi_n^{\text{spin}}$ ) must be antisymmetric.

As the nuclear spin wave function of the singlet state  $|S_0\rangle$  is antisymmetric under the exchange of two identical fermions, the rotational wave function of  $\text{pH}_2$  is symmetric with its rotational ground state quantum number  $j = 0$ . On the contrary, the spin wave functions of the triplet states  $|T_{+1}\rangle$ ,  $|T_0\rangle$ , and  $|T_{-1}\rangle$  are symmetric. Thus, the rotational wave functions of  $\text{oH}_2$  are antisymmetric with its rotational ground-state quantum number  $j = 1$ . The rotational ground state of  $\text{pH}_2$  has a lower energy than the ground state of  $\text{oH}_2$  by about  $120 \text{ cm}^{-1}$  (173 K) [39]. As a result, at room temperature (293 K), the composition of hydrogen gas is  $\approx 25\%$   $\text{pH}_2$  and  $\approx 75\%$   $\text{oH}_2$ , as the four states, following the Boltzmann distribution, are almost equally populated. At lower temperatures, the equilibrium distribution shifts in favor of the lower energy  $\text{pH}_2$ , even  $\text{H}_2$  at its boiling point (20.3 K) exists as almost 100%  $\text{pH}_2$  (Fig. 2.18). However, the transition between the singlet and triplet states is strictly forbidden due to quantum mechanics. Thus, the spontaneous conversion between isomers is extremely slow, unless the symmetry in the hydrogen molecule is temporarily broken. This can be done by exposing hydrogen molecules to a paramagnetic catalyst (such as charcoal or iron(III) oxide) so that the spins experience a strong magnetic field. On the other hand, once the  $\text{pH}_2$  is generated at low temperature, it can be stored for several weeks even at room temperature [39].

### 2.3.2 Hydrogenative Parahydrogen-Induced Polarization

The alignment of the nuclear spins in parahydrogen can be transferred to other molecules, resulting in drastically enhanced NMR signals. This allows one to overcome the limitation of low sensitivity in NMR. In order to exploit the spin order of parahydrogen, the symmetry of the parahydrogen molecule must be broken. In the classical version of the PHIP method, it is achieved via the addition of parahydrogen to an unsaturated substrate as a consequence of catalytic hydrogenation. Because two protons become chemically and/or magnetically inequivalent in the product molecule. It is worth pointing out that the hydrogenation reaction must be performed in a pairwise manner. This means that two atoms of the hydrogen molecule are added to the substrate together, maintaining the spin order. Otherwise, the uncoupling of protons during the reaction causes the singlet order

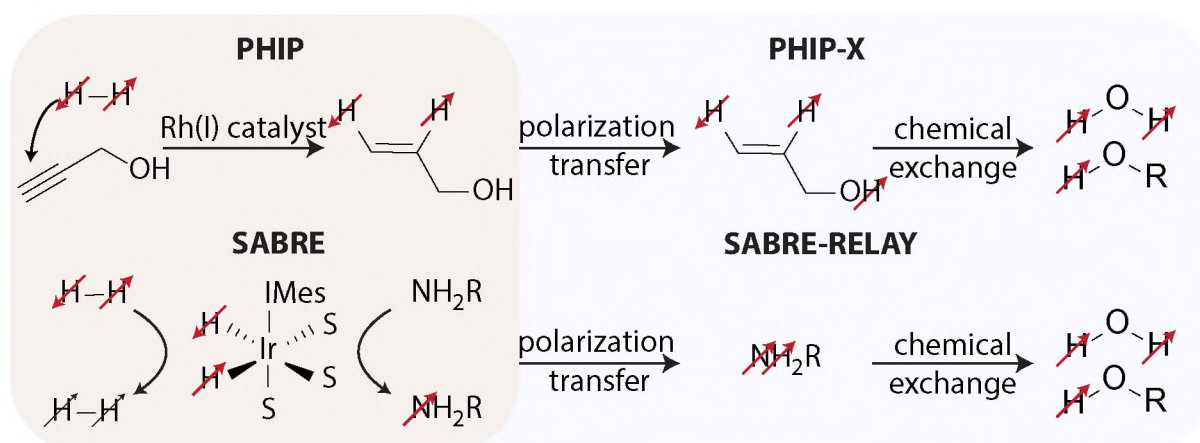


Figure 2.19: Top: Hydrogenative PHIP and PHIP-X reactions. In the presented scheme, propargyl alcohol is hydrogenated using Rh-catalyst, yielding hyperpolarized allyl alcohol (PHIP). This hyperpolarized product can be used as a transfer agent to hyperpolarize a target molecule with exchangeable protons (PHIP-X). Bottom: SABRE and SABRE-RELAY reactions. Parahydrogen and a compound with an amine group are bound to an iridium catalyst, transferring the polarization to the amine group (SABRE). In the following step, the amine group can be used as a source of hyperpolarized exchangeable protons, thus polarization can be transferred to another compound through a chemical exchange (SABRE-RELAY).

to disappear and unequal population of the Zeeman sublevels of the target molecule are not achieved (molecule is not polarized).

In conventional NMR, there are two well-known procedures for performing PHIP experiments: PASADENA (Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment), where the hydrogenation reaction is conducted in a high magnetic field, and ALTADENA (Adiabatic Longitudinal Transport After Dissociation Engenders Net Alignment), where the reaction is carried out in a low field and then the product is transferred adiabatically to the magnet for the NMR experiment [44]. These methods are discussed to some extent below.

To understand the mechanism of PHIP experiments in an intuitive way, the population approach is often considered [40, 44]. In this context, as the parahydrogen protons are magnetically inequivalent in the target molecule, the protons can be considered as a weakly coupled two-spin system (AX) isolated from the rest of the molecule. If the hydrogenation reaction is performed using thermal hydrogen at room temperature ( $p\text{H}_2/o\text{H}_2 = 1/3$ ), the energy levels of this AX spin system ( $|\alpha\alpha\rangle$ ,  $|\alpha\beta\rangle$ ,  $|\beta\alpha\rangle$ , and  $|\beta\beta\rangle$ ) are almost equally populated with a minor difference arising from the Boltzmann distribution, which

is of the order of  $10^{-5}$ . As shown in Fig. 2.20, the four transitions between the states give rise to two doublets with the same amplitude proportional to the Boltzmann factor.

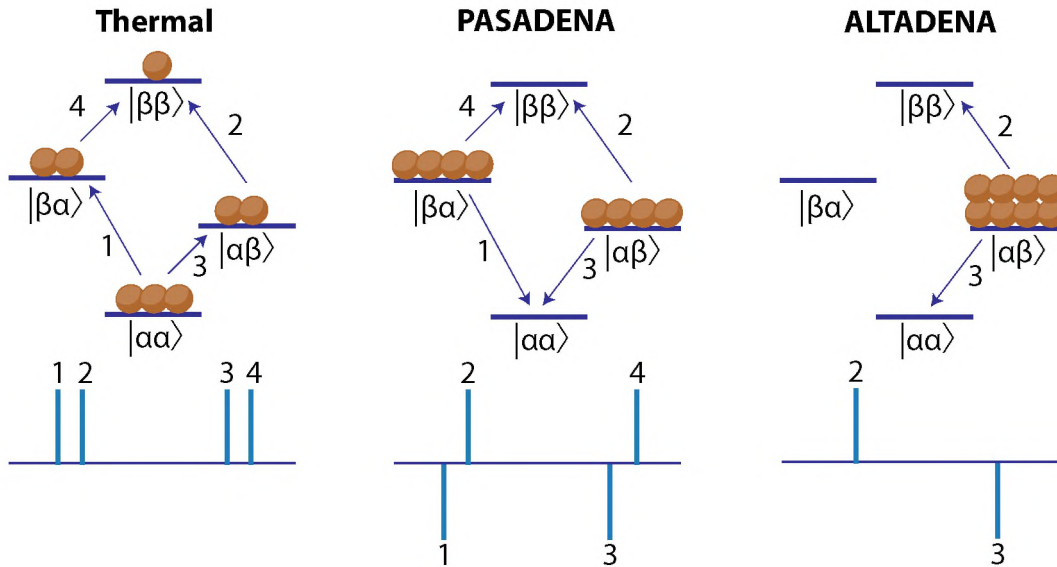


Figure 2.20: Population models used to explain PHIP experiments (thermal polarization, PASADENA, and ALTADENA). The energy levels and spectra are not drawn with scale.

When the hydrogenation reaction is conducted in a high magnetic field with 100% enriched parahydrogen, only the singlet state is populated in the PASADENA approach. Thus, the states with a partially singlet character  $|\alpha\beta\rangle$  and  $|\beta\alpha\rangle$  become populated. Due to the selective population of these states, the NMR spectrum consists of two antiphased doublets with great intensity arising from the large population difference (Fig.2.20).

On the other hand, if the hydrogenation reaction with 100% enriched parahydrogen is carried out in a low field (outside the strong NMR magnet), the evolution occurs in the strongly coupled spin system (in the singlet-triplet eigenbasis). Then only the singlet states become populated. The adiabatic transfer to a high field ensures that the system remains in the state corresponding to the high-field state  $|\alpha\beta\rangle$ . Therefore, under ALTADENA conditions, two antiphase peaks appear in the NMR spectrum due to the transition between  $|\beta\beta\rangle$  and  $|\alpha\beta\rangle$  and  $|\alpha\alpha\rangle$  and  $|\alpha\beta\rangle$  (Fig. 2.20).

Even though the population-difference approach allows us to simply predict the peaks and their signs, it is not adequate to describe the whole process. This is particularly true in more complex spin systems, where the density-operator formalism must be employed. A further discussion on the subject can be found in Ref. [39–41].

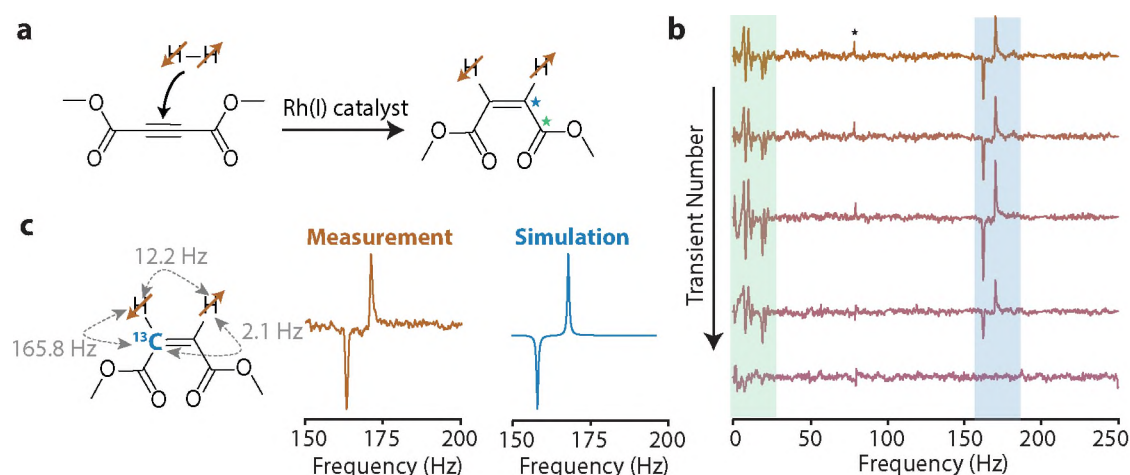


Figure 2.21: (a) Pairwise addition of parahydrogen to dimethyl acetylenedicarboxylate to form dimethyl maleate catalysed by [1,4-Bis(diphenylphosphino)butane](1,5-cyclooctadiene)rhodium(I) tetrafluoroborate. The positions of  $^{13}\text{C}$  in the carboxyl and vinyl groups at 2.2% natural abundance are indicated by green and blue stars. (b) Zero-field spectra of hyperpolarized dimethyl maleate via repeated (five transients) PHIP (see main text for the experimental details). The green and blue boxes highlight the peaks arising from hyperpolarized dimethyl maleate with  $^{13}\text{C}$  in the carboxyl group and the vinyl group, respectively. The black star indicates the position of noises stemmed from the experimental setup. (c) Experimental and simulated spectra of hyperpolarized dimethyl maleate with  $^{13}\text{C}$  in the vinyl group. The  $J$ -coupling values used for simulations with SpinDynamica are given with structural formula.

The major limitation of PHIP is the necessity to use dedicated compounds with unsaturated bonds. This shortcoming may be addressed by the exchange-based polarization method called PHIP-X [45]. This opens means for hyperpolarization of many biomolecules. The main principle of the method is the polarization of a suitable transfer molecule (e.g., propargyl alcohol) and then successive transfer of the polarization to a target molecule via a proton-exchange process, occurring at a low magnetic field (Fig. 2.19). PHIP-X has been implemented to hyperpolarize water, ethanol, pyruvic acid, and lactic acid [45]. In Chapter 7, this method is further discussed with exciting results for hyperpolarization of important biomolecules such as urea, glycine, and ammonium.

As first presented in Ref. [46], the hydrogenative-PHIP method can be employed for spin polarization in zero-field NMR, yielding substantial signal enhancement. Unlike conventional NMR, in ZULF NMR experiments, the spherical symmetry of parahydrogen is broken by a DC pulse that induces the precession of different nuclear species at different angles (evolution under the  $J$ -couplings can be ignored during the pulse). Taking

advantage of the absence of magnetic susceptibility, zero-field NMR has been used to monitor the heterogeneous/biphasic hydrogenation reaction with continuous bubbling. Furthermore, due to the greater penetration of the electromagnetic field into metals at lower frequencies, monitoring chemical reactions inside metal containers becomes possible [4]. It should be noted that for PHIP, ZULF NMR commercial atomic magnetometers are advantageous because of their temperature stability, i.e., chemical reactions enabling hyperpolarization are sensitive to temperature variation [6].

As an example of a preliminary study, zero-field spectra of dimethyl maleate hyperpolarized with the use of PHIP are presented in Fig. 2.21. A sample of 1 M natural isotopic abundance dimethyl acetylenedicarboxylate (DMAD) and 7 mM [1,4-Bis(diphenylphosphino)butane] (1,5-cyclooctadiene) rhodium(I) tetrafluoroborate  $[(\text{Rh}(\text{dppb})(\text{COD}))\text{BF}_4]$  dissolved in 500  $\mu\text{L}$  of acetone was subjected to parahydrogen bubbling ( $\approx 100$  ml/min flow at 6 bar) for about 20 s at zero field (to form hyperpolarized dimethyl maleate) followed by a sharp DC pulse and then data acquisition. The sequence was repeated five times until no clear spectral pattern remained. After four transients, no distinguishable signal was obtained from the NMR sample. It can be shown that a concentration limit for the detection of the compound using the method is at a level of tens of mM, however, a high concentration of the substrate in the solution allowed us to obtain numerous transients which can be averaged to increase the signal-to-noise ratio, as shown in Fig. 2.21(c). The experimental setup and data analysis for zero-field PHIP NMR are explained in Chapter 6. A more detailed discussion of the zero-field NMR using PHIP can be found in Ref. [47].

### 2.3.3 Non-hydrogenative Parahydrogen Induced Polarization: Signal Amplification by Reversible Exchange

An alternative parahydrogen-based polarization technique is non-hydrogenative PHIP, also known as SABRE (Signal Amplification By Reversible Exchange), where a spin order is transferred catalytically from parahydrogen to a molecule of interest without chemical alteration of the target molecule (Fig. 2.19). Polarization transfer occurs through a

scalar coupling when the hydrogen molecule and the substrate bind to the metal center of the catalyst (e.g., Ir complex) simultaneously. The hydrogen molecule is attached to the metal center in a dehydrated form. Since this bilateral exchange is reversible, after the polarization transfer, derived orthohydrogen and hyperpolarized substrates dissociate from the metal complex. This allows the SABRE procedure to be repeated by requiring only parahydrogen as the consumable reactant within the process.

Polarization transfer can be conducted either in the low or high field. If this step is performed at a low magnetic field, spontaneous polarization transfer occurs without the need for nuclear spin manipulation by rf pulses [48]. The optimum polarization-transfer field depends on a  $J$ -coupling network between the attached substrate and the parahydrogen-derived hydride in the metal complex. Generally, the transfer of polarization to the  $^1\text{H}$  nuclei of the substrate is observed in 5–8 mT field (e.g., in pyridine through four-bond  $^1\text{H}$ - $^1\text{H}$  coupling), while  $^{15}\text{N}$  nuclei are polarized in 0.2–0.4  $\mu\text{T}$  field through two-bond  $^1\text{H}$ - $^{15}\text{N}$  coupling [49, 50]. To provide  $\mu\text{T}$ -field conditions, a mu-metal shield is used, and this approach is named SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei) [51]. However, as a limitation, after polarization in a low field, a sample must be transported quickly to the detection high-field region, where NMR measurements are performed. However, the main advantage of ZULF NMR here is its ability to perform *in situ* SABRE experiments at a low-field regime without the need for sample shuttling [52].

Until now, SABRE has only been successfully implemented in zero-field NMR spectroscopy for the  $^{15}\text{N}$ -pyridine molecule by achieving four orders of magnitude signal enhancement with respect to thermal prepolarization by a permanent magnet [6, 52, 53].

As a preliminary study, the catalyst activation process was evaluated by obtaining zero-field NMR spectra of  $^{15}\text{N}$ -labelled pyridine [Fig. 2.22(a)]. A sample with 70 mM  $^{15}\text{N}$ -pyridine (CAS# 34322-45-7) and 2 mM Crabtree’s catalyst, (1,5-cyclooctadiene)(pyridine)-(tricyclohexylphosphine)-iridium(I) hexafluorophosphate (CAS# 64536-78-3), dissolved in 300  $\mu\text{L}$  methanol was bubbled with parahydrogen ( $\approx 100$  ml/min flow at

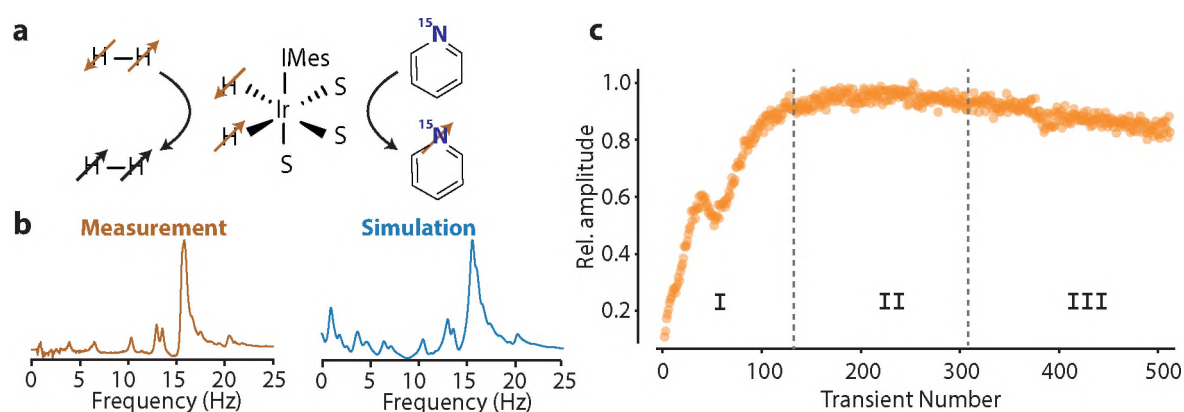


Figure 2.22: (a) Schematic of the SABRE reaction. Parahydrogen and target compound (pyridine) are temporarily bounding to an iridium catalyst, transferring the polarization from parahydrogen to the molecule. (b) Experimental (512 averaged transients) and simulated zero-field spectra of SABRE-hyperpolarized  $^{15}\text{N}$ -labelled pyridine. The  $J$ -coupling values used in simulations are given in Table A.1. (c) Relative amplitude of the peaks in zero-field spectra of hyperpolarized  $^{15}\text{N}$ -pyridine versus transient number. Initially, the upward trend in peak amplitudes is observed due to more potent activation of the Ir-catalyst through continuous reversible bilateral exchange reactions (stage I). After the amplitude of zero-field peaks remains constant for a while (stage II), the evaporation of the sample over time due to continuous bubbling, reducing the amount of  $^{15}\text{N}$ -pyridine in the sample, results in a decrease in the NMR signal (stage III).

6 bar) for 6 s at the zero field. After bubbling, a sharp DC pulse was applied to transform the spin order to observable magnetization by breaking the symmetry of the initial state for heteronuclear couples because nuclei with different gyromagnetic ratios rotate at a different angle during the pulse. And then signal acquisition was started. The sequence was repeated 512 times. As shown in Fig. 2.22(c), during repeated measurements, the increment in peak amplitudes was observed in the zero-field spectrum of  $^{15}\text{N}$ -pyridine hyperpolarized by SABRE due to the elevated efficiency of catalyst activation over time [54]. As a consequence of proper catalyst activation, the curve of relative amplitude exhibits a plateau. Following the plateau, the relative amplitude of each transient gradually decreases because of sample evaporation under continuous bubbling. This issue is discussed in more detail in Chapter 5 with an experimental design to prevent evaporation of a sample during the SABRE experiments.

The main limitation of SABRE is the range of molecules that are amenable to hyperpolarization due to the need for the target molecule to reversibly bind to the SABRE catalyst on a suitable timescale [55]. To improve the applicability of the technique,

SABRE-RELAY is introduced which is based on a similar principle with PHIP-X explained above. The hyperpolarized substrate via the SABRE approach can be used as a transfer molecule to yield a hyperpolarized target molecule through the chemical-exchange process (Fig. 2.19). For conventional NMR spectroscopy, SABRE-RELAY has been used to hyperpolarize various amides, carboxylic acids, alcohols, phosphates, and carbonates by transferring polarization from hyperpolarized amine and ammonia [56]. Moreover, recently, zero-field NMR has been combined with SABRE-RELAY technique to detect hyperpolarized [ $^{13}\text{C}$ ]-methanol, [ $1\text{-}^{13}\text{C}$ ]-ethanol, and [ $2\text{-}^{13}\text{C}$ ]-ethanol [57].

In this chapter, the basic principle of conventional NMR is discussed to provide a background that enables explaining ZULF NMR. While introducing ZULF NMR spectroscopy on a theoretical level, the interpretation of the  $J$ -spectrum, which is an essential tool to comprehend further chapters, is studied in detail. Additionally, the theoretical explanations are supported with experimental examples. Furthermore, parahydrogen-based hyperpolarization methods are examined for conventional NMR as well as ZULF NMR under the scope of the dissertation.

# Chapter 3

## Zero-field NMR of Organophosphorus Compounds

The work in this chapter is based on material presented in the following publication [58]:

**S. Alcicek**, P. Put, V. Kontul, and S. Pustelny, "Zero-Field NMR J-Spectroscopy of Organophosphorus Compounds", *The Journal of Physical Chemistry Letters* **12**, 787–792 (2021).

### 3.1 Motivation and Overview

As mentioned in Chapter 2 previously, zero-field  $J$ -spectroscopy has been used successfully for thermal studies of isotopically enriched compounds containing  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclei. In this chapter, the study of the naturally abundant phosphorus-31 ( $^{31}\text{P}$ ) nucleus is presented using zero-field NMR.

Although some of the organophosphorus compounds are highly toxic chemicals utilized as herbicides, insecticides, or fungicides; natural phosphorus-containing compounds play vital roles in cell structure, energy metabolism, pH homeostasis, bone mineralization, etc. [59–61]. The naturally abundant isotope of phosphorus ( $^{31}\text{P}$  accounts for nearly 100% of the total phosphorus population) is an attractive target for NMR, due to its spin-1/2 property [62]. This is a particular reason why  $^{31}\text{P}$  magnetic resonance spectroscopy is used for *in vivo* detection of phosphorus-containing metabolites such as inorganic phosphate, adenosine triphosphate (ATP), and phosphocreatine, yielding information on intracellular pH, cellular energy metabolism, and phospholipid metabolism in cells [62, 63]. This information is valuable especially for the evaluation of tumor prognosis [64, 65]. However, there are some difficulties with the technique, including long measurement time and

complex data analysis [66].

This chapter presents theoretical and experimental studies on organophosphorus compounds using zero-field NMR. In order to investigate relatively large spin systems, compared to those previously studied, trimethyl phosphate and trimethyl phosphite were measured and their  $J$ -spectra were analyzed and identified using a straightforward interpretation method. To study yet slightly more complex molecules, dichlorvos and dimethyl phosphite were successively used. With the  $J$ -spectrum of dimethyl phosphite, we demonstrate the applicability of zero-field NMR in detecting compounds with a wide range of  $J$ -coupling values. In particular, the spectrum of the molecule with the largest  $J$ -coupling values ever reported in zero-field NMR was measured. An analysis of the spin system was performed using the first-order perturbation theory. The spectra of the compounds were also simulated to support the theoretical investigations. Finally, the  $J$ -spectrum of dimethyl methylphosphonate is reported.

## 3.2 Material and Method

### 3.2.1 Sample Preparation

The experiments reported in this dissertation were performed using liquid NMR samples. The general description of the sample preparation process for the studies presented in Chapters 3, 4 and 7 can be found below:

For ZULF NMR experiments using thermal prepolarization, the NMR samples are degassed and sealed to eliminate the paramagnetic effect of oxygen, which causes faster relaxation (for a detailed discussion of this effect, see Chapter 7). This is achieved by the freeze-pump-thaw method, which uses a glass vacuum manifold (Fig. 3.1). Standard 5 mm tubes can be attached to the manifold through a piston PTFE valve. A liquid-nitrogen cold trap is placed between the glass vacuum manifold and a vacuum pump to protect the pump from condensable vapors. High-vacuum J. Young needle valves allow one to perform freeze-pump-thaw cycles. A cycle starts with cooling an NMR sample by immersion of the tube in a dry ice-acetone mixture ( $-78^{\circ}\text{C}$ ) or liquid nitrogen ( $-196^{\circ}\text{C}$ ).

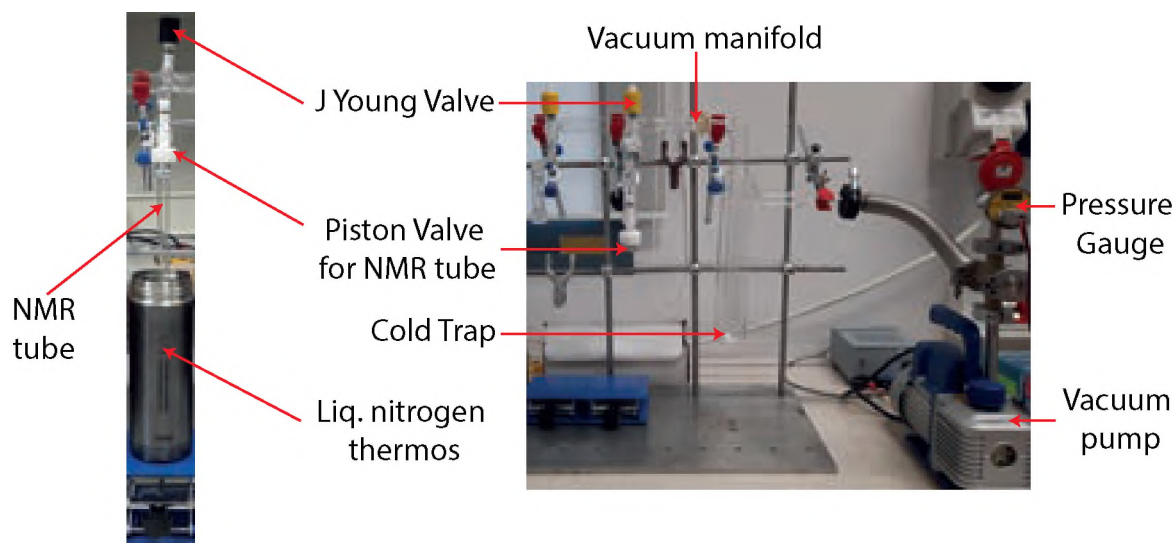


Figure 3.1: Photographs of the sample-preparation setup. A glass vacuum manifold with multiple channels is connected to a vacuum pump through a cold trap. During the sample preparation, the cold trap is held in a liquid nitrogen container. A pressure gauge is placed on top of the vacuum pump line to monitor the system pressure. High-vacuum valves (J. Young and piston valves) are employed to perform freeze-pump-thaw cycles. To freeze NMR samples, liquid nitrogen is used. A detailed explanation of the procedure can be found in the main text.

The dry ice-acetone mixture cannot be used when the melting point of the sample of interest is below  $-78^{\circ}\text{C}$ . On the other hand, the use of liquid nitrogen to freeze samples, particularly those with water content, might break the NMR tubes because of rapid cooling. To avoid the problem, such samples can be cooled down with nitrogen vapor by keeping the samples above the liquid nitrogen. When the sample is frozen, the valves are opened to vacuum the NMR tube. The pressure drop is observed using a pressure gauge. When the pressure stabilizes, the valves are closed. Then, the liquid nitrogen container is moved away and the sample begins to melt, freeing gas bubbles from the solution. However, if we let the frozen sample thaw itself, the bottom of the sample melts before the top part, which might cause the tube to break. Therefore, to control the procedure, the tube can be heated from the top during melting by squirting it with tepid water or acetone. The cycles are repeated until the pressure over frozen liquid reached  $<10^{-4}$  mbar. The tubes are then flame-sealed under vacuum, while the valves are closed. It is recommended that NMR tubes with flammable and/or temperature-sensitive samples are flame-sealed while the samples are frozen.

For this study, trimethyl phosphate (CAS# 512-56-1), trimethyl phosphite (CAS# 121-45-9), dimethyl phosphite (CAS# 868-85-9), dichlorvos (CAS# 62-73-7), and dimethyl methylphosphonate (CAS# 756-79-6) were purchased from Sigma-Aldrich. Dimethyl methylphosphonate was also sourced from Thermo Scientific to verify the reliability of the method. The neat liquid samples (0.15 - 0.2 ml) were transferred into standard 5 mm NMR tubes and degassed multiple times using the freeze-pump-thaw method as explained above. The tubes were then flame-sealed under vacuum.

### 3.2.2 Experimental Setup

A single-beam spin-exchange relaxation free (SERF) atomic magnetometer [35] is employed for zero-field NMR measurements. In Fig. 3.2, a schematic of our zero-field NMR setup is shown. The three-layer mu-metal magnetic shields attenuate external magnetic fields to a level of  $10^{-6}$ . Additionally, an innermost ferrite layer (not shown in Fig. 3.2) is used to reduce magnetic noise stemming from thermal Johnson currents induced in mu-metal. A set of coils located within the shielding is used to provide an absolute-zero-magnetic-field region. Field shimming at the position of the NMR sample was performed by measuring a neat  $^{13}\text{C}$ -formic acid, until a single narrow line at  $\approx 222$  Hz is observed in the  $J$ -spectrum. A rubidium-87 ( $^{87}\text{Rb}$ ) vapor cell ( $3\times 3\times 3\text{ mm}^3$ ), containing about 300 Torr of  $\text{N}_2$  as buffer gas, is placed on a resistively heated ceramic element. To maintain a sufficient density of alkali vapor and operate in the SERF regime, the cell temperature is maintained at  $160^\circ\text{C}$  [67]. To optically pump and probe the alkali polarization and thus measure magnetic field, circularly-polarized, resonant (795 nm) light is employed to illuminate the cell. After passing through the cell, the intensity of the light is measured by a photodiode. In this setup, the magnetic field is modulated along the  $z$ -direction at high frequency (14 kHz) and the photodiode signal is demodulated at the frequency. The phase-sensitive signal from a lock-in amplifier is used for magnetometric purposes, providing sensitivity along the  $z$ -axis. The magnetometric sensitivity of this setup reaches  $60\text{ fT}/\sqrt{\text{Hz}}$  level, while the measurement bandwidth is above 400 Hz meaning that sensitivity drops significantly above this frequency (for a detailed description of

the experimental setup, see Ref. [18]).

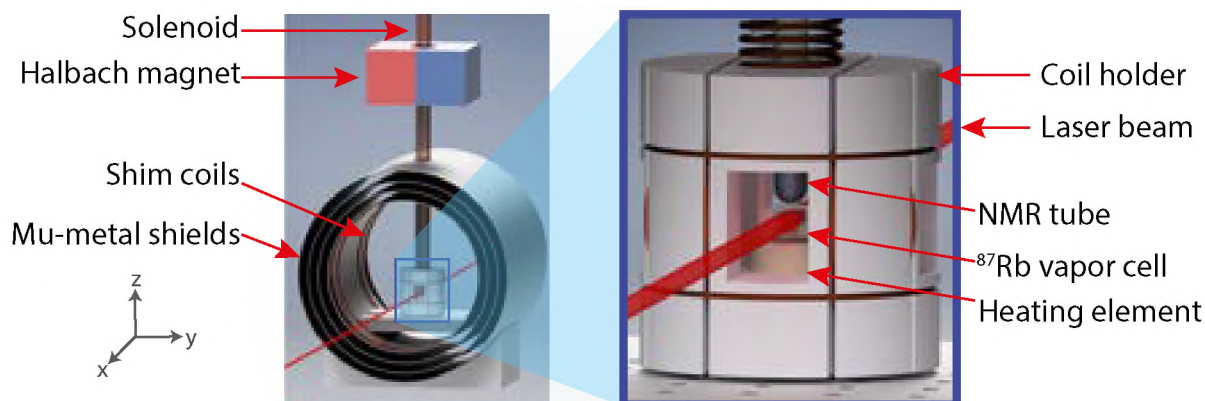


Figure 3.2: Schematics of the experimental setup. After thermal prepolarization in a 1.8 T Halbach magnet, a sample is transferred to the magnetic shield, to the vicinity of the  $^{87}\text{Rb}$  vapor cell. The  $z$ -component of the magnetic field, originating from the sample, is measured by monitoring the intensity of light traversing a vapor cell. Adapted from Ref. [58].

The samples are thermally polarized for about 20 s by a 1.8 T Halbach magnet [18] located above the magnetic shield, and mechanically shuttled into a zero-field detection region, inside the shield. During the transfer, taking about 300 ms, a guiding field of roughly 100  $\mu\text{T}$  is applied by a solenoid coil. When the sample reaches the detection region, the guiding field is suddenly turned off to generate an oscillating zero-field NMR signal [1]. As an alternative approach, a sharp transverse DC magnetic-field pulse along the  $y$ -axis, following turning off the solenoid, is used to acquire signals with higher amplitude (see Sec. 3.3.3).

### 3.2.3 Data Processing and Simulation

Zero-field signal is often saved in the time domain and then converted to a function of frequency by Fourier transform following the digital signal processing. A signal-baseline drift, which results in a large, non-linear background, present in the magnetometer read-out is removed by subtracting a high-order polynomial from the signal. The initial part of the raw data obtained via the magnetometer consists of corrupted data points that originate from the saturation of the magnetometer by a guiding field and a DC pulse (see Sec. 2.2.5). Therefore, this initial recuperation period of approximately 30-50 ms of the

signal is dropped. The missing data points are replaced by backward prediction using Burg’s autoregressive all-pole model method [68]. To correct for non-optimal acquisition time and minimize spectral leakage, the signals are apodized by decaying exponential and subsequently zero-padded to provide sufficient spectral resolution. Additionally, a digital notch filter is employed to remove 50 Hz magnetic noise and its harmonics. As the last step of data processing, an ACMA algorithm was used for automatic phase correction [69]. The entire data processing is performed using a digital signal processing software (ZULF-DSP) on Python [70].

Simulations of zero-field NMR spectra are performed by numerical diagonalization of the density matrix, using the high-performance library for spin simulations [3, 26]. The  $J$ -coupling values for the spin simulations are taken from the literature, providing a match between the simulations and the experimental data.

## 3.3 Results and Discussion

### 3.3.1 Simple $\text{XA}_n$ Spin Systems

Trimethyl phosphate and trimethyl phosphite are  $\text{XA}_9$  spin systems, where spin dynamics are dominated by the  $^1\text{H}$ - $^{31}\text{P}$  three-bond  $J$ -coupling (Fig. 3.3a). In the molecule, the effect of the  $^{13}\text{C}$  nucleus is negligible due to its low abundance (1.1%). As in these compounds all  $^1\text{H}$  nuclei are magnetically equivalent, using the rules described in Sec. 2.2, the allowed-transition frequencies and hence the NMR-line positions are  $J_{\text{PH}}$ ,  $2J_{\text{PH}}$ ,  $3J_{\text{PH}}$ ,  $4J_{\text{PH}}$  and  $5J_{\text{PH}}$  (Fig. 3.3b). Yet, due to their unique electronic environment, the  $J_{\text{PH}}$ -coupling values of trimethyl phosphate and trimethyl phosphite are slightly different (11.0 Hz and 10.5 Hz, respectively) and hence the resonances are at slightly different frequencies (Fig. 3.3c). The experimental spectra (Fig. 3.3c) agree nicely with the results of the simulations (Fig. 3.3d). Owing to narrow linewidths, all peaks are clearly distinguishable, allowing for chemical differentiation of such compounds despite their identical spin topology.

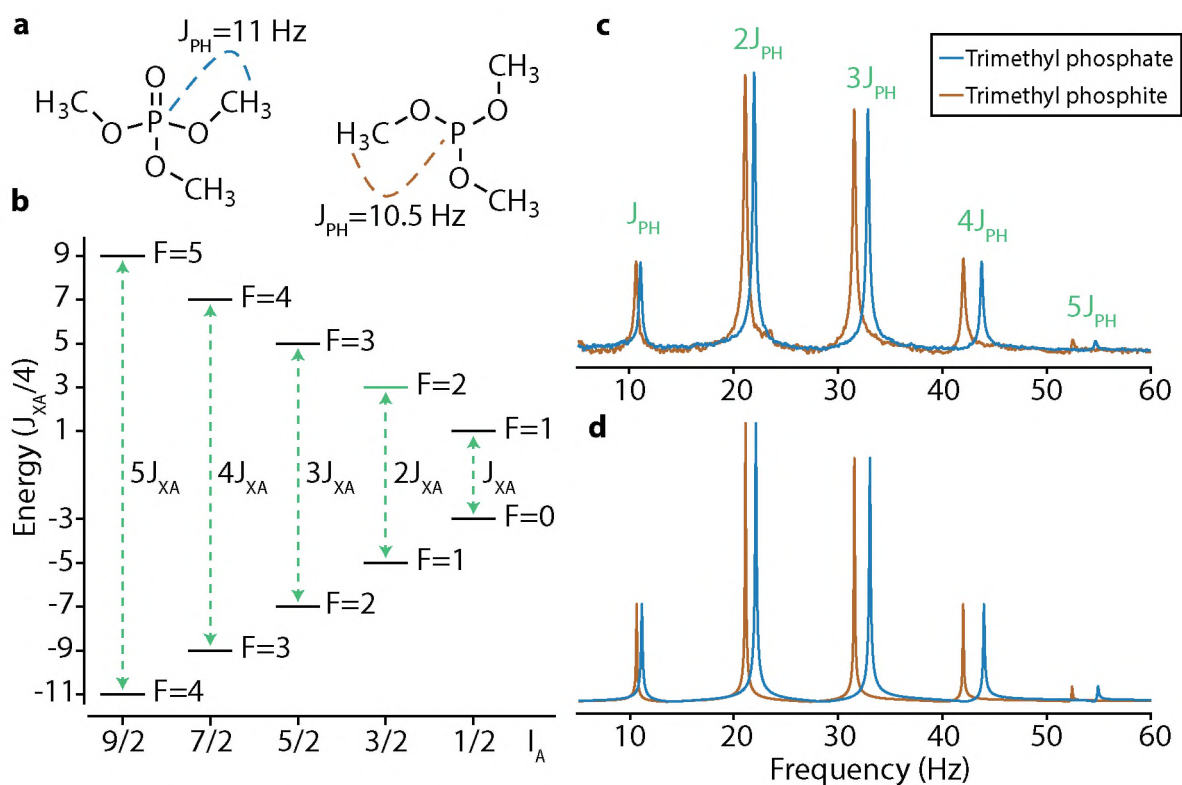


Figure 3.3: (a) Structural formulas of trimethyl phosphate (left) and trimethyl phosphite (right) with heteronuclear  $J$ -coupling. (b) Energy-level diagram along with the observable transitions of the  $\text{XA}_9$  spin system. The manifolds are grouped by the quantum number  $I_A$ , and each manifold is labelled by its quantum number  $F$  (the magnetic-sublevel structure is omitted for clarity). (c) Experimental and (d) simulated  $J$ -spectra of trimethyl phosphate and trimethyl phosphite. The experimental spectra are the result of 128 averaged signals. Adapted from Ref. [58].

### 3.3.2 $(\text{XA}_n)\text{B}_m$ Spin Systems

Dichlorvos is an example of a  $(\text{XA}_6)\text{B}$  spin system, which consists of one  $^{31}\text{P}$  spin and six equivalent  $^1\text{H}$  spins coupled with three-bond couplings  $J_{\text{PH}}^{\text{A}} = 11.4\text{ Hz}$  and another, single  $^1\text{H}$  spin with a three-bond coupling  $J_{\text{PH}}^{\text{B}} = 5.2\text{ Hz}$  [60, 71]. Weak ( $<0.1\text{ Hz}$ ) six-bond homonuclear  $J$ -couplings between inequivalent  $^1\text{H}$  nuclei are negligible in such a system. Also, quadrupolar nuclei,  $^{35/37}\text{Cl}$ , is neglected from spin system analysis, due to their fast relaxations (see discussion in Chapter 4) [72]. In Fig. 3.4a, the zero-order energy-level structure is demonstrated. The levels are split and shifted due to the weak  $J_{\text{XB}}$ -coupling and the frequencies of the observable transitions are calculated using the first-order perturbation (Fig. 3.4b). The reason for the small systematic difference ( $\approx 0.42\text{ Hz}$ ) between estimated and experimental peak positions is the inadequate difference between the weak and strong  $J$ -coupling values, which reduces the precision of the first-order correction provided by a perturbative calculation. The low-frequency ( $<3.5\text{ Hz}$ )  $\Delta F_T = \pm 1$  transitions are not evident in the experimental spectra due to their spectral overlap. The spectral overlap in the low-frequency range is a result of existing 6 observable transitions within a 2 Hz window, short relaxation times of dichlorvos (it reduces spectral resolution), and the presence of excessive flicker ( $1/f$ ) noise. The peaks resulting from  $\Delta F_T = 0$  transitions are scarcely visible in the  $J$ -spectra due to their relatively low amplitudes besides spectral broadening stemming from fast spin relaxation rate (Fig. 3.4c). On the other hand, as shown in Fig. 3.4c, a good agreement is presented between measurement and numerical simulation. The 2.5 times broader linewidth (0.85 Hz) in the measured dichlorvos spectra compared to trimethyl phosphate is a result of a faster relaxation rate. This may originate from the longer rotational correlation time of this larger compound [73].

Dimethyl phosphite is a  $(\text{XA})\text{B}_6$  spin system with a coupling topology similar to dichlorvos. The main difference from dichlorvos is the strongest  $J$ -coupling between  $^{31}\text{P}$  and single  $^1\text{H}$  instead of six equivalent  $^1\text{H}$  nuclei. The one-bond coupling between  $^1\text{H}$  and  $^{31}\text{P}$  is  $J_{\text{PH}}^{\text{A}} = 695\text{ Hz}$  and the three-bond coupling between six  $^1\text{H}$  and  $^{31}\text{P}$  equals  $J_{\text{PH}}^{\text{B}} = 11.7\text{ Hz}$  [60, 74]. The zero-order energy-level structures of the  $\text{XA}$  and  $(\text{XA})\text{B}_6$  spin

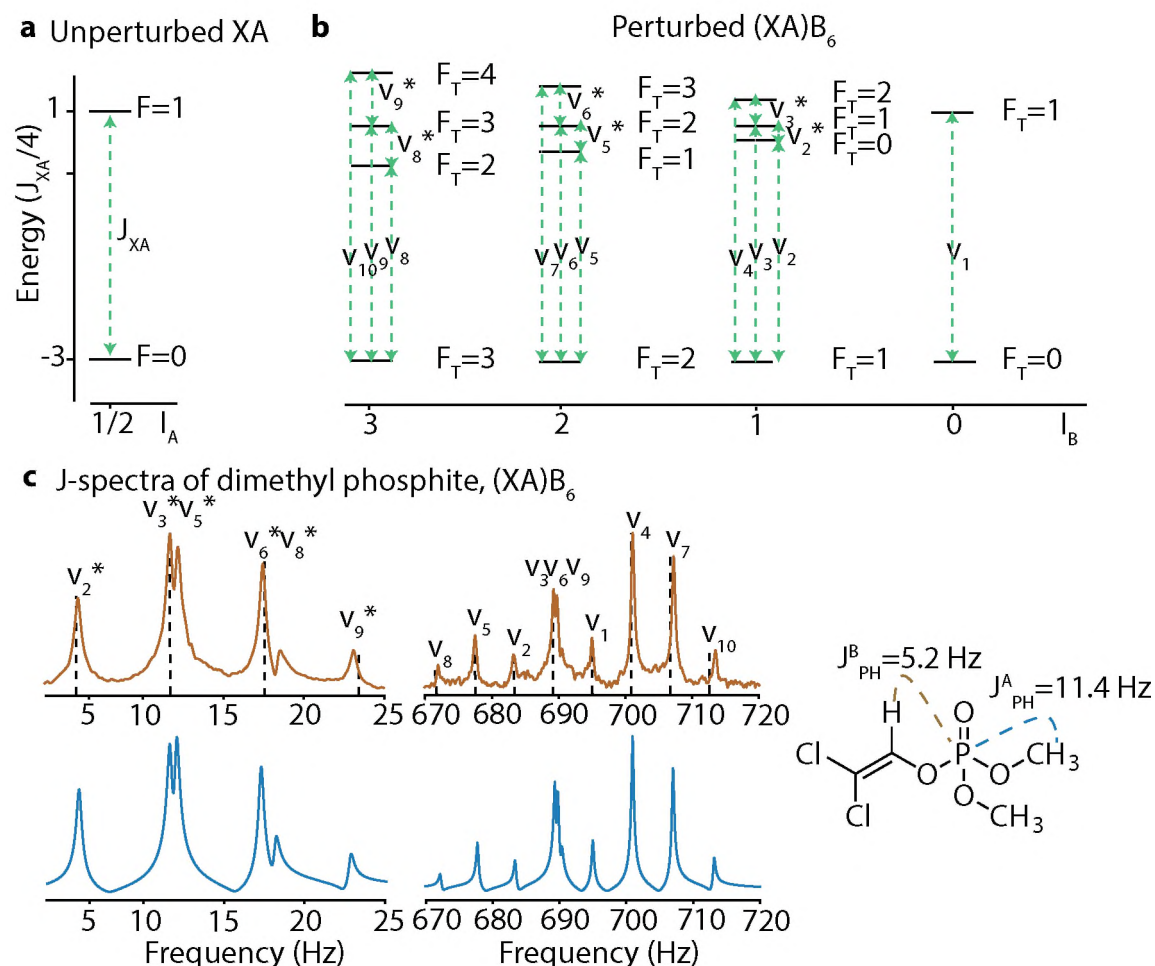


Figure 3.4: Energy-level diagrams of the (a)  $\text{XA}_6$  to (b)  $(\text{XA}_6)\text{B}$  spin system and related observable transitions. For the perturbed  $\text{XA}_6$  spin system,  $v_{0-6}$  denote the high frequency ( $>3.5 \text{ Hz}$ )  $\Delta F_T = \pm 1$  transitions. The manifolds are grouped by the quantum number  $I_A$  and each manifold is labelled by its quantum number  $F$  or  $F_T$ . For clarity, the magnetic-sublevel structure is omitted. (c) Experimental (solid orange line) and simulated (solid blue line) dichlorvos spectra. The dashed lines are line positions ( $v_{0-6}$ ) predicted by the first-order perturbation theory. The experimental spectrum is the result of 128 averaged transients. The heteronuclear  $J$ -coupling values used for simulation are given with the structural formula. Adapted from Ref. [58].

systems are shown in Fig. 3.5a and Fig. 3.5b, respectively. Weak four-bond homonuclear  $J$ -coupling ( $<0.5$  Hz) between inequivalent  $^1\text{H}$  nuclei are neglected in this analysis.

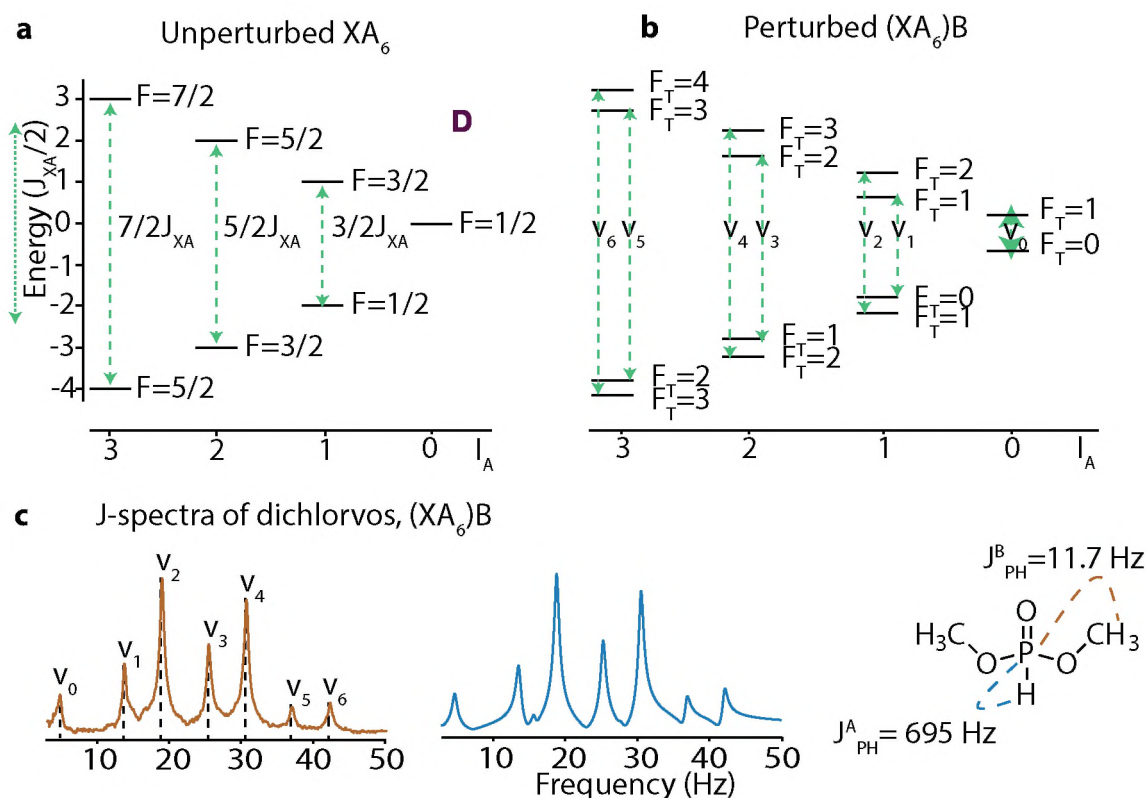


Figure 3.5: Energy-level diagram of the (a)  $\text{XA}$  and (b)  $(\text{XA})\text{B}_6$  spin systems along with transitions observable at the zero field (dashed arrows). The high and low-frequency transitions are denoted by  $v_{1-10}$  and  $v_{2-9}^*$ , respectively. The manifolds are grouped by the quantum numbers  $I_A$  and  $I_B$  and each manifold is labelled by its quantum number  $F$  or  $F_T$ . (c) Experimental and simulated (blue solid line) spectra of dimethyl phosphite along with transition predicted by the first-order perturbation theory (black dashed lines). The heteronuclear  $J$ -coupling values used for simulation are given with the structural formula of dimethyl phosphite. The experimental spectrum is the result of 128 averaged transients. Adapted from Ref. [58].

The  $J$ -spectrum of dimethyl phosphite can be divided into two frequency ranges, which arise from the presence of weak and strong couplings present in the molecule. The high-frequency (670-720 Hz) transitions, denoted as  $v_{1-10}$ , and the low-frequency (5-25 Hz) transitions, represented as  $v_{2-9}^*$ , were calculated using the  $J$ -coupling values given above (Fig. 3.5c). It is noteworthy that the first-order calculation still produces systematic line shifts and more notably does not reproduce additional line splitting seen both in the experiment and exact numerical calculations (Fig. 3.5c). The spectrum of dimethyl phosphite contains the highest frequency lines reported to date in zero-field NMR. After

averaging 128 transients, the signal-to-noise ratio (SNR) of the strongest line in the  $J$ -spectrum in the low and high-frequency ranges are  $\sim 100$  and  $\sim 25$ , respectively. While both signals should have similar amplitudes, the difference in the signals originates from a reduced sensitivity of the magnetometer at higher frequency (measurements beyond the bandwidth). The simulated and experimental  $J$ -spectra yet again present an adequate agreement at both low and high frequencies (Fig. 3.5c).

The detailed results of the first-order perturbative calculation for dichlorvos and dimethyl phosphite spin systems can be found below. The values of all observable transitions are presented in Tables 3.1 and 3.2. Note that not all of the transitions in the case of dichlorvos are visible in the experimental spectra, as explained above.

Table 3.1: Frequencies ( $\nu$ ) of the allowed transitions between the energy levels characterized with the quantum numbers  $F$  and  $F_T$ , corresponding to the dominant spin subsystem  $\text{XA}_6$ , and the total spin  $F_T$  of the whole  $(\text{XA}_6)\text{B}$  system, obtained using the first-order perturbation theory. The  $J$ -couplings used for the perturbative calculations are  $J_{\text{PH}}^{\text{A}} = 11.4 \text{ Hz}$  and  $J_{\text{PH}}^{\text{B}} = 5.2 \text{ Hz}$ . Adapted from Ref. [58].

| $F_T$         | $F$               | $\nu$                      | $\nu \text{ (Hz)}$ |
|---------------|-------------------|----------------------------|--------------------|
| $0 \mapsto 1$ | $1/2 \mapsto 1/2$ | $J_{XB}$                   | 5.2                |
| $1 \mapsto 2$ | $1/2 \mapsto 3/2$ | $1/3 J_{XB} + 3/2 J_{XA}$  | 18.8               |
| $1 \mapsto 1$ | $1/2 \mapsto 3/2$ | $-1/3 J_{XB} + 3/2 J_{XA}$ | 15.4               |
| $0 \mapsto 1$ | $1/2 \mapsto 3/2$ | $-2/3 J_{XB} + 3/2 J_{XA}$ | 13.6               |
| $1 \mapsto 0$ | $1/2 \mapsto 1/2$ | $1/3 J_{XB}$               | 1.7                |
| $1 \mapsto 2$ | $3/2 \mapsto 3/2$ | $2/3 J_{XB}$               | 3.5                |
| $2 \mapsto 3$ | $3/2 \mapsto 5/2$ | $2/5 J_{XB} + 5/2 J_{XA}$  | 30.6               |
| $2 \mapsto 2$ | $3/2 \mapsto 5/2$ | $-1/5 J_{XB} + 5/2 J_{XA}$ | 27.5               |
| $1 \mapsto 2$ | $3/2 \mapsto 5/2$ | $-2/5 J_{XB} + 5/2 J_{XA}$ | 25.4               |
| $2 \mapsto 3$ | $5/2 \mapsto 5/2$ | $3/5 J_{XB}$               | 3.1                |
| $2 \mapsto 1$ | $3/2 \mapsto 3/2$ | $2/5 J_{XB}$               | 2.1                |
| $3 \mapsto 4$ | $5/2 \mapsto 7/2$ | $3/7 J_{XB} + 7/2 J_{XA}$  | 42.1               |
| $3 \mapsto 3$ | $5/2 \mapsto 7/2$ | $-1/7 J_{XB} + 7/2 J_{XA}$ | 39.2               |
| $2 \mapsto 3$ | $5/2 \mapsto 7/2$ | $-4/7 J_{XB} + 7/2 J_{XA}$ | 36.9               |
| $3 \mapsto 4$ | $7/2 \mapsto 7/2$ | $4/7 J_{XB}$               | 3.0                |
| $3 \mapsto 2$ | $5/2 \mapsto 5/2$ | $3/7 J_{XB}$               | 2.2                |

For molecules with more complex spin topologies, the prediction of spectral patterns via the perturbation theory might be challenging. Dimethyl methylphosphonate, being the  $(\text{XA}_6)\text{B}_3$  spin system is shown as an example for complex  $^1\text{H}$ - $^{31}\text{P}$  spin systems (Fig. 3.6a). This chemical is composed of a single  $^{31}\text{P}$  spin, three equivalent  $^1\text{H}$  spins

Table 3.2: Frequencies  $\nu$  of the allowed transitions between the energy levels characterized with the quantum numbers  $F$  and  $F_T$ , corresponding to the dominant spin subsystem XA, and the total spin  $F_T$  of the whole (XA) $B_6$  system, obtained using the first-order perturbation theory. The  $J$ -couplings used for perturbative calculation are  $J_{PH}^A = 695$  Hz and  $J_{PH}^B = 11.7$  Hz. Adapted from Ref. [58].

| $F_T$         | $F$           | $\nu$                  | $\nu$ (Hz) |
|---------------|---------------|------------------------|------------|
| $0 \mapsto 1$ | $0 \mapsto 1$ | $J_{XA}$               | 695.0      |
| $1 \mapsto 2$ | $0 \mapsto 1$ | $1/2 J_{XB} + J_{XA}$  | 700.9      |
| $1 \mapsto 1$ | $0 \mapsto 1$ | $-1/2 J_{XB} + J_{XA}$ | 689.2      |
| $1 \mapsto 0$ | $0 \mapsto 1$ | $-J_{XB} + J_{XA}$     | 683.3      |
| $0 \mapsto 1$ | $1 \mapsto 1$ | $1/2 J_{XB}$           | 5.85       |
| $1 \mapsto 2$ | $1 \mapsto 1$ | $J_{XB}$               | 11.7       |
| $2 \mapsto 3$ | $0 \mapsto 1$ | $J_{XB} + J_{XA}$      | 706.7      |
| $2 \mapsto 2$ | $0 \mapsto 1$ | $1/2 J_{XB} + J_{XA}$  | 689.2      |
| $2 \mapsto 1$ | $0 \mapsto 1$ | $-3/2 J_{XB} + J_{XA}$ | 677.5      |
| $1 \mapsto 2$ | $1 \mapsto 1$ | $J_{XB}$               | 11.7       |
| $2 \mapsto 3$ | $1 \mapsto 1$ | $3/2 J_{XB}$           | 17.6       |
| $3 \mapsto 4$ | $0 \mapsto 1$ | $3/2 J_{XB} + J_{XA}$  | 712.6      |
| $3 \mapsto 3$ | $0 \mapsto 1$ | $-1/2 J_{XB} + J_{XA}$ | 689.9      |
| $3 \mapsto 2$ | $0 \mapsto 1$ | $-2 J_{XB} + J_{XA}$   | 671.6      |
| $2 \mapsto 3$ | $1 \mapsto 1$ | $3/2 J_{XB}$           | 17.6       |
| $3 \mapsto 4$ | $1 \mapsto 1$ | $2 J_{XB}$             | 23.4       |

with two-bond couplings of  $J_{PH}^A = 17.3$  Hz, and six equivalent  $^1H$  spins with three-bond couplings of  $J_{PH}^B = 11$  Hz [75]. The proximity of heteronuclear  $J$ -coupling strength in compound influences the precision of the first-order perturbation calculation as mentioned above. In the  $J$ -spectrum of dimethyl methylphosphonate, numerous multiplets are observed in a 0-75 Hz window, resulting in a unique spectral pattern shown in Fig. 3.6b. Even though the elucidation of each peak in  $J$ -spectra of such compounds is not simple, their specific spectral patterns can be used for chemical fingerprinting by serving our primary goal. The consistency of the  $J$ -spectral pattern is crucial for the reliability of the chemical fingerprinting determined by the zero-field NMR technique. To test it, dimethyl methylphosphonate was purchased from two different suppliers and measured many times using three different experimental zero-field NMR setups. Subsequent to employing two different software for post-processing, we obtained the same spectral pattern and proved the applicability of zero-field NMR as a tool for chemical fingerprinting and pattern recognition.

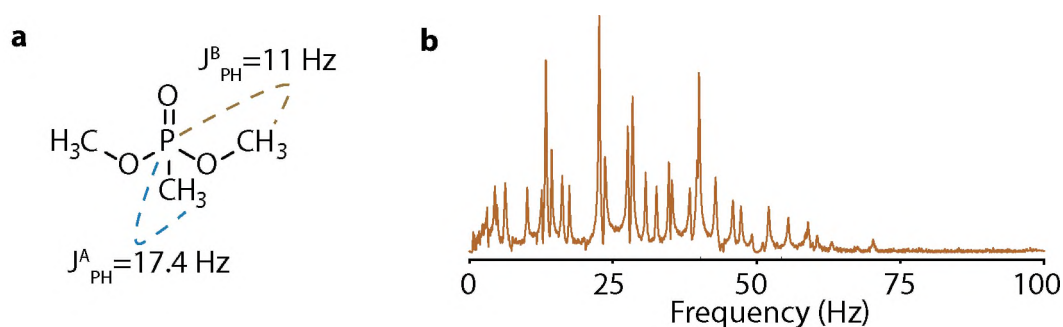


Figure 3.6: (a) Structural formula of dimethyl methylphosphonate with the heteronuclear  $J$ -couplings reported given in the literature [75]. (b) Experimental  $J$ -spectrum of dimethyl methylphosphonate. The experimental spectrum is the result of 512 averages.

### 3.3.3 Generating Zero-field NMR Signal in $^1\text{H}$ - $^{31}\text{P}$ Spin Systems

There are few ways to generate zero-field NMR signals as explained at length in Sec. 2.2  $^1\text{H}$ - $^{13}\text{C}$  spin systems. In this subsection, the generation of zero-field NMR signals originating from  $^1\text{H}$ - $^{31}\text{P}$  spin systems is comprehensively studied for different experimental sequences.

Let us consider a zero-field NMR experiment of thermal-prepolarized sample that undergoes non-adiabatic transfer from high field (polarization field) to the ultralow field (guiding field). Since in our setup, the NMR tube is positioned above a sensor, i.e., along the  $z$ -direction that is a magnetometer sensitivity axis, we analyze only the detection of the  $z$ -component of magnetization  $\hat{M}_z$ . In this case, after the transfer of the NMR sample to the detection region (inside the shield), the guiding field applied by the solenoid is suddenly switched off. Then the NMR signal is either acquired immediately (Fig. 2.11a) or the signal acquisition follows a transverse DC pulse (Fig. 2.11b).

The calculation of the optimum transverse pulse angle is discussed in Sec. 2.2 for the  $^1\text{H}$ - $^{13}\text{C}$  spin system. If we use the same argument for the  $^1\text{H}$ - $^{31}\text{P}$  spin system, where  $\gamma_P/\gamma_H \approx 2/5$ , the optimal condition is achieved when rotation phase is  $\theta_H \approx 5\pi$  and  $\theta_P \approx 2\pi$ .

As derived in Ref. [17] and also in Sec. 2.2, applying the transverse DC pulse enhances the NMR signal by  $\frac{(\gamma_X + \gamma_A)}{(\gamma_X - \gamma_A)}$  for the XA spin system, compared to the NMR signal obtained without the transverse pulse. Therefore, for the  $^1\text{H}$ - $^{31}\text{P}$  system, the achievable maximum

signal enhancement is

$$\frac{(\gamma_H + \gamma_P)}{(\gamma_H - \gamma_P)} \approx 2.36. \quad (3.1)$$

To support our inference and calculations, we simulate zero-field NMR signals of the  $^1\text{H}$ - $^{31}\text{P}$  system using the SpinDynamica package for Mathematica [19]. The calculations are performed using a finite-length pulse with an amplitude of 50  $\mu\text{T}$ , which closely matches the experimental conditions (in the simulation, the  $J$ -coupling evolution is present during the pulse). The amplitudes of such signals for different pulse lengths are shown as red points in Fig. 3.7a. The signal-pulse dependence is then compared with a more general analytical formula (solid line in Fig. 3.7a):

$$M_z(\theta_H) \propto (\gamma_H + \gamma_P) \left( \cos \theta_H - \cos \frac{\gamma_P}{\gamma_H} \theta_H \right) + (\gamma_H - \gamma_P) \left( \cos \theta_H + \cos \frac{\gamma_P}{\gamma_H} \theta_H \right), \quad (3.2)$$

derived in Ref. [76] for a similar  $^1\text{H}$ - $^{13}\text{C}$  spin system as mentioned in Sec. 2.2. Figure 3.7b shows simulated spectra for a simple  $^1\text{H}$ - $^{31}\text{P}$  system, following the sudden transition to the zero field with and without the optimal pulsing. The experimental signal enhancement using a transverse DC pulse obtained for trimethyl phosphate is shown in Fig. 3.7c.

### 3.3.4 Limitations and Outlook

A major limitation of measurements presented in this work is the low thermal polarization provided by the permanent magnet and hence low amplitudes of the observed ZULF NMR peaks. This implies application of highly concentrated samples or, in many cases, averaging over many transients (long measurement time). For instance, the detectable concentration limit of the molecules in this work is about 1 M in a single scan. This limitation needs to be overcome to be able to obtain zero-field spectra of diluted phosphorus compounds, e.g., such as those present in biological samples. The hyperpolarization methods such as PHIP [46, 47] and SABRE [6, 53], but also dynamic nuclear polarization (DNP) [77], etc., which already have been combined with zero-field NMR spectroscopy, have the potential to facilitate the observation of the important molecules in living cell metabolism [55, 78, 79]. In this context, hyperpolarization, providing about

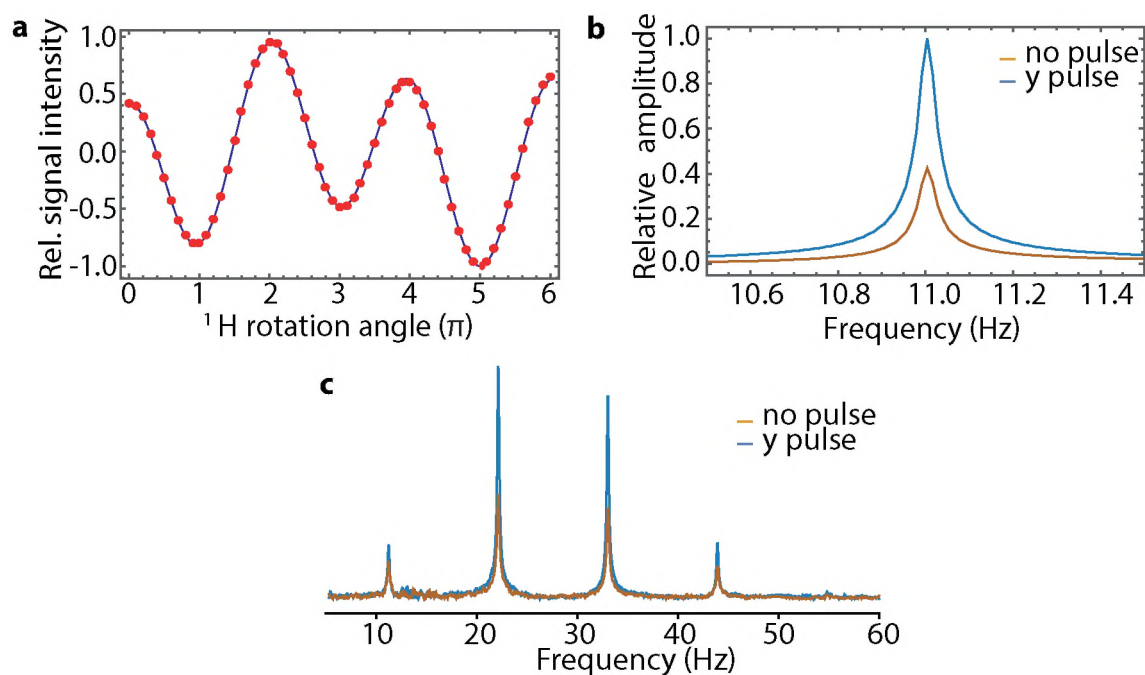


Figure 3.7: (a) Amplitude of the zero-field NMR signal of the  $^1\text{H}$ - $^{31}\text{P}$  nuclear system versus the  $^1\text{H}$  rotation angle  $\theta_H$  induced by the field applied along the  $y$ -axis. The red dots are results of numerical simulations (SpinDynamica), while the solid line corresponds to the signal amplitude determined based on Eq. (3.2). The signal is normalized to the maximum amplitude. (b) Simulated  $J$ -spectra of the  $^1\text{H}$ - $^{31}\text{P}$  spin system with  $J_{\text{PH}} = 11$  Hz, following sudden transition to zero-field with/without the optimum  $y$ -pulse. (c) Experimental  $J$ -spectra of trimethyl phosphate obtained after the sudden transition to zero-field with/without optimum the  $y$ -pulse. The spectra are the result of 32 averages. Adapted from Ref. [58].

four orders of magnitude signal enhancement, reduces the detectable concentration limit to under the millimolar level [53]. The hyperpolarization of  $^{31}\text{P}$  nuclei in phosphocreatine and inorganic phosphate was shown in physiological aqueous solution by dissolution DNP [80]. In addition,  $^{31}\text{P}$  hyperpolarization in a phosphine, a phosphine oxide, a phosphine sulfide, and a phosphonate ester, achieved using SABRE, resulted in a substantial signal enhancement [81, 82].

Another drawback of the presented approach is the finite bandwidth of the magnetometer, which reduces sensitivity of measurements of phosphorus compounds with stronger one-bond  $^1\text{H}$ - $^{31}\text{P}$   $J$ -couplings (see Fig. 3.5c). This limitation may be addressed by detecting an oscillating field in a given frequency range. Numerous atomic-magnetometer schemes allow to detect oscillating magnetic fields in a tunable frequency range [83, 84].

### 3.4 Conclusion

The organophosphorus compounds were investigated using a novel modality of NMR, zero-field  $J$ -spectroscopy.  $J$ -spectra of basic organophosphorus molecules were analyzed using straightforward interpretation methods and demonstrated the ability of chemical identification of compounds forming spin systems of the same spin topologies and only small differences in  $J$ -coupling constants (at a level of 0.5 Hz). In addition to the first measurements of zero-field spectra of the  $^{31}\text{H}$ - $^{31}\text{P}$  systems, the broadest frequency-range  $J$ -spectrum measurements were demonstrated. This allowed us to investigate the chemicals with weak and strong  $J$ -couplings in a single transient. A good agreement between high-spectral-resolution measurements and numerical simulations confirmed that the zero-field NMR can be used to identify the chemical fingerprinting of phosphorus-containing compounds. However, the analysis and identification of more complex molecules might require additional tools (e.g. a dedicated software) to facilitate further investigations.

Zero-field NMR techniques, with their promise of portability and low cost, combined with the ability to study naturally abundant phosphorus compounds, can be attractive for the cell metabolism studies. In particular, the use of zero-field NMR either to assess

energy metabolism via measurement of the important phosphorylated metabolites such as ATP, phosphocreatine, and glucose-phosphate or to evaluate tumor differentiation by analysis of phospholipids is an exciting subject for future research [64, 66, 85, 86].

# Chapter 4

## Zero-field NMR of Biomolecules: Spin Topology Modification Under Chemical Exchange

The work in this chapter is based on material presented in the following publication [87]:

**S. Alcicek**, P. Put, D. Barskiy, V. Kontul, and S. Pustelny, "Zero-Field NMR of Urea: Spin-Topology Engineering by Chemical Exchange", *The Journal of Physical Chemistry Letters* **12**, 10671–10676 (2021).

### 4.1 Motivation and Overview

As chemical exchange alters spin-spin couplings and NMR relaxation rates, ZULF NMR is capable of monitoring this process. In particular, chemical reactions (bond breaking, bond making) or conformational modifications (bond rotation) of molecules can be investigated [77]. Until now, the application of ZULF NMR has been demonstrated in the context of biomolecules consisting of 2–5 coupled nuclear spins [4, 7]. However, for larger spin systems, ZULF NMR spectra become complicated as a result of the increased number of coupled nuclei, making the spectral analysis challenging. In this chapter, the study reporting various approaches to modifying and simplifying zero-field spectra of molecules consisting of a large number of spins, some of which undergo chemical exchange, is presented. Specifically, ZULF NMR *J*-spectroscopy is used to investigate solutions of urea, a molecule with a large coupling network and exchangeable protons.

Urea is a prominent biomolecule that plays a key role in amino-acid and protein metabolism, enabling 80-90% of nitrogen excretion from human body. It is produced

in the liver through the urea cycle, transported through the bloodstream, and excreted into urine by the kidneys [88]. Therefore, measuring the urea level in urine and blood is a routinely used medical diagnostic technique to examine liver and kidney function [89–91]. Furthermore,  $[^{13}\text{C}]$ -urea and  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea have recently become attractive contrast agents for magnetic resonance imaging, as urea is a highly biocompatible and valuable marker for the evaluation of myocardial perfusion and renal physiology. [92–94]. Finally, interest in urea is also stimulated by a growing demand for robust and reliable compound detection in fields such as environmental monitoring, agricultural and food chemistry [95].

In this study,  $[^{15}\text{N}_2]$ -urea and  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea are investigated in various solution environments by observing an alteration in the zero-field NMR  $J$ -spectra. First, the effect of the proton-exchange process on spectra is reported by measuring  $[^{15}\text{N}_2]$ -urea and  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea in an aprotic solvent, dimethyl sulfoxide (DMSO), and a protic solvent, water ( $\text{H}_2\text{O}$ ). Since the proton-exchange rate in urea is both acid- and base-catalyzed, aqueous solutions of urea at various pH levels are studied. The results are explained by zero-field NMR simulations, considering the combined effects of chemical exchange and nuclear spin dynamics using a simple theoretical model.  $[^{15}\text{N}_2]$ -urea was also measured in mixtures of  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  to study the influence of deuterium nuclei on zero-field  $J$  spectra. The experimental findings are supported by simulations that take into account the proportion of  $^1\text{H}/\text{D}$  isotopologues of urea in solution. By analyzing the energy-level structures of isotopologues using a perturbation theory, all spectral lines, arising from  $J$ -coupling interactions ( $^{15}\text{N}-^1\text{H}$ ,  $^{15}\text{N}-\text{D}$ , and  $^1\text{H}-\text{D}$ ) in spin subsystems, are identified. Based on the results, straightforward ways to study complex biomolecules with ZULF NMR are possible by taking advantage of the chemical-exchange process. Lastly, this approach is used to study zero-field NMR spectra of amino acids.

## 4.2 Material and Methods

### 4.2.1 Sample Preparation

For the measurements presented, all chemicals were purchased from Sigma-Aldrich and used without further purification.  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea (CAS# 58069-83-3) and  $[^{15}\text{N}_2]$ -urea (CAS# 2067-80-3) solutions at various pH values were prepared in 8 M concentration by dissolving them in sodium hydroxide (CAS# 1310-73-2) or hydrochloric acid (CAS# 7647-01-0). The pH of each sample was measured at room temperature by a portable pH meter (Mettler Toledo Seven2Go) with a micro electrode (Mettler-Toledo InLab Pro-ISM). For the study of the  $^1\text{H}$ -D exchange, 8 M  $[^{15}\text{N}_2]$ -urea solutions were prepared by dissolving urea in distilled water,  $\text{D}_2\text{O}$  (CAS# 7789-20-0), and 25%, 50%, 75% distilled water- $\text{D}_2\text{O}$  (CAS# 7789-20-0) mixtures. For the preparation of urea solutions in an aprotic solvent,  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea (CAS# 58069-83-3) and  $[^{15}\text{N}_2]$ -urea were dissolved in DMSO (CAS# 67-68-5) with a final concentration of 5.4 M.

For the amino-acid study, 3 M  $[2\text{-}^{13}\text{C}]$ -glycine (CAS# 20220-62-6) solution was prepared by dissolving the compound in distilled water.  $[2\text{-}^{13}\text{C}]$ -L-alanine (CAS# 62656-85-3) and  $[2,3\text{-}^{13}\text{C}_2]$ -L-alanine were dissolved in a diluted hydrochloric acid (CAS# 7647-01-0) solution making 2 M alanine solutions with pH of 2.38.

Each sample (0.15 ml) was placed inside a standard 5 mm NMR tube and then flame sealed under vacuum ( $<10^{-4}$  mbar) following degassing by several freeze-pump-thaw cycles (see Sec. 3.2.1 for detail regarding the sample preparation).

### 4.2.2 Zero-field NMR Experiments

While a comprehensive description of the experimental setup can be found in Sec. 3.2.2, here we just recall its crucial elements. The NMR samples were thermally polarized for 20 s using the 1.8 T Halbach magnet placed above the magnetic shield, and mechanically shuttled into the zero-field detection region, where the magnetic field of the sample is measured using the home-built alkali-vapor atomic magnetometer. During sample trans-

portation, which takes about 300 ms (plus an additional 700 ms delay after reaching the final position), a guiding field of 10  $\mu$ T is applied by a solenoid wrapped along the whole length of the shuttling path. When the sample reaches the detection region, the guiding field is turned off suddenly to generate an oscillating zero-field NMR signal [1]. Each zero-field NMR spectra is the result of averaging 2048 transients.

### 4.2.3 Data Processing and Simulations

The entire data processing was performed using Python as described in Sec. 3.2.3. A high-performance spin simulation library **Spintrum** was employed to simulate zero-field NMR spectra through numerical diagonalization of density matrices describing spin systems [3, 26]. The  $J$ -coupling values used in the simulations were taken directly from the literature or estimated using the gyromagnetic ratios of the nuclei (see discussion below). Simulations of chemical-exchange effects on the zero-field spectra of urea were obtained using an approach presented in Ref. [77]. Details of the calculations can be found in Ref. [87]

## 4.3 Results and Discussion

### 4.3.1 Zero-field $J$ -spectra of Urea in Protic and Aprotic Solvents

The urea molecule consists of two  $-\text{NH}_2$  groups joined by a carbonyl ( $\text{C}=\text{O}$ ) functional group. To analyze the general structure of  $J$ -spectra of  $^{15}\text{N}$ -urea and  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea, first, numerical simulations were performed using  $J$ -coupling constants presented in Fig. 4.1 and taken from Refs. [96, 97]. Since one-bond  $^1\text{H}$ - $^{15}\text{N}$  coupling in the  $-\text{NH}_2$  group, marked by a dashed line in Fig. 4.1, is the strongest interaction in this system, the main features in the  $J$ -spectra of both isotopologues of urea are centered around  $(3/2)|^1J_{\text{NH}}| \approx 133.65$  Hz. This is expected for an  $\text{XA}_2$  nuclear spin system, corresponding to the transitions in the manifold with the total proton spin  $F$  of a unity (see Sec. 2.2). Hereafter, we refer to this group of peaks as high-frequency peaks. Weaker homonuclear and heteronuclear interactions result in the appearance of low-frequency peaks ( $<10$  Hz),

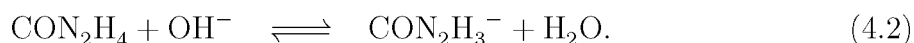
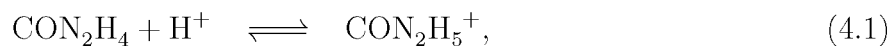
as well as further modifications (splitting and shifting) of the high-frequency peaks. The presence of an additional, relatively strong  $^{13}\text{C}$ - $^{15}\text{N}$  interaction in  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea increases the shifts in corresponding energy manifolds, giving rise to a wider span of low- and high-frequency peaks compared to the spectrum of  $[^{15}\text{N}_2]$ -urea.

In Fig. 4.1, the  $J$ -spectra of  $[^{15}\text{N}]$ -urea and  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea in dimethyl sulfoxide and water are compared. The experimental spectrum of urea in DMSO agrees well with the simulation, particularly in terms of line positions. The peaks become substantially (3 times) broader when water is used as a solvent (Fig. 4.1). This influence is expected since amide protons are known to undergo chemical exchange with water protons, and this process contributes to the nuclear-spin relaxation rate [98]. Nevertheless, the mere fact of being able to observe the multiplets in  $J$ -spectra indicates that the proton-exchange rate in urea at a neutral pH level is slow compared to the  $J$ -coupling evolution. This is confirmed by a measured chemical-exchange rate value of  $\approx 1.9\text{ s}^{-1}$ , being considerably slower than the dominant interaction in the system ( $^1J_{\text{NH}}$ ) [98].

Since, at truly zero field, water contributes only to a ZULF NMR spectrum via a peak at 0 Hz (absence of heteronuclear coupling), the water signal does not overlap with high-frequency part of the  $J$ -spectra of target molecules. This feature makes zero-field NMR an attractive modality for the analysis of biological samples with a high concentration of water (e.g., blood, urine, or cell cultures), eliminating the necessity for water solvent suppression [99].

### 4.3.2 Effects of pH-dependent Chemical Exchange on Zero-field Spectra

The proton-exchange process in aqueous solutions of urea is pH-dependent and both acid- and base-catalyzed. These two variants of the process can be written as [100–103]:



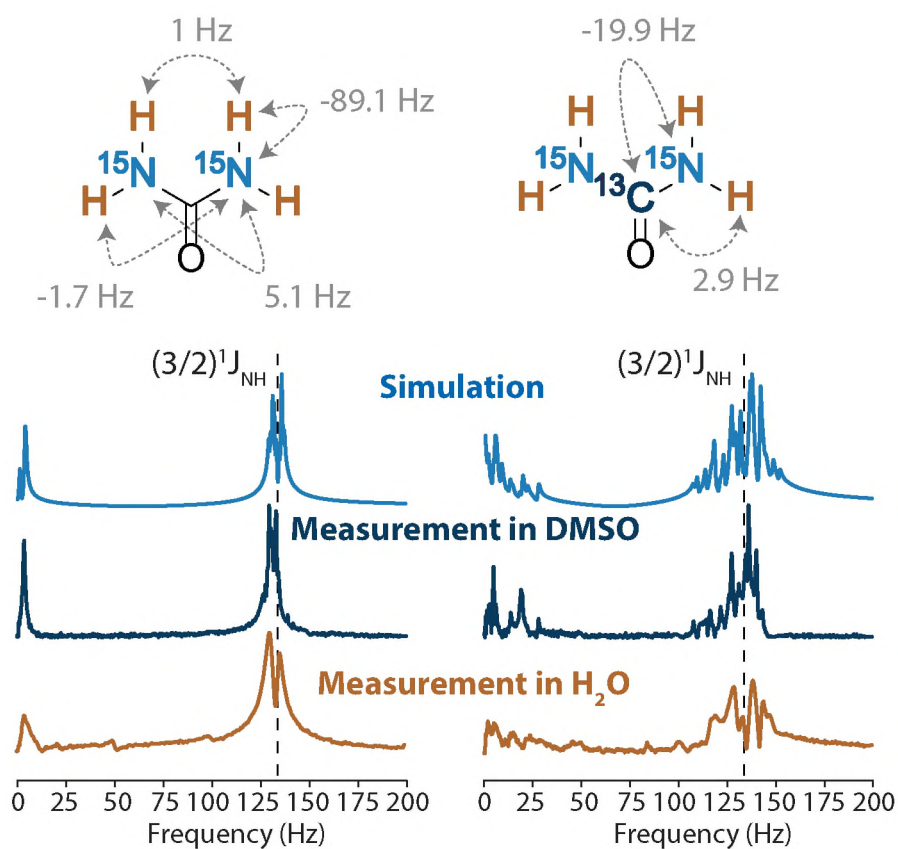


Figure 4.1: Simulated and experimental zero-field spectra of  $^{15}\text{N}_2$ -urea and  $^{13}\text{C}, ^{15}\text{N}_2$ -urea in aprotic (DMSO) and protic ( $\text{H}_2\text{O}$ ) solvents. The structural formulas are shown along with the  $J$ -coupling constants used for the simulations. Adapted from Ref. [87].

For the presented pH study, the effect of the proton-exchange rate on  $J$ -spectra was studied by varying pH of the solution while maintaining the same concentration of urea (8 M). As shown in Fig. 4.2, due to the increased proton-exchange rate, the amplitudes of high-frequency peaks (120–150 Hz) observed in the spectra gradually decrease without considerable line broadening at both low and high pH values. It is evident that, when the proton-exchange rate was much higher than the  $J$ -coupling ( $k_{\text{ex}} \gg J_{\text{NH}}$ ), the  $^1\text{H}$  nuclei were effectively decoupled from the rest of the spin system and the  $J_{\text{NH}}$ -coupling did not contribute to the observed zero-field spectrum. Thereby, the high-frequency peaks vanished in the spectra of highly acidic (pH = 1.4) and highly basic (pH = 14) solutions. This effect was also confirmed by simulations of the urea spin system (Fig. 4.2). The disappearance of high-frequency peaks under highly acidic/basic conditions resembles the results reported in a work on zero-field NMR of ammonium under highly acidic conditions [77]. The authors of the work demonstrated that an increase in the proton-exchange rate resulted in the vanishing of the zero-field NMR signals. This was explained by the nature of the experiment; after thermal prepolarization, a sample spends a significant amount of time (1 s) in the low-field region (tens of  $\mu\text{T}$ ) before being detected in zero field. In the experiment, the time between prepolarization and signal acquisition was down-limited due to the necessity to reduce vibration noise, stemming from NMR-tube transport, which had a detrimental effect on the spectra. For such a delay, the water protons depolarized despite a guiding field of 10  $\mu\text{T}$ . In the case of a faster proton exchange, unpolarized protons are more often involved in the chemical-exchange process. This affects the “memory” of nuclear spin orders, causing a reduction in the peak amplitudes.

To investigate the influence of the guiding-field strength on the peak amplitudes, we applied a magnetic field along the shuttling path and varied its magnitude from 0.01 mT to 0.1 mT (note that outside of the magnetic shield, the Earth’s magnetic field coincided with the solenoid guiding field, effectively increasing the magnitude of transfer field, but inside the shield, the Earth’s magnetic field is completely attenuated). As shown in Fig. 4.3, increasing the field strength by an order of magnitude results in  $\approx 25\%$  enhancement of the signal due to the slower proton relaxation time  $T_1$  (Fig. 4.3). In this way, we verified

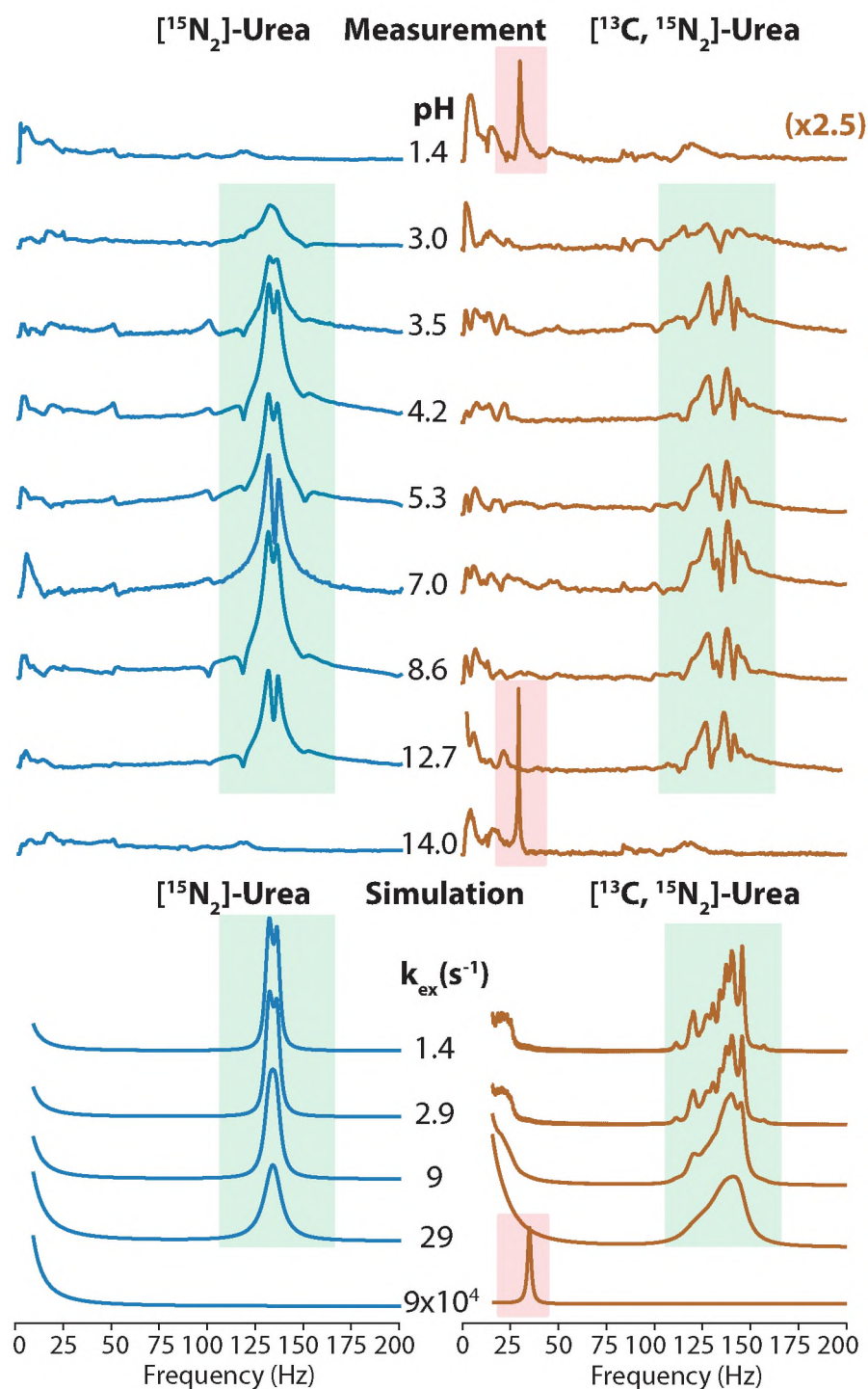


Figure 4.2: Experimental (top) and simulated (bottom) zero-field  $J$ -spectra of  $[\text{N}_2^{15}]\text{-urea}$  and  $[\text{C}^{13}, \text{N}_2^{15}]\text{-urea}$  in aqueous solutions at various pH values. The lines arising from mainly one-bond, strong  $J$ -coupling interaction between  $^{15}\text{N}$  and  $^1\text{H}$  are green shaded (120–150 Hz), while the narrow peaks (around 30 Hz), originating from one-bond  $J$ -coupling between  $^{13}\text{C}$  and  $^{15}\text{N}$ , are highlighted in red. Adapted from Ref. [87].

that guiding-field strength had a significant influence on the amplitude of the zero-field NMR signal of molecules under the chemical exchange.

The boost of the signal amplitude in the stronger guiding field is predicted to be universal for molecules under rapid chemical exchange and can be exploited in measuring zero-field spectra of such molecules. The enhancement in the signal, originating from a stronger transfer field, may not only be beneficial for remote prepolarization experiments, but also find use in ZULF hyperpolarization techniques, which are based on chemical exchange [45, 56]; a stronger magnetic field slows relaxation of protons in a solution, which yields higher signal amplitudes of zero-field NMR signals.

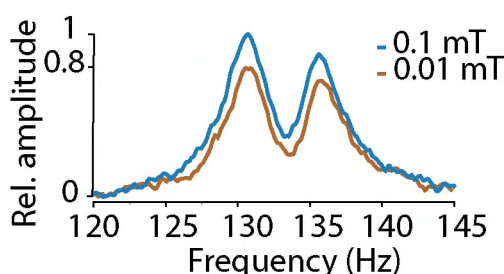


Figure 4.3: Zero-field  $J$ -spectra of  $[^{15}\text{N}_2]$ -urea solutions measured for guiding fields of 0.01 mT and 0.1 mT. NMR signals were acquired after the field was switched off. Adapted from Ref. [87].

On the other hand, a rapid proton exchange leads to a modification of the effective spin system, which can thoroughly facilitate the interpretation of the obtained spectra. This was demonstrated in the spectra of  $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea in highly acidic and basic solutions (red boxes in Fig. 4.2), where a narrow peak arose at about 30 Hz. This signal appeared at  $(3/2)J_{\text{CN}}$  and originated from the  $J$ -coupling between  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclei in the  $\text{CN}_2$  spin system, where, due to fast exchange, the protons were effectively decoupled from the rest of the spin system. The emergence of the low-frequency peak was also supported by the numerical simulations for the spin system under rapid chemical exchange, as shown at the bottom of Fig. 4.2. It should be emphasized that, by taking advantage of the accelerated chemical exchange, a narrower single peak (1 Hz width) with higher amplitude arose in the zero-field spectrum due to a modification of the spin topology from the complex  $\text{XAB}_2\text{A}'\text{B}'_2$  system to the simple  $\text{XA}_2$  system. This simplified spectrum still relied on the molecule-specific combination of the  $J$ -coupling strength and coupling pattern, enabling

chemical fingerprinting.

### 4.3.3 Zero-field $J$ -spectra of Deuterium Isotopologues of Urea

As the next stage of the study,  $^1\text{H}/\text{D}$  isotopologues of urea were investigated. In such a case, the spin coupling network was modified, replacing  $^1\text{H}$  (spin-1/2) with deuterium (spin-1), by dissolving urea in  $\text{D}_2\text{O}/\text{H}_2\text{O}$  mixture. To simulate the spectra of urea solutions with various  $\text{D}_2\text{O}/\text{H}_2\text{O}$  ratios, the proportion of each isotopologue in the solution was calculated using a binomial distribution. As the probability of an amide-proton site being occupied by deuterium depends on the fraction  $p$  of deuterium in the solution, the molar fraction  $x$  of each isotopologue is given by:

$$x = \binom{n}{k} p^k (1-p)^{n-k}, \quad (4.3)$$

where  $n$  is the number of possible sites where deuterium nuclei can reside and  $k$  is the number of deuterium nuclei that each isotopologue contains. The simulation spectra of all isotopologues shown in Fig. 4.4 were then summed after weighing each spectrum with an appropriate binomial coefficient. The  $^{15}\text{N}$ -D coupling constants were estimated using the appropriate  $J_{\text{NH}}$  constants and the gyromagnetic ratios of deuterium and hydrogen, where the  $J_{\text{ND}}$  coupling constant was equal to  $J_{\text{ND}} \approx (\gamma_{\text{D}}/\gamma_{\text{H}})J_{\text{NH}}$ . This approach neglected secondary isotope effects, but still offered the good approximation [104]. As shown in Fig. 4.5, a good agreement was obtained between the experimental spectra of urea solutions with the various ratios of  $\text{D}_2\text{O}/\text{H}_2\text{O}$  and their simulated counterparts.

In the analysis of  $J$ -spectra of urea  $^1\text{H}/\text{D}$  isotopologues, two nitrogen atoms were treated as magnetically equivalent. Hence the isotopologues consist of three different spin subsystems:  $-\text{NH}_2$  – an  $\text{XA}_2$  spin system,  $-\text{NHD}$  – an  $(\text{XA})\text{B}$  spin system, and  $-\text{ND}_2$  – an  $\text{XB}_2$  spin system. As shown in Fig. 4.6, the lines originating from  $-\text{NH}_2$  and  $-\text{NHD}$  groups are predicted using the first-order perturbation theory (for detailed calculation, see Table 4.1). It should also be noted that signals from the  $-\text{ND}_2$  group were not observed in the spectra. This resulted from the fact that the amplitude of the zero-field NMR signal

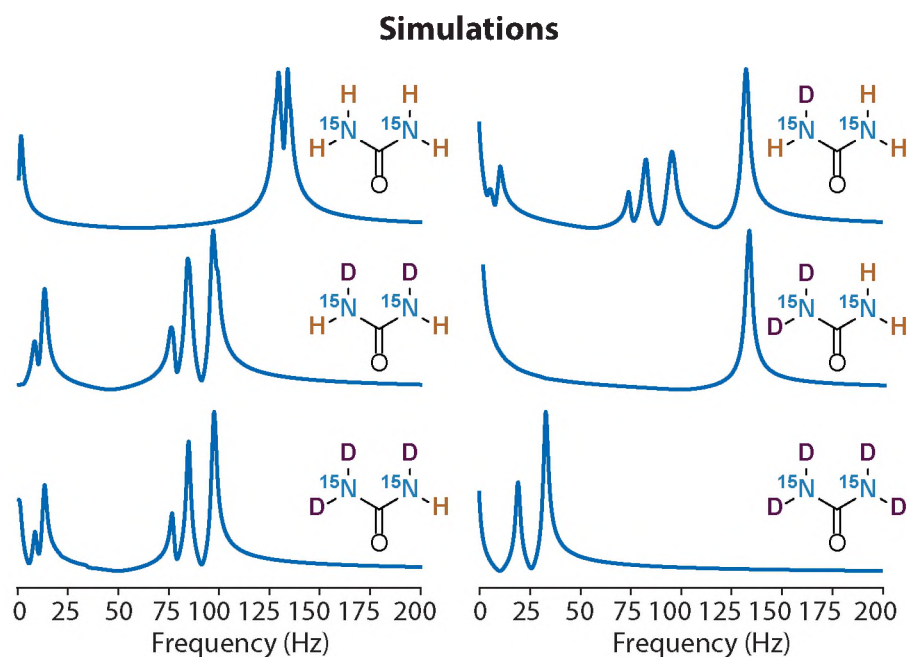


Figure 4.4: Simulated zero-field  $J$ -spectra of deuterated  $[^{15}\text{N}_2]$ -urea isotopologues with corresponding structural formulas. A unique zero-field spectra of isotopologues were averaged with an appropriate weight to generate the resultant spectrum (see main text). Adapted from Ref. [87].

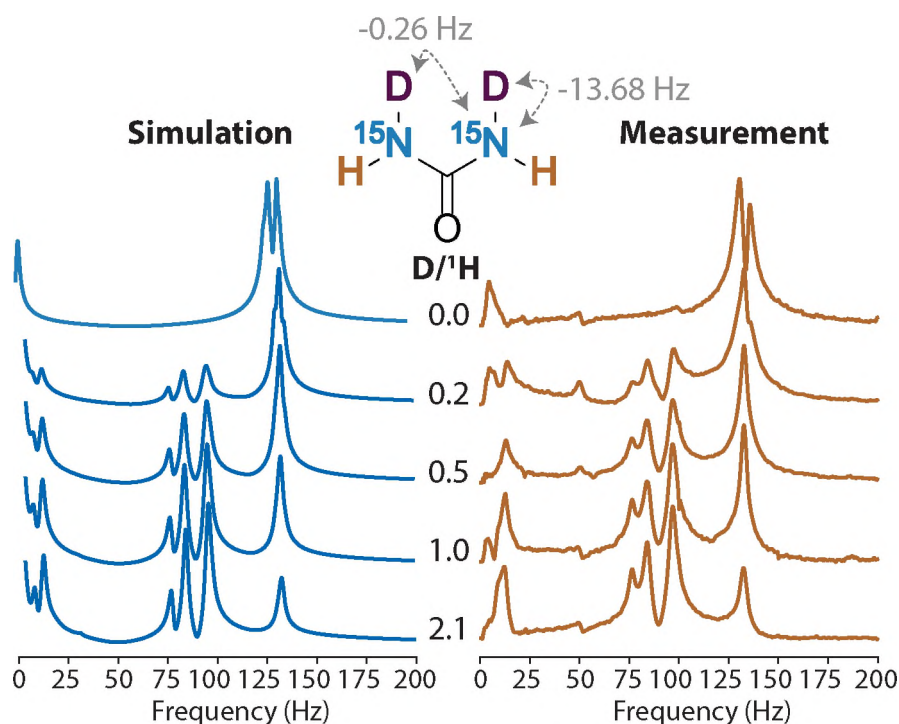


Figure 4.5: Experimental and simulated zero-field  $J$ -spectra of  $[^{15}\text{N}_2]$ -urea in aqueous solutions with various  $^1\text{H}/\text{D}$  ratios. The  $J$ -coupling values used in simulations are given with the chemical structures of a representative  $^1\text{H}/\text{D}$  isotopologue of urea. Adapted from Ref. [87].

was proportional to the square of the difference between the gyromagnetic ratios of the  $J$ -coupled nuclei [1], which equals to  $(\gamma_D - \gamma^{15N})^2/(\gamma^1H - \gamma^{15N})^2 \approx 0.0025$ .

Table 4.1: Allowed transition frequencies ( $\nu$ ) between levels characterized with the quantum numbers  $F$  and  $F_T$ , obtained using first-order perturbation theory, in the (XA)B spin subsystem. The last column gives the numerical values of the corresponding transitions in (NH)D group, using the  $J$ -coupling constants of  $J_{NH} = 89.1$  Hz and  $J_{ND} = 13.68$  Hz [96, 97]. The table is taken from Ref. [87]

| $F_T \mapsto F'_T$ | $F \mapsto F'$ | $\nu_{(XA)B}$         | $\nu_{(NH)D}$ (Hz) |
|--------------------|----------------|-----------------------|--------------------|
| $1 \mapsto 0$      | $0 \mapsto 1$  | $-J_{XB} + J_{XA}$    | 75.42              |
| $1 \mapsto 1$      | $0 \mapsto 1$  | $-1/2J_{XB} + J_{XA}$ | 82.26              |
| $1 \mapsto 2$      | $0 \mapsto 1$  | $1/2J_{XB} + J_{XA}$  | 95.94              |
| $0 \mapsto 1$      | $1 \mapsto 1$  | $1/2J_{XB}$           | 6.84               |
| $1 \mapsto 2$      | $1 \mapsto 1$  | $J_{XB}$              | 13.68              |

In ZULF NMR, the observation of quadrupolar nuclei (spin larger than 1/2) is challenging because of their additional electric influence on nuclei reorientation that can lead to faster relaxation [8]. Some studies reported that although the  $J$ -couplings to deuterium,  $^{14}\text{N}$ , and  $^{35/37}\text{Cl}$  nuclei may not be directly visible as peaks in zero-field spectra, they may cause additional line fracturing [58, 105]. On the contrary, in the study on zero-field NMR of quadrupolar nuclei, peaks originating from  $J$ -coupling interactions of  $^1\text{H}$ -D and  $^1\text{H}$ - $^{14}\text{N}$  are presented in  $J$ -spectra of ammonium isotopologues [106]. Due to high local symmetry of  $^{14}\text{N}$ -ammonium and a relatively small electric moment of deuterium, resulting in small nuclear quadrupolar interactions, the detection of  $J$ -coupling interactions of such nuclei using zero-field NMR is feasible [8].

These findings show that zero-field NMR is qualified to detect  $^1\text{H}$ /D isotopologues of urea molecules, as well as provide information on the  $^1\text{H}$ /D ratio in solution due to the simulation of the  $J$ -spectrum. It was also demonstrated with our research that the  $J$ -spectra of complex molecules with more than two heteronuclei can be easily interpreted by analyzing the energy structure of each small spin subgroup separately.

#### 4.3.4 Zero-field $J$ -spectra of Amino acids in Aqueous Solutions

By exploiting the manipulation of the effective spin system through the chemical-exchange process, which was proven with the urea work, the zero-field NMR study was next ex-

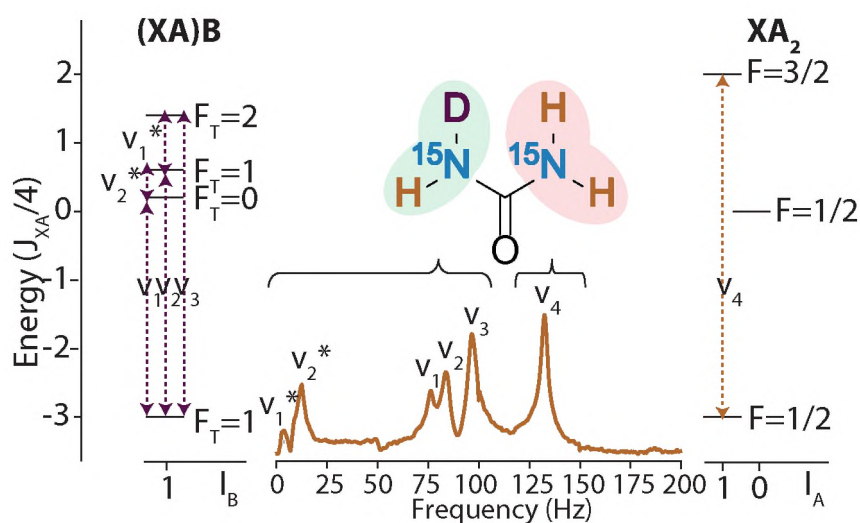


Figure 4.6: Energy-level structures of the (XA)B, and  $\text{XA}_2$  spin subsystems. High and low-frequency transitions in the (XA)B spin system are denoted by  $v_{1-3}$  and  $v_{1-2}^*$ , respectively. The transition in the  $\text{XA}_2$  spin system is represented as  $v_4$ , which is given by  $3/2J_{XA}$ . The manifolds are grouped by the quantum numbers  $I_A$  and  $I_B$  that denote the spin of the A and B nuclei, respectively. Each manifold is labelled by its total spin quantum number  $F$  or  $F_T$  (see Table 4.1 for the detailed energy-level calculations). For clarity, only a single sublevel in each manifold and a single transition at each frequency are shown. Middle: Experimental spectra of  $[^{15}\text{N}_2]\text{-urea}$  in the mixture of  $\text{D}_2\text{O}/\text{H}_2\text{O}$  (1:1). For all peaks in the spectrum, the corresponding transitions ( $v_{1-4}$ ,  $v_{1-2}^*$ ) are determined by the first-order perturbation theory. Adapted from Ref. [87].

tended to the detection of amino acids. Amino acids play an essential role in regulation and metabolism as building blocks of proteins. Proteins consist of 20 different amino acids, half of which are synthesized endogenously (non-essential), while the remaining amino acids are obtained from dietary sources (essential). The analysis of amino acid is one of the most commonly used analytical procedures [107]. It is utilized for many purposes, such as the elucidation of the structure of proteins, the diagnosis of disease states, the determination of the nutritional value of foods, etc. [108]. Thus far, the simplest amino acid, [1- $^{13}\text{C}$ ]-glycine, is the only amino acid detected using zero-field NMR with thermal prepolarization approach [7]. The zero-field spectrum of [1- $^{13}\text{C}$ ]-glycine displays a peak at low frequency ( $<10$  Hz), where detection can suffer from flicker noise.

Here, we discuss the zero-field NMR spectra of a group of  $^{13}\text{C}$ -labelled amino acids from the simplest spin systems to the more complex systems. Since one-bond  $J$ -coupling interactions between  $^{13}\text{C}$  and  $^1\text{H}$  are in the range of 120-250 Hz, where the zero-field NMR exploiting optical magnetometer provides the highest sensitivity (below  $60 \text{ fT}/\sqrt{\text{Hz}}$  for this setup), the amino acids with  $^{13}\text{C}$ -labeling at the carbon position  $\text{C}_2$  were investigated.

As the first stage of the ZULF NMR amino-acid studies, [2- $^{13}\text{C}$ ]-glycine was measured. Glycine molecule consists of a single  $^{13}\text{C}$  spin and two  $^1\text{H}$  spins with one-bond couplings  $J_{\text{CH}}^1 = 143.6 \text{ Hz}$ . In the molecule, the  $J$ -coupling interactions of exchangeable-proton groups -OH and -NH average out under rapid chemical exchange. Hence, one may predict the existence of a single peak in zero-field spectra arising at  $3/2 J_{\text{XA}}$ , which originates from the transition between the  $F=1/2$  and  $F=3/2$  state of the  $\text{XA}_2$  spin system. As shown in Fig. 4.7, the experimental and simulated spectra of [2- $^{13}\text{C}$ ]-glycine reveals good agreement.

The next amino acid is [2- $^{13}\text{C}$ ]-L-alanine being the  $\text{XAB}_3$  spin system. The zero-field NMR spectrum of this biomolecule (Fig. 2.15) could be explained based on Sec. 2.2 using first-order perturbation calculations. In its zero-field spectrum a multiplet appears in 140-155 Hz range. This multiplet originated from the single peak associated with the one-bond  $J$ -coupling interaction between  $^{13}\text{C}$  and  $^1\text{H}$  ( $J_{\text{CH}}^1 = 146.1 \text{ Hz}$ ) that was split due to additional heteronuclear two-bond couplings ( $J_{\text{CH}}^2 = -4.5 \text{ Hz}$ ) and homonuclear

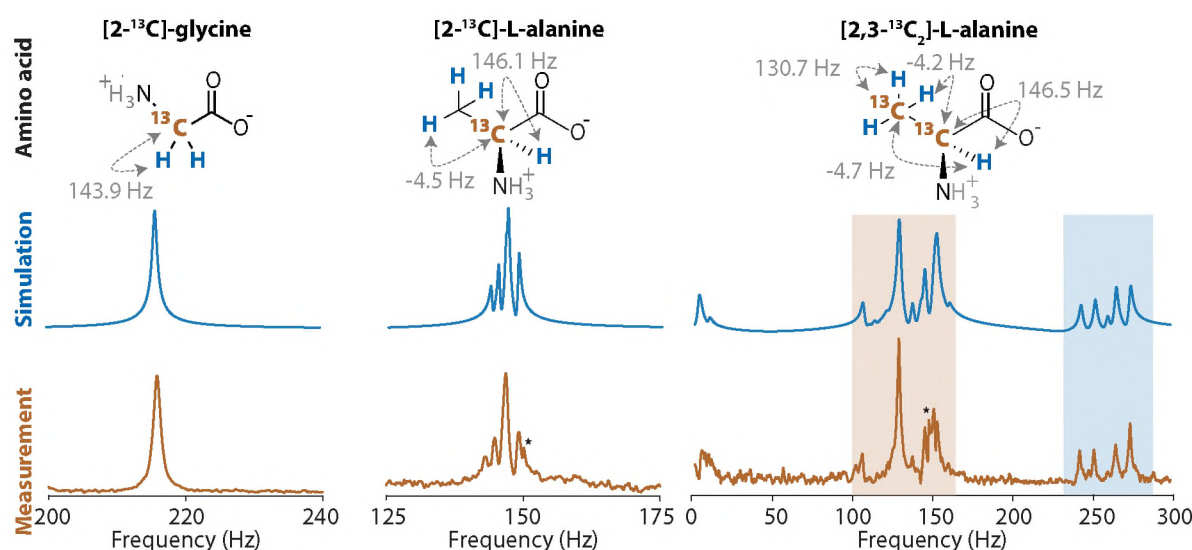


Figure 4.7: Simulated and experimental zero-field NMR spectra of  $[2-^{13}\text{C}]$ -glycine,  $[2-^{13}\text{C}]$ -alanine, and  $[2,3-^{13}\text{C}_2]$ -L-alanine in aqueous solutions. The structural formulas are shown with the  $J$ -coupling constants used in the simulations. To clear up yet not to affect the spectra of interest, AC-line noise at 50 Hz and its harmonics were filtered out during post-processing, with an exception of the 150 Hz line (the resonance is marked by black stars). The orange and blue shades in zero-field NMR spectra of  $[2,3-^{13}\text{C}_2]$ -L-alanine (100-160 Hz and 240-290 Hz frequency ranges, respectively) are used to facilitate the spectral interpretation (see main text for details).

couplings ( $J_{\text{HH}}^3 = 7 \text{ Hz}$ ) [24, 25]. This was confirmed by numerical simulations both in terms of splitting pattern and position of peaks (Fig. 4.7).

Next, L-alanine with  $^{13}\text{C}$ -labelling at the  $\text{C}_2$  and  $\text{C}_3$  carbon position, i.e.,  $[2,3-^{13}\text{C}_2]$ -L-alanine, was measured by zero-field NMR. The presence of an additional  $^{13}\text{C}$  spin (denoted by Y) modifies the spin topology, forming an  $\text{XYAB}_3$  spin system. A simple interpretation of the experimental spectrum of this compound can be as follows: In the absence of weak  $J$ -coupling interactions, the one-bond  $J$ -coupling interaction between a single  $^{13}\text{C}_2$  spin and a single  $^1\text{H}$  spin results in a main peak at 146.5 Hz, as expected from the  $\text{XA}$  spin subgroup [24, 25]. On the other hand, the one-bond  $J$ -coupling between a single  $^{13}\text{C}_3$  spin and three  $^1\text{H}$  spins produced two peaks at  $J_{\text{CH}}^1 = 130.7 \text{ Hz}$  and  $2J_{\text{CH}}^1 = 261.4 \text{ Hz}$ , as expected for the  $\text{YB}_3$  spin subgroup. In specified frequency regions, the multiplets arising due to more complex spin topology leading to splitting and shifting of the main peaks are expected. As shown in Fig. 4.7, in the L-alanine zero-field spectrum, the peaks in the 100-160 Hz frequency range (highlighted in Fig. 4.7 with orange) are determined by both  $\text{XA}$  and  $\text{YB}_3$  spin subsystems, while the peaks in the 240-290 Hz range (blue highlight in

Fig. 4.7) stem predominantly from the  $\text{YB}_3$  spin subsystem. As also shown, the simulated spectrum was in good agreement with the experimental spectrum (Fig. 4.7). Even though for such a not-too-simple system, the prediction of positions and amplitudes of all peaks using the first-order perturbation approach was time-consuming and impractical, the overall interpretation of the spectrum was straightforward and, good agreement can be obtained with numerical simulations.

## 4.4 Conclusion

To conclude, urea, one of the key biomolecules, was investigated under various solution conditions using ZULF NMR. It is reported that the compound can be readily detected in water by modifying spin topology under the chemical-exchange process. Then the research was extended to other key biomolecules with similar structures (i.e., amino acids). It is also reported that the  $J$ -spectra of complex molecules, such as urea and alanine isotopologues, can be clearly interpreted by identifying simple subgroups in the system and analysing their energy structures independently. All the experimental results are congruent with simulations, confirming our theoretical interpretation.

This study could allow future *in vivo/in vitro* investigations of complex biomolecules, especially, in biofluids with a high concentration of water such as blood, urine, etc. Specifically, one of the prominent clinical-analysis methods, the determination of urea level in urine and blood, can be a subject of the future research. On the other hand, in the presented study, highly concentrated (2-8 M), isotopically-enriched biomolecule solutions were harnessed. Even with such high concentrations, low thermal prepolarization with the use of 1.8 T magnet, results in low amplitude of zero-field NMR signals. As mentioned previously, to address this issue, zero-field NMR is combined with hyperpolarization methods such as parahydrogen-induced polarization (PHIP) [46, 47], signal amplification by reversible exchange (SABRE) [53], dynamic nuclear polarization (DNP) [77], etc. However, as these methods are limited to just a selection of molecules, widely-applicable exchange-based polarization methods such as SABRE-RELAY and PHIP-X

may be preferable for a diverse set of biomolecules [45, 56, 57, 109]. Therefore, as the next step, we used PHIP-X to hyperpolarize biomolecules such as urea, ammonium and glycine. The results of the PHIP-X study can be found in Chapter. 6.

# Chapter 5

## Zero-field NMR of Natural Abundance $^{15}\text{N}$ -Pyridine Derivatives with In Situ SABRE Hyperpolarization

### 5.1 Motivation and Overview

Despite its various advantages, ZULF NMR spectroscopy suffers from low sensitivity, originating from extremely low spin polarization achieved with thermal prepolarization by a permanent magnet (see Chapters 3 and 4). To improve sensitivity, ZULF NMR has been combined with various hyperpolarization methods such as PHIP [46], SABRE [53], d-DNP [77], SABRE-RELAY [57] as mentioned in Sec. 2.3. Among them, SABRE is of particular interest due to its cost effectiveness and ability to continuously source hyperpolarization without altering the chemical structure of the target molecule [55].

In zero-field NMR, SABRE has been only employed to detect the molecule of  $^{15}\text{N}$ -pyridine [6, 52, 53]. In this chapter, we present our research that aims at extending these studies by investigations of pyridine derivatives. The molecules are interesting because of their antimicrobial, antiviral, or anticancer properties, which are determined by the attached substitutes and their positions. Due to these properties, they play a crucial role in medicine, particularly in drug development [110, 111]. Furthermore, one of the pyridine derivatives, nicotinamide (a form of vitamin B<sub>3</sub>), is a potential contrast agent for magnetic resonance imaging due to its biocompatibility and long relaxation time [111–114].

In the presented ZULF NMR studies, investigations of  $^{15}\text{N}$ -pyridine derivatives with the same substitute attached at different positions are presented. Additionally, the

molecules with various substitutes placed at given positions on pyridine ring were measured. To be able to perform the measurements, an experimental setup was constructed. It provides detection of signals with a high signal-to-noise ratio by repeatable/long-term *in situ* SABRE measurements. With this method, we present the experimental zero-field NMR spectrum for each natural abundance  $^{15}\text{N}$ -pyridine derivative (contains 0.36% of  $^{15}\text{N}$ , spin=1/2) and report the distinguishability of such compounds by means of  $J$ -spectroscopy-based chemical fingerprinting. The analysis of pyridine derivatives with similar strength of dominant interactions also revealed the influence of even weak  $J$ -coupling interactions in the zero-field spectrum. The experimental results were supported by numerical simulations.

## 5.2 Material and Methods

### 5.2.1 Parahydrogen Generator

For this research a home-built parahydrogen ( $\text{pH}_2$ ) generator was constructed (Fig 5.1). The generator consists of several gas cylinders, containing hydrogen and nitrogen, and a cylinder to be filled with  $\text{pH}_2$  that are connected with stainless tubings, Swagelok connectors, and valves. Additionally, there is a cold-finger system that enables orthohydrogen-to-parahydrogen conversion and a vacuum pump.

As oxygen has a crucial effect on the relaxation time of parahydrogen, prior to parahydrogen production, it must be removed from the system (tubings and cylinders). To address this problem, a nitrogen gas was used to purge the entire parahydrogen system and then the system was vacuumed to  $10^{-3}$  mbar. The generator can produce theoretically up to 50%-enriched parahydrogen. by passing pure hydrogen gas through a chamber (cold finger made of 2 m of tubing) filled with an iron (III) oxide catalyst (CAS # 371254-50G) immersed in 77 K liquid nitrogen. Subsequently, a 10 L vacuumed aluminum cylinder was filled with  $\text{pH}_2$ . During the process, the cylinder was slowly filled until the pressure inside the cylinder reached 5-6 bar. The filling of the 10 L cylinder usually takes about 5 h with a maximum gas flow of  $\approx 100$  ml/min. The fill flow was set using a needle valve mounted

on top of the parahydrogen cylinder. Following production, the parahydrogen cylinder was detached from the generator and transported to the main experiment, where it was connected to a bubbling system used for samples hyperpolarization.

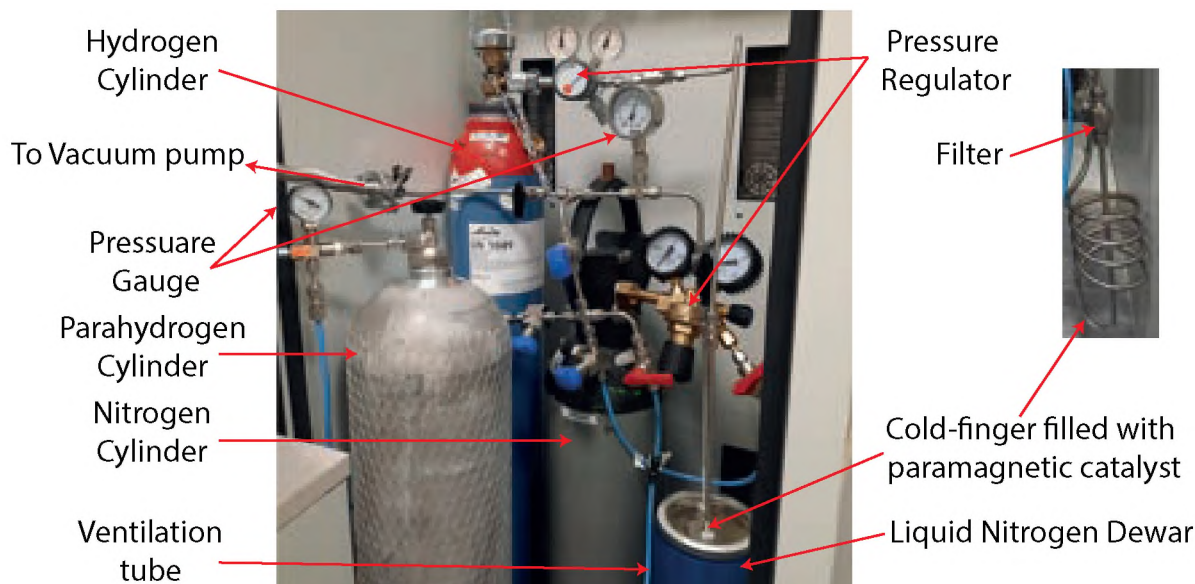


Figure 5.1: Photograph of the home-built parahydrogen generator with additional image of the cold-finger part. The cold finger was filled with a paramagnetic catalyst ( $\text{Fe}_2\text{O}_3$ ) and filters were attached to both ends of the cold finger to hold the catalyst in place. For parahydrogen production, the cold finger was immersed inside a liquid nitrogen dewar (77 K). Hydrogen pressure in the system was controlled by a pressure regulator attached to a hydrogen cylinder. A needle valve was mounted on top of the parahydrogen cylinder to restrict filling flow. Nitrogen gas was employed to purge the line. The system was also connected to a vacuum pump, which allows removing other gases (e.g., oxygen) from the system. Following the production, a line with PTFE tubing was used to ventilate the system with high-pressure hydrogen. The procedure of parahydrogen production can be found in the main text.

### 5.2.2 Bubbling System

The bubbling system was constructed using Swagelok 1/4 inch tubes, connectors, and valves (Fig. 5.2). It has two inlets for  $\text{pH}_2$  and  $\text{N}_2$  gases and one outlet where the flow is controlled by a needle valve and a mass flow controller (Aalborg GFC17A-VAL6-A0). This arrangement enables us to purge the system with only nitrogen gas before parahydrogen bubbling. A manual short valve is implemented between the inlet and outlet valves to bypass the NMR tube; hence, the system can be pressurized without bubbling into the NMR sample. Additionally, during the experiment, an automatic short valve

allows us to turn off bubbling quickly (less than 0.2 ms) before starting data acquisition.

When the manual and automatic short valves were closed, gas (during purging: nitrogen, during measurement: para-enriched hydrogen) passes through Teflon capillary (1/16 inch O.D.  $\times$  0.012 inch I.D.), which was inserted into a 1/4 inch PTFE tube and stabilized by the surrounding holder. The NMR tube (5 mm diameter, 14 inch long) with a sample of interest was attached to the system by stretching a PTFE tube around it. In the system, the capillary reached almost to the bottom of the NMR tube for efficient bubbling.

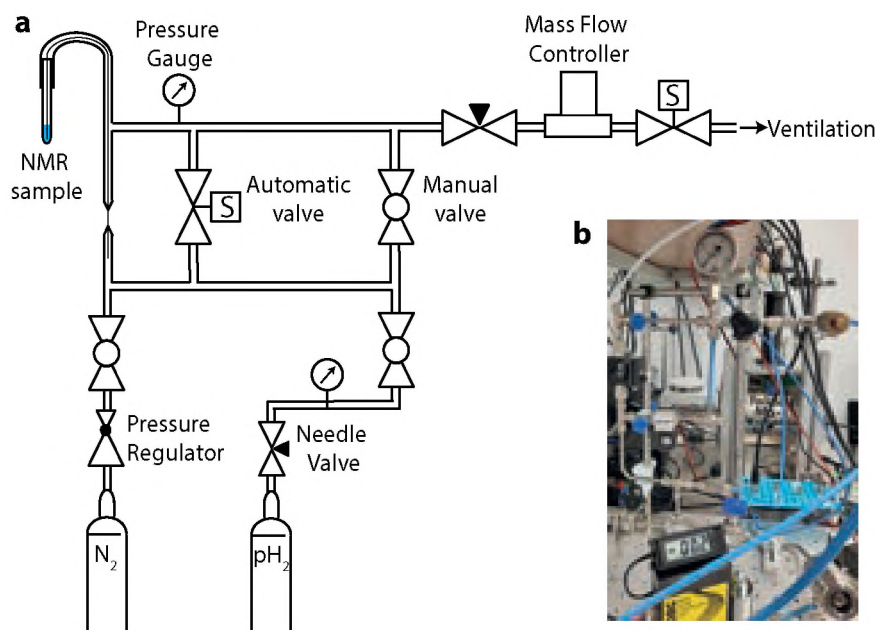


Figure 5.2: Schematic (a) and photograph (b) of the bubbling system. The nitrogen gas, which was used to purge the NMR sample prior to the measurement, was supplied by a high-pressure nitrogen cylinder through a pressure regulator. Using the manual valves, the gas in the system can be switched from nitrogen and parahydrogen. Then, the needle valve attached to the parahydrogen cylinder was used to pressurize the system slowly. By means of two solenoid automatic valves, the experimental sequence of the *in situ* measurements was fully automatized. The bubbling system was mounted nearby the ZULF NMR device. Before the measurements, the NMR tube with a sample of interest was placed inside the shield. Only one of the symbols is named in the figure for the sake of clarity.

For SABRE experiments, a vapor condenser was placed on top of the mu-metal shield, surrounding the upper part of the NMR tube (Fig. 5.3). The liquid-nitrogen vapor was used to cool the NMR tube by means of the condenser to prevent vaporization of volatile compounds in a sample such as methanol. By placing a heater inside the liquid nitrogen

container, the flow of the liquid-nitrogen vapor could be increased and controlled. During the SABRE measurements, the temperature inside the condenser was held around 0°C.

### 5.2.3 Sample Preparation

Chemicals: pyridine (CAS# 110-86-1), 3,5 dichloropyridine (CAS# 2457-47-8), nicotinamide (CAS# 98-92-0), 3-methoxypyridine (CAS# 7295-76-3), 4-methoxypyridine (CAS# 620-08-6), 3-methylpyridine (CAS# 108-99-6), 4-methylpyridine (CAS# 108-89-4), methanol (CAS# 67-56-1) were purchased from Sigma-Aldrich and used without further purification. NMR samples consisted of 25 mM an air-stable SABRE pre-catalyst 'IrCl(COD)(IMes)' (COD = 1,5-cyclooctadiene, IMes= 1,3-bis(2,4,6-trimethyl-phenyl)-imidazolium) mixed with 2 M pyridine derivatives (except for nicotinamide) in 500- $\mu\text{L}$  methanol. Due to the low solubility of nicotinamide in methanol, 1 M nicotinamide was prepared by adding 20  $\mu\text{L}$  of DMSO and 480  $\mu\text{L}$  of methanol additional to 25 mM pre-catalyst.

Since the pre-catalyst forms to an active catalyst in the presence of  $\text{pH}_2$  and a substrate, the activation process was performed in the Earth field by bubbling  $\text{pH}_2$  through the samples for a few minutes. The change in the color of the sample (the solution becoming transparent) indicated catalyst activation (see Sec. 2.3.3 for detailed description of the process). Then, the sample was placed in a magnetic shield near the atomic magnetometer. The SABRE experiments were carried out by bubbling  $\text{pH}_2$  through sample solutions located in the zero-field region for 15 s at a flow rate of 80 sccm and a pressure of 5 bar, followed by a DC magnetic-field pulse, aligned with the sensitive axis of the magnetometer (Fig. 5.4a and b). The optimal pulse angle for the  $^{15}\text{N}$ - $^1\text{H}$  spin system is  $\pi/2 = \theta_H - \theta_N$  [53], which can be explained as in the case of applying a pulse parallel to the sensitive axis of the magnetometer to generate an oscillating magnetization after thermal prepolarization (see Sec. 2.2).

### 5.2.4 Experimental Setup

A zero-field NMR setup with two commercial ready-to-use optically-pumped magnetometers (QuSpin QZFM) was used for SABRE experiments (Fig. 5.3). Although the magne-

tometers were sensitive for both transverse axes ( $x$  and  $y$ ), a zero-field signal, originating from oscillating magnetization, can be detected by only one channel of each sensor, depending on the applied DC pulse direction. The magnetometers were placed oppositely in the coil holder, near the piercing solenoid coil, where the bottom of a 5 mm NMR tube was located. The coils wired around the coil-holder were used to apply DC magnetic pulses. The piercing solenoid coil can provide a guiding field for spins during a parahydrogen-gas bubbling through an NMR sample. The three-layer mu-metal shields were used to attenuate external magnetic fields with an additional ferrite cylinder as the innermost layer (compare to the home-built setup described in Chapter 3). The magnetic-field coils, located within inside the shield, enabled us to achieve a truly zero magnetic field ( $< 0.1$  nT). A 16-bit DAQ card (NI USB-6002) was employed to acquire the magnetometer signals and control the experimental sequence (e.g., to control automatic valves in the bubbling system and magnetic-field pulses). The experimental sequence was pre-programmed using LabView software, which also provided data acquisition (for a detailed description of the setup see Ref. [18]).

As described in Sec. 3.2.3, Python was used for data processing while the simulations of zero-field NMR spectra were performed by **Spintrum**, a high-performance library for spin simulations [3, 26]. The  $J$ -coupling values provided in the literature were used in the simulations (Tables A.1, A.2, A.3, A.4).

## 5.3 Results and Discussion

Zero-field spectra of natural abundance 2 M  $^{15}\text{N}$ -pyridine,  $^{15}\text{N}$ -3,5-dichloropyridine,  $^{15}\text{N}$ -3-methoxypyridine,  $^{15}\text{N}$ -4-methoxypyridine,  $^{15}\text{N}$ -3-methylpyridine,  $^{15}\text{N}$ -4-methylpyridine, and 1 M  $^{15}\text{N}$ -nicotinamide are presented in Fig. 5.4. Since in the molecules, the strongest  $J$ -coupling occurs between  $^{15}\text{N}$  and two ortho protons on the pyridine ring  $J_{\text{NH}}$  (see Fig. 5.4c), the main peaks in their spectra are located at  $3/2J_{\text{NH}}$ , which is about 15 Hz. Even though the strengths of the dominant  $J$ -couplings are similar for the molecules, the spectral patterns of the molecules are different due to the weaker hetero- and homonuclear

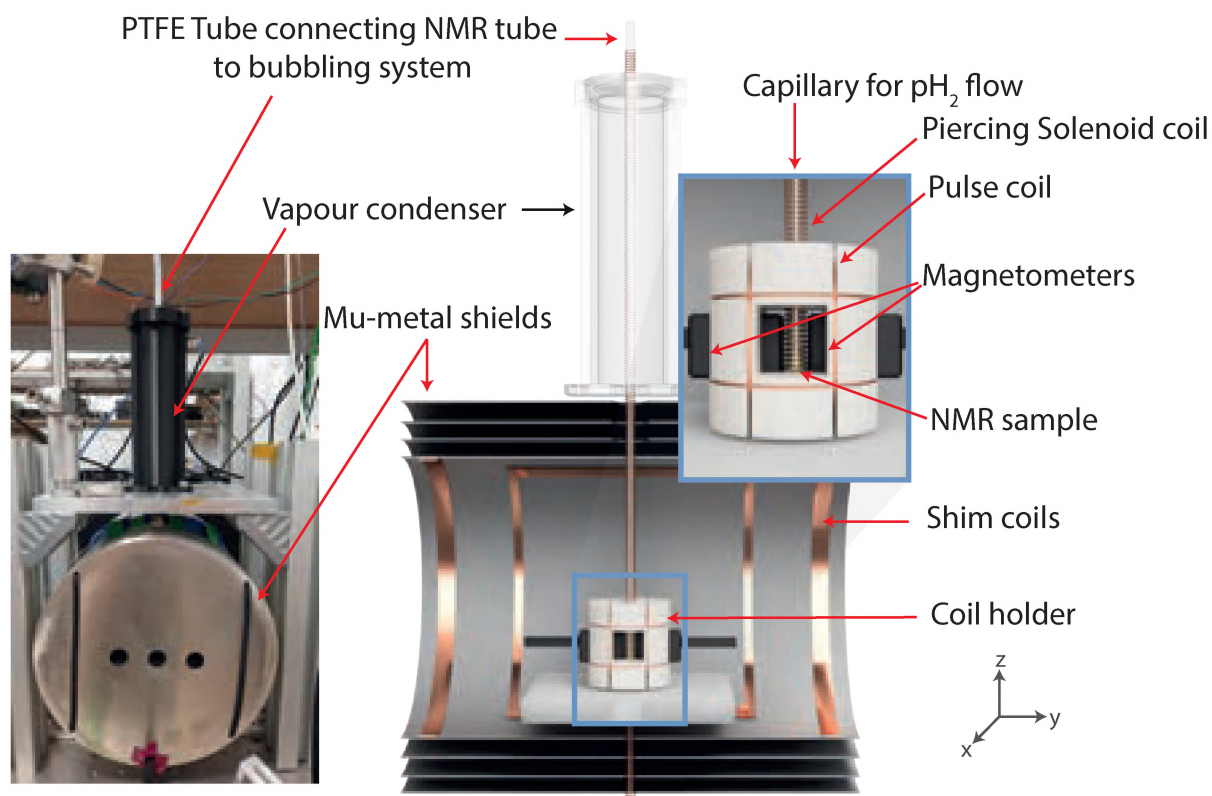


Figure 5.3: Photograph from the outside and schematics of the inside of the zero-field NMR setup for SABRE measurements, using the commercial optically-pumped magnetometers. An NMR tube, containing a SABRE sample, was attached to the bubbling system via a Teflon tubing. A capillary inside the NMR tube allowed the exposure of the sample to  $\text{pH}_2$ . The top of the long NMR tube was surrounded by a vapor condenser supplied by liquid nitrogen vapor (black cylinder on the top). The bottom of the tube is inserted into the magnetic shielding (silver cylinder at the bottom) to achieve zero-field conditions (attenuating the Earth and other uncontrollable fields). Additionally, the shim coils (goldish stripes in the schematics) were employed to provide an absolute zero-field detection region. The NMR tube surrounded by a piercing solenoid reached the detection region (marked in blue), where two commercial magnetometers were oppositely positioned in the coil holder (white element). The pulse coils, wound around the coil holder, were used to generate DC magnetic field to the NMR sample.

$J$ -couplings ( $^{15}\text{N}$  and  $^1\text{H}$ ,  $^1\text{H}$  and  $^1\text{H}$ ) of bonds larger than 2.

In the zero-field spectrum of  $^{15}\text{N}$ -3,5-dichloropyridine, we observe a narrower line at 15.7 Hz compared to the broader peak at 15.4 Hz in the  $^{15}\text{N}$ -pyridine spectrum. This can be explained by the presence of Cl atoms that self-decouple due to rapid relaxation under the influence of the quadrupole interaction, rather than protons with weak  $J_{\text{NH}}$  interaction which has a significant effect on the main peak, as presented in this study. This argument can also be used for  $^{15}\text{N}$ -nicotinamide where a substitute is an amide group. If ortho or meta protons are not chemically equivalent by symmetry, as in the case of  $^{15}\text{N}$ -nicotinamide,  $^{15}\text{N}$ -3-methoxypyridine, and  $^{15}\text{N}$ -3-methylpyridine, the  $J$ -coupling constants within the neighboring spins differ, resulting in the peaks with relatively high amplitude around the main lines. It should be noted that even though in this study, the solubility issue of nicotinamide in methanol was considerably overcome by using DMSO as a co-solvent, the concentration of nicotinamide was two times less than other substrates. Therefore, the lower signal-to-noise ratio in the  $^{15}\text{N}$ -nicotinamide spectrum is observed compared to other spectra. Nevertheless, the first hyperpolarized nicotinamide spectrum ever reported in this regime indicates the prospect of the use of this molecule for ultralow-field imaging in the future [115]. Because, according to the *in vivo* research presented in Ref. [113] that evaluated the pharmacokinetics of SABRE hyperpolarized 4,6-d $_2$ -nicotinamide in the plasma of healthy rats, it is a promising contrast agent with potential use for imaging studies in humans.

When a substitute is a methyl group, consisting of three  $^1\text{H}$ , the system forms a more complex spin network with additional weak  $J$ -couplings. This results in a broader peak, as seen in the zero-field spectra of  $^{15}\text{N}$ -3-methylpyridine and  $^{15}\text{N}$ -4-methylpyridine. In the simulations, this is taken into account as an additional light broadening. On the other hand, narrower spectral patterns appear in the spectra of  $^{15}\text{N}$ -3-methoxypyridine and  $^{15}\text{N}$ -4-methoxypyridine, even though the methoxy group also consists of three  $^1\text{H}$ . The reason for such behavior is the existence of weaker  $J$ -couplings to the methoxy protons with the presence of an additional oxygen nucleus in between. As each pyridine derivative has a different spin system, modified mainly by weak  $J$ -coupling interactions, the zero-

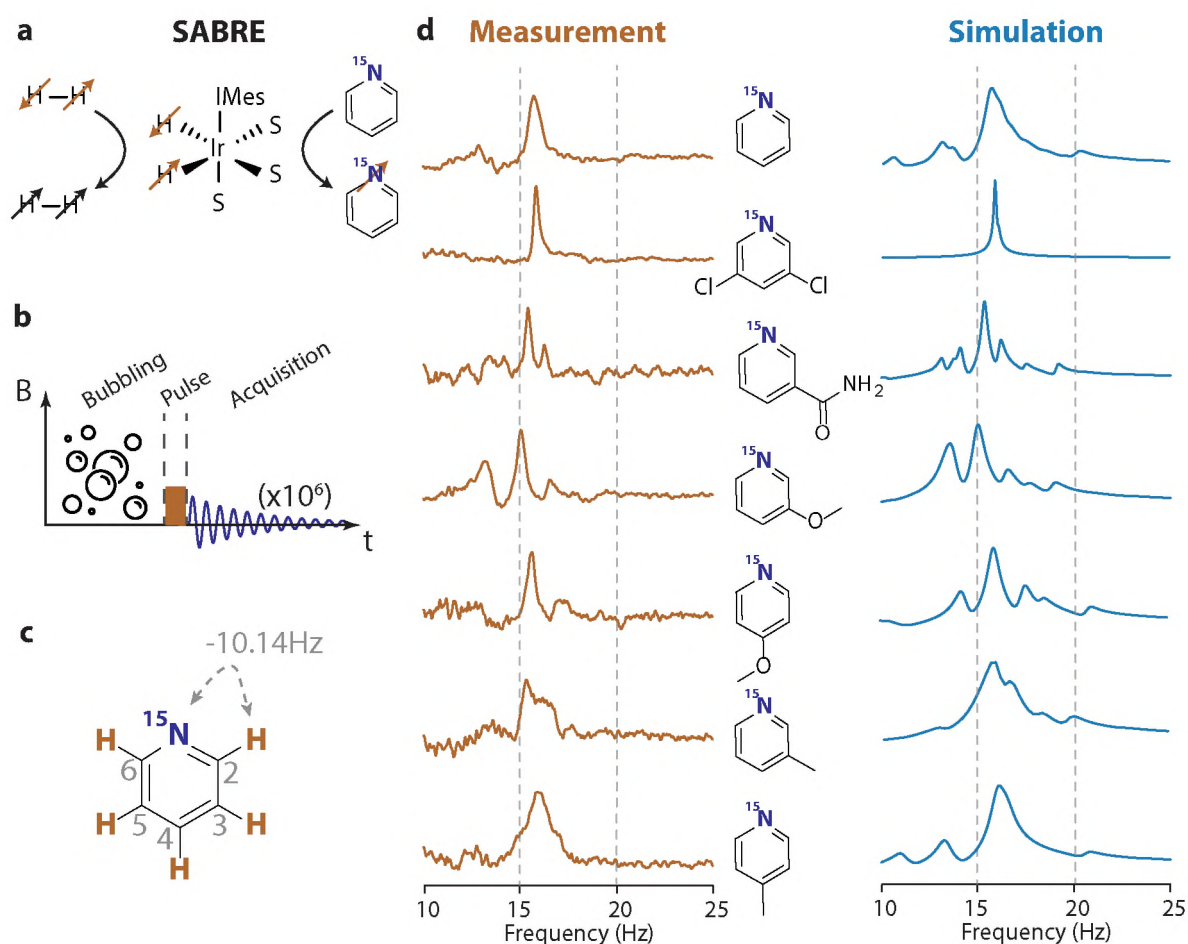


Figure 5.4: (a) Schematic of the SABRE reaction. Parahydrogen and target substrate (e.g.,  $^{15}\text{N}$ -pyridine) are temporarily bounding to an iridium catalyst, transferring the spin order from parahydrogen to the molecule of interest. (b) Scheme of the experimental sequence for the SABRE experiments. (c) Structural formula of  $^{15}\text{N}$ -pyridine with the strongest  $J_{\text{NH}}$  coupling interaction  $J_{\text{NH}}$  marked with dashed, double-headed gray arrow. Ortho, meta and para positions on  $^{15}\text{N}$ -pyridine ring are denoted with (2,6), (3,5), and (4), respectively. (d) Experimental (left) and simulated (right) zero-field spectra of natural abundance  $^{15}\text{N}$ -pyridine (128 averaged transients) and its derivatives:  $^{15}\text{N}$ -3,5-dichloropyridine (128),  $^{15}\text{N}$ -nicotinamide (256),  $^{15}\text{N}$ -3-methoxypyridine (128),  $^{15}\text{N}$ -4-methoxypyridine (82),  $^{15}\text{N}$ -3-methylpyridine (256), and  $^{15}\text{N}$ -4-methylpyridine (238) hyperpolarized by SABRE.

field spectrum is unique for each compound. This is confirmed by numerical simulations (Fig. 5.4d, right column).

As demonstrated above, SABRE can be successfully implemented in ZULF NMR for sensitive and selective detection of various molecules, with a particular example of heterocycle compounds containing an electron-donating atom (e.g., pyridine derivatives). However, the range of molecules suitable for SABRE hyperpolarization is limited due to the necessity of reversibly binding of the molecules to the SABER catalyst on an appropriate time scale [49]. In order to make this method widely applicable, a novel hyperpolarization technique, referred to as SABRE-RELAY approach, has recently emerged [56]. This method is based on polarization transfer under chemical exchange from the SABRE-hyperpolarized transfer molecule to a target molecule with an exchangeable proton (Fig. 2.19). As shown in Ref. [57], SABRE-RELAY has been successfully combined with zero-field NMR to detect  $^{13}\text{C}$ -labelled methanol and ethanol, as well as vodka. Even though the SABRE-RELAY approach cannot provide a signal enhancement as strong as in the traditional SABRE method fundamentally, the development of *in situ* SABRE-RELAY technique can still improve its efficiency which suffers from the relaxation of magnetization during the transfer from an external solenoid into the detection area.

For this study, as the long-time bubbling (15 s for each transient) during SABRE experiments results in the evaporation of an NMR sample, a vapor condenser, maintained by liquid-nitrogen vapor, was invented to enable many measurements with a single sample. Additionally, when an NMR sample consists of a high-amount solid substrate dissolved in a solvent, the evaporation of solvents causes precipitation of the solid substrate. This precipitation can cause capillary obstruction, resulting in non-efficient parahydrogen bubbling throughout the SABRE experiment. In our experimental setup, by holding the condenser temperature at  $0^\circ\text{C}$ , a sample with 0.5 ml can be used for measurements of 1024 transients ( $\approx 7.5$  h). Furthermore, if lower temperatures were provided (e.g., by increasing the amount of liquid-nitrogen vapor), the measurements could be performed even for longer times. It should be noted that even though a higher signal-to-noise ratio is easily achievable by means of this design, in our study with 1-2 M natural abundance

$^{15}\text{N}$ -pyridine derivatives, an experiment with less than 200 transients was adequate to observe the spectral pattern of each compound. On the other hand, this design enable us to measure ZULF NMR spectra of naturally abundant  $^{15}\text{N}$  compounds at the millimolar level. In Ref. [52], an alternative method has been introduced to prolong the SABRE experiments for more than 5 h by supplying solvent vapor with a methanol presaturator during the bubbling of the parahydrogen through the solution. Despite the success of the technique presented in Ref. [52], no need for additional solvent consumption, purposing longer measurement time by controlling the condenser temperature easily, could be mentioned as advantages of the method introduced in our study.

## 5.4 Conclusion

To conclude, we investigated naturally abundant  $^{15}\text{N}$ -pyridine derivatives using zero-field NMR and analyzed their spectral patterns for chemical fingerprinting. In this study, zero-field NMR has been combined for the first time with SABRE hyperpolarization to detect nicotinamide, which is a vitamin B<sub>3</sub> amide and a potential contrast agent for imaging, and many other  $^{15}\text{N}$ -pyridine derivatives. With the study, the significant effect of even weak  $J$ -coupling interactions on zero-field spectra was demonstrated. This enabled high-precision chemical analysis even for the group of molecules derived from the same compound, i.e.,  $^{15}\text{N}$ -pyridine. All acquired spectra contribute to the ZULF-NMR library, which can be used for future pattern recognition. This has great potential in chemical analysis and homeland security. Moreover, the developed experimental setup allowed multiple/long-term, *in situ* SABRE experiments to be performed under the ZULF conditions leading to detection of signals with a higher signal-to-noise ratio. However, it would also be of interest to extend this work to develop *in situ* SABRE-RELAY hyperpolarization for ZULF NMR. Owing to this research, we improve the applicability of ZULF NMR spectrometer as a highly specific, sensitive, compact, and cheap sensor for chemical and biochemical analysis. This paves the way for further applications of ZULF NMR for biochemical *in vivo* analysis and fundamental-physics investigations (e.g., spin-gravity

searches).

# Chapter 6

## Proton-Exchange-Based Hyperpolarization for Biomolecules

The material presented in this chapter is being prepared for publication:

**S. Alcicek**, E. Van Dyke, J. Xu, S. Pustelny, D. Barskiy, "Parahydrogen-based Hyperpolarization of Biomolecules via Chemical Exchange", In preparation.

### 6.1 Motivation and Overview

As explained above many times, the main sensitivity limitation of NMR/MRI can be overcome using hyperpolarization techniques. Parahydrogen-based methods are advantageous because of their low cost, technical simplicity, and ability to quickly produce large volumes of hyperpolarized material. Integration of such techniques with portable (benchtop or zero- and ultralow-field) NMR spectrometers holds great promise for democratizing NMR equipment and improving its sensitivity. A major drawback of parahydrogen-induced polarization is the need for a dedicated compound (precursor), which is modified during the reaction to produce a hyperpolarized target molecule. Recently, this problem has been addressed through the relay of hyperpolarization to the target by chemical exchange (PHIP-X) [45]. This approach might extend the applicability of PHIP toward biomolecules with exchangeable protons, without affecting the target molecule. In the original work, PHIP-X was used to hyperpolarize such biomolecules with -OH groups as pyruvic acid, lactic acid, and D-[ $^{13}\text{C}_6$ ]-glucose, achieving maximum  $^1\text{H}$  polarization level of the target biomolecule of 0.07%, while a polarization level of 13% was reported in a transfer molecule [45]. Even though PHIP-X is a newly emerged and attractive method in numerous aspects, optimization studies are required for an efficient polarization transfer

to the molecule of interest.

Here, we expand PHIP-X research to (bio-)molecules with -NH/-NH<sub>2</sub> groups, such as urea. This was done using an NMR benchtop spectrometer (1 T) combined with a robotic arm, which allowed automated optimization studies. As a transfer molecule, allyl alcohol, produced from propargyl alcohol, was used with a subsequent polarization transfer via the proton-exchange mechanism at low magnetic fields. In the molecule, all protons interact strongly and polarization can be distributed through all coupled nuclear spins. To maximize the efficiency of the polarization transfer, key parameters such as catalyst concentration, parahydrogen bubbling time, polarization transfer field, and transfer time were studied. A specific NMR pulse sequence (insensitive nuclei enhanced by polarization transfer – INEPT) was used to enhance the <sup>15</sup>N-NMR signal of hyperpolarized target molecules: [<sup>15</sup>N<sub>2</sub>]-urea, [<sup>13</sup>C, <sup>15</sup>N<sub>2</sub>]-urea, [<sup>15</sup>N]-glycine, [<sup>15</sup>N]-ammonium nitrate, and [<sup>15</sup>N]-benzamide. In addition, D-[1-<sup>13</sup>C]-glucose was hyperpolarized via PHIP-X and detected using <sup>1</sup>H-<sup>13</sup>C INEPT NMR spectroscopy (1.4 T). In order to introduce PHIP-X for ZULF NMR, hyperpolarization of the transfer molecule and polarization transfer to water protons were investigated by means of ZULF NMR spectroscopy. This study aims at developing a widely applicable ZULF NMR *in situ* detection scheme.

## 6.2 PHIP-X in Conventional NMR

In this section, high-field NMR detection via PHIP-X technique is examined comprehensively with optimization studies.

### 6.2.1 Material and Methods

All chemicals: propargyl alcohol (CAS# 107-19-7) acetone (CAS# 67-64-1), [<sup>13</sup>C, <sup>15</sup>N<sub>2</sub>]-urea (CAS# 58069-83-3), [<sup>15</sup>N<sub>2</sub>]-urea (CAS# 2067-80-3), [<sup>15</sup>N]-glycine (CAS# 7299-33-4), [<sup>15</sup>N]-benzamide (CAS# 31656-62-9), and [<sup>15</sup>N]-ammonium nitrate (CAS# 31432-46-9), D-[1-<sup>13</sup>C]-glucose (CAS# 40762-22-9), and [1,4-Bis(diphenylphosphino)butane] (1,5-cyclooctadiene)rhodium(I) tetrafluoroborate ([Rh(dppb)(COD)]BF<sub>4</sub>) (CAS# 79255-71-

3) were purchased from Sigma Aldrich and used without further purification. High-field NMR measurements were performed using bench-top NMR spectrometers: a SpinSolve Nitrogen ULTRA 43 MHz and a SpinSolve Carbon 60 MHz, Magritek.

To study hyperpolarization of the transfer agent, solutions of 0.5 M propargyl alcohol and various concentrations of Rh-catalyst (5, 10, 15 mM) were prepared in acetone. For PHIP-X experiments, 0.5 M propargyl alcohol, 5 mM Rh-catalyst, and the labelled target compound were mixed. Due to the poor solubility of biomolecules in acetone, 50  $\mu$ L of dimethyl sulfoxide (DMSO) was used for each NMR sample to increase the amount of dissolved molecule of interest. In this way, 100 mM [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea and [ $^{15}\text{N}_2$ ]-urea; 60 mM D-[1- $^{13}\text{C}$ ]-glucose (CAS#40762-22-9); 50 mM [ $^{15}\text{N}$ ]-ammonium nitrate and [ $^{15}\text{N}$ ]-benzamide (CAS# 31656-62-9); and 15 mM [ $^{15}\text{N}$ ]-glycine solutions were prepared.

For each experiment, a 650  $\mu$ L sample was transferred to a pressurizable 5 mm NMR tube. The NMR tube was then attached to a bubbling system, described in Ref. [57], for parahydrogen exposition. Parahydrogen-enriched hydrogen gas was prepared by passing high-purity hydrogen over a hydrated iron(III) oxide catalyst at 30 K. Each NMR sample was bubbled with para-enriched hydrogen gas via a thin capillary (PTFE, OD: 0.9 mm) for a given time (detailed information is provided below for each experiment). The flow rate of parahydrogen was held at 80 sccm at 6 bar using a mass flow controller. Subsequent to the bubbling with parahydrogen, the NMR tube was placed in a magnet bore of a benchtop NMR spectrometer using a robotic arm (Fig. 6.4a). A whole experimental sequence, including NMR-signal acquisition, was controlled electronically using an Arduino Uno microcontroller board. This automated experimental setup ensures consistency, which is essential to carry out optimization studies with high accuracy. Additionally, using a magnet with adjustable field strength (1-100 mT) designed by Dr. Peter Blümli, various magnetic fields during bubbling were studied to investigate the influence of magnetic field over polarization transfer through exchangeable protons.

## 6.2.2 Results and Discussion

PHIP-X approach can be described as a two-stage process. Initially, a transfer molecule, e.g., propargyl alcohol, is hyperpolarized by adding parahydrogen to its structure. Then, polarization is transferred from the hyperpolarized transfer agent (e.g., hyperpolarized allyl alcohol) to a target molecule at low magnetic field via the proton-exchange mechanism (Fig. 6.2a). The first step, which is the well-known hydrogenative-PHIP-ALTEDENA experiment [116], has significant effect on target-compound hyperpolarization. Thus, the efficiency of the parahydrogenation reaction of propargyl alcohol to hyperpolarized allyl alcohol and then propanol was studied for various amounts of Rh(I)-catalyst (5, 10, 15 mg) and different parahydrogen bubbling times, the result is presented in Fig. 6.1. An NMR sample was bubbled with parahydrogen for a given time (5-60 s) at the Earth's field, above the NMR spectrometer for each data point shown in Fig. 6.1b and c. The sample was then transferred to the detection region with the robotic arm to obtain an  $^1\text{H}$ -NMR spectrum of hyperpolarized products: allyl alcohol ( $\text{B}^*$ ) and propanol ( $\text{C}^*$ ). After acquisition, the NMR tube was detached from the bubbling setup and purged with nitrogen gas to prevent further hydrogenation with the remaining dissolved parahydrogen. When the hyperpolarized products in the solution were relaxed to thermal equilibrium, a thermal  $^1\text{H}$ -NMR spectrum was obtained to estimate the relative concentrations of the substrate (A) and the products (B and C). The peak assignments used for  $^1\text{H}$ -NMR spectral analysis in this kinetic study can be found in Fig. A.1 of Appendix A.4.

As presented in Fig. 6.1b, the high amount of catalyst induced a fast consumption of propargyl and allyl alcohols. In the experiments with 5 mM catalyst, propanol production is shown after 40 s of hydrogen bubbling, while for higher catalyst concentration, propanol appeared in the solution after 20-30 s of bubbling with hydrogen. Since the gradual production of allyl alcohol might allow us to perform many transients, thus achieving a higher SNR, the 5 mM catalyst was used in further experiments of this study. As demonstrated in Fig. 6.1, allyl alcohol have the highest polarization after 20-30 s bubbling, and propanol begin to be hyperpolarized more efficiently after 50-60 s of exposure to parahydrogen.

However, during the first 60 s of bubbling, the polarization level of propanol is never as high as the maximum polarization of allyl alcohol. Even though propanol could also be considered as a transfer agent for the PHIP-X technique, allyl alcohol seems to be more suitable for high-polarization transfer to a target molecule.

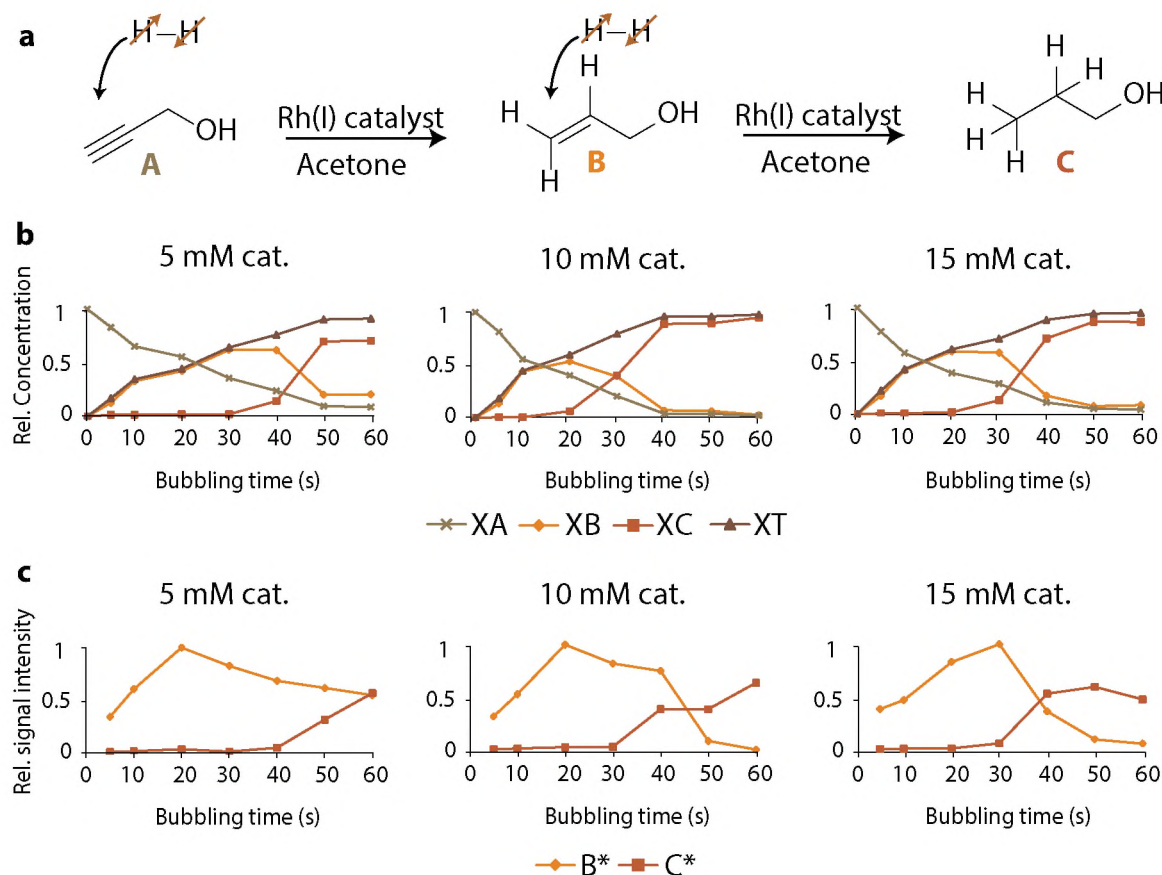


Figure 6.1: (a) Scheme of two-steps hydrogenation of propargyl alcohol in acetone catalyzed by [1,4- bis(diphenylphosphino)butane] (1,5-cyclooctadiene)rhodium(I) tetra fluoroborate. Parahydrogen undergoes pairwise addition to propargyl alcohol to first form allyl alcohol and then propanol. (b) Kinetic of propargyl alcohol hydrogenation reaction versus hydrogen bubbling time for the various concentrations of Rh(I)-catalyst. The initial substrate, propargyl alcohol, and products, allyl alcohol and propanol are denoted by A, B, and C, respectively, while XA, XB, and XC represent their fractions in the solution after hydrogenation reaction. (c) The relative intensity of signal arising from hyperpolarized allyl alcohol (B\*) and propanol (C\*) via ALTADENA-PHIP for various parahydrogen bubbling times (see main text for experimental detail).

As the next step, the polarization transfer to a target molecule was investigated. Due to the complexity of the  $^1\text{H}$ -NMR spectrum, with many peaks originating from the transfer and target compounds, the  $^1\text{H}$ - $^{15}\text{N}$  INEPT technique was used to facilitate spectral analysis.  $^1\text{H}$ - $^{15}\text{N}$  INEPT is a specific pulse sequence used in NMR to enhance signal reso-

lution by transferring polarization from the coupled proton (here, hyperpolarized protons via PHIP-X) to  $^{15}\text{N}$ . While the basic INEPT sequence generates anti-phase magnetization, the refocused INEPT produces in-phase magnetization [15]. If decoupling is applied with basic INEPT, the anti-phase peaks cancel each other. However, with refocused INEPT, decoupling is applied to obtain singlet in-phase peaks (for more details, see Sec. 2.1.9). Optimal spin echo delays for the INEPT pulse sequence for each molecule were calculated based on numerical simulations (for details see Sec. A.3).

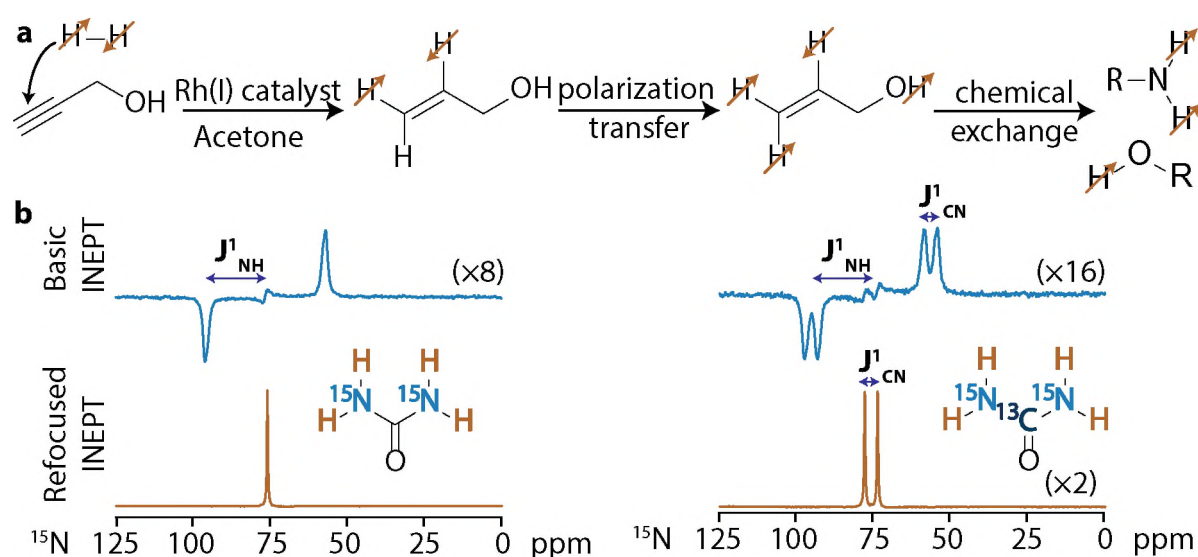


Figure 6.2: (a) Schematic representation of the PHIP-X process and (b) basic and refocused  $^1\text{H}$ - $^{15}\text{N}$  INEPT NMR spectra of hyperpolarized  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea and  $^{15}\text{N}_2$ -urea via PHIP-X.

Figure 6.2b presents the single-transient basic and refocused  $^1\text{H}$ - $^{15}\text{N}$  INEPT spectra of hyperpolarized  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea and  $^{15}\text{N}_2$ -urea via PHIP-X. The NMR samples (0.5 M propargyl alcohol, 5 mM Rh(I)-catalyst, 100 mM urea in DMSO and acetone mixture) were bubbled with parahydrogen at the Earth's field for 20 s before acquiring the  $^{15}\text{N}$ -NMR spectra. As expected, in spectra of  $^{15}\text{N}_2$ -urea subsequent to refocused INEPT (with proton decoupling), a single line appears at  $\approx 75$  ppm, while in the basic INEPT spectrum (no proton decoupling), the line is split by the  $J_{\text{NH}}$ -coupling between  $^1\text{H}$  and  $^{15}\text{N}$ . The existence of an additional  $^{13}\text{C}$ - $^{15}\text{N}$   $J$ -coupling interaction in  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea results in further splitting of the peaks into doublets, possessing half the signal intensity. Narrower line/lines in the refocused INEPT spectra, compared to the basic INEPT spectra, resulted

from decoupling, eliminating the effect of proton exchange on line broadening.

After hyperpolarizing urea using PHIP-X, optimization of the bubbling field (0.05-80 mT) and bubbling time (5-120 s) was performed. To study the influence of the parahydrogen bubbling time on the signal enhancement, four identical NMR samples (0.5 M propargyl alcohol, 5 mM Rh(I)-catalyst, 100 mM [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea dissolved in DMSO and acetone mixture) were prepared. For each bubbling time interval (5, 20, 40, and 60 s), one of the samples was bubbled with parahydrogen in the Earth's field and then the NMR tube was transferred into the spectrometer via the robotic arm to obtain the  $^1\text{H}$ - $^{15}\text{N}$  refocused INEPT NMR spectrum of hyperpolarized [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea. This process was repeated for each sample several times: 24, 6, 3, and 2, respectively. Owing to the high concentration of the substrate, the production of hyperpolarized transfer agents carries on for many transients, allowing repolarization of the target molecule in each transient. This approach enables us to acquire many NMR signals originating from the repolarized target molecule as shown in Fig. 6.3a. The highest signal enhancement is observed after 60 s of bubbling, when allyl alcohol and propanol exhibit almost the same polarization level (Fig. 6.1). This may lead to the conclusion that the effect of propanol as a transfer agent is not insignificant. Although the long bubbling time appears more efficient, the averaged spectra of repeated measurements with various bubbling time intervals show that PHIP-X repetitions with short bubbling time can provide an overall higher signal-to-noise ratio (Fig. 6.3b).

In order to investigate the influence of the parahydrogen bubbling field on the signal enhancement, ten different magnetic fields (0.05-80 mT) were used to investigate the NMR spectra of samples (0.5 M propargyl alcohol, 5 mM Rh(I)-catalyst, 100 mM [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea in DMSO and acetone mixture) exposed to parahydrogen for 20 s (Fig. 6.3d). Immediately after bubbling, the sample was transferred to a robotic arm to record the refocused  $^1\text{H}$ - $^{15}\text{N}$  INEPT NMR spectrum of hyperpolarized [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea. For each sample, the measurement was repeated three times, as a result, the calculated mean values and standard deviations of the relative signals are presented in Fig. 6.3c. When the bubbling field was around 2 mT, the highest signal enhancement was observed. As a con-

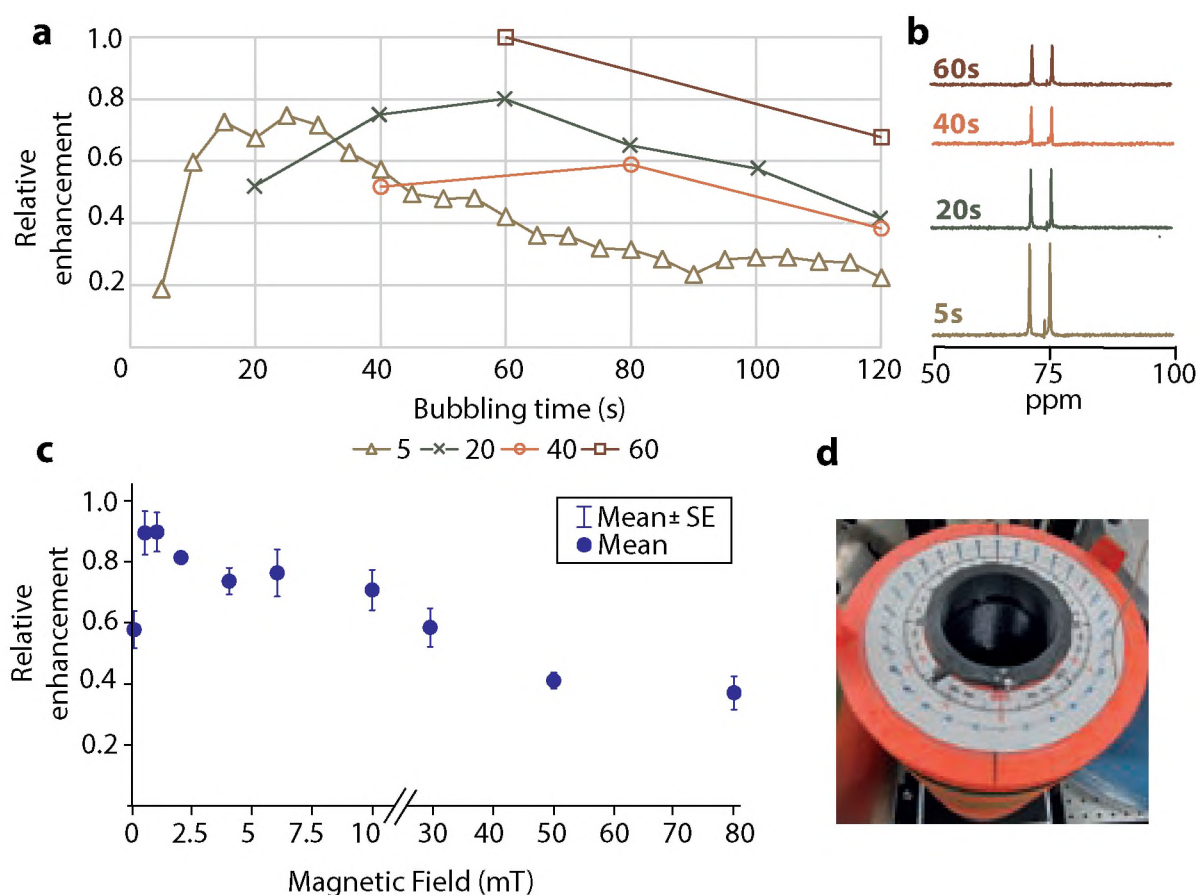


Figure 6.3: (a) Effect of parahydrogen bubbling time on peak intensity in the  $^1\text{H}$ - $^{15}\text{N}$  INEPT NMR spectra of hyperpolarized  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea. The graph corresponds to four NMR samples of urea which were bubbled with parahydrogen several times (24, 6, 3, and 2) in different time intervals (5, 20, 40, and 60 s, respectively). (b) Averaged  $^1\text{H}$ - $^{15}\text{N}$  refocused INEPT NMR spectra of hyperpolarized  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea for each bubbling time interval. For instance, the average of 24 signal transitions, each obtained after 5 s of parahydrogen exposure in the Earth's field. (c) The magnetic field (0.05-80 mT) in which the parahydrogen bubbling was conducted versus signal enhancement in the  $^{15}\text{N}$ -NMR spectrum of hyperpolarized  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea. Each data point is a consequence of averaged three transients after repeated 20 s of the parahydrogen supply. Error bars represent standard errors. (d) The image of the adjustable magnet consisting of transversely oriented Halbach arrays that was used for adjusting bubbling field. This constructed magnet is capable of generating a field from 1 mT to 101 mT depending on the relative angle between Halbach magnets.

sequence of the bubbling field rising up to 80 mT, a gradual decrease in signal intensity was observed. However, in the previous PHIP-X study [45], the numerical simulations show that if the magnetic fields during parahydrogenation increase up to 80 mT, the proton polarization in the -OH group of the transfer molecule increases significantly. On the other hand, the polarization transfer from the -OH group of the transfer molecule to the target molecule is the key step that can be influenced by magnetic field strength. Therefore, to explain this disagreement, further simulations could be performed to evaluate the polarization level of a target molecule.

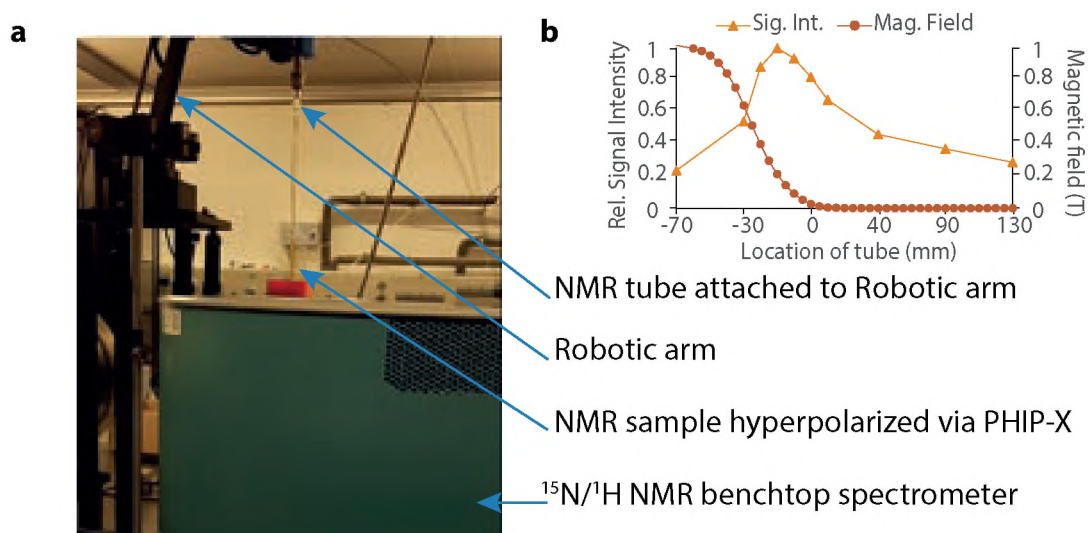


Figure 6.4: (a) Experimental setup used for the ALTADENA-PHIP and PHIP-X experiments. The NMR tube was connected to the parahydrogen bubbling system and hooked to the robotic arm from the top part. The robotic arm transferred the NMR sample between the region with a low magnetic field (0.05-80 mT), where parahydrogen bubbling occurs, and the detection region in the spectrometer. Thus, the parahydrogenation reaction could conduct in a given magnetic field before the signal was measured inside the magnet. (b) Intensity of hyperpolarized  $^{13}\text{C}$ ,  $^{15}\text{N}_2$ -urea signal versus the position of NMR tube inside the bore of spectrometer during parahydrogenation. Each data point corresponds to a measurement, following a transfer of the sample into the detection region after 10 s of parahydrogen bubbling in a given position inside the spectrometer. Magnetic field profile inside the bore of spectrometer was measured using a gaussmeter. The location of the tube determined based on '0' position of robotic arm (see Fig. A.5).

Although hyperpolarization of the target molecule by PHIP-X becomes more efficient by applying an optimal magnetic field during parahydrogenation, relaxation throughout the transfer, taking roughly 3 s, lowered the signal intensity significantly. However, above the detection region of the  $^{15}\text{N}/^1\text{H}$  NMR spectrometer, where 1 T magnetic field

was applied for thermal polarization, the magnetic field varied from 1 T towards to the Earth’s field. By taking advantage of this field profile inside the bore of the spectrometer, we performed parahydrogenation at different positions in the bore to vary the bubbling field, as well as to shorten the transfer time. For this purpose, an NMR sample (0.5 M of propargyl alcohol, 5 mM of Rh(I)-catalyst, of 100 mM [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea dissolved in the DMSO and acetone mixture) was exposed to parahydrogen for 10 s in a given location inside the bore and, subsequently, the NMR tube was moved to the detection region by robotic arm to acquire the refocused  $^1\text{H}$ - $^{15}\text{N}$  INEPT NMR spectrum of hyperpolarized [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea. This experiment was repeated for ten different locations inside the bore. When the NMR tube was in the middle of magnetic bore during the parahydrogenation, a single-scan enhancement of 17,100 was observed for [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea. This corresponded to a polarization level of 1.2% per molecule. In this way, with a short relaxation time, the most enhanced [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea signal was detected due to the reduction of relaxation in the course of the sample transfer. When the sample was parahydrogenated at a magnetic field closer to the Earth’s field or high field (1 T), drops in signal intensity were observed. By taking into account the effect of the differences in the sample transfer times for each measurement, these results are congruent with the previous result concerning optimization of the applied magnetic field during parahydrogenation.

In addition to urea, [ $^{15}\text{N}$ ]-glycine, [ $^{15}\text{N}$ ]-ammonium, and [ $^{15}\text{N}$ ]-benzamide were hyperpolarized to demonstrate the potential of PHIP-X for hyperpolarization of a wide range of chemicals. Samples of 15 mM [ $^{15}\text{N}$ ]-glycine, 50 mM [ $^{15}\text{N}$ ]-ammonium and 50 mM [ $^{15}\text{N}$ ]-benzamide in the DMSO and acetone mixtures were prepared with additional 0.5 M propargyl alcohol and 5 mM Rh(I)-catalyst. Each sample was subjected to parahydrogen bubbling for 20 s in the Earth’s field and transferred to the spectrometer to obtain the refocused  $^1\text{H}$ - $^{15}\text{N}$  INEPT NMR spectrum of hyperpolarized  $^{15}\text{N}$ -labelled compounds. Spin-echo delays for INEPT experiments were calculated using numerical simulations (see Sec. A.3 for details). The peaks in spectra of [ $^{15}\text{N}$ ]-glycine [ $^{15}\text{N}$ ]-ammonium, and [ $^{15}\text{N}$ ]-benzamide were observed with a few orders of magnitude signal enhancement, particularly, corresponding to 0.1%, 0.13% and 0.02% polarization levels per spin, respectively

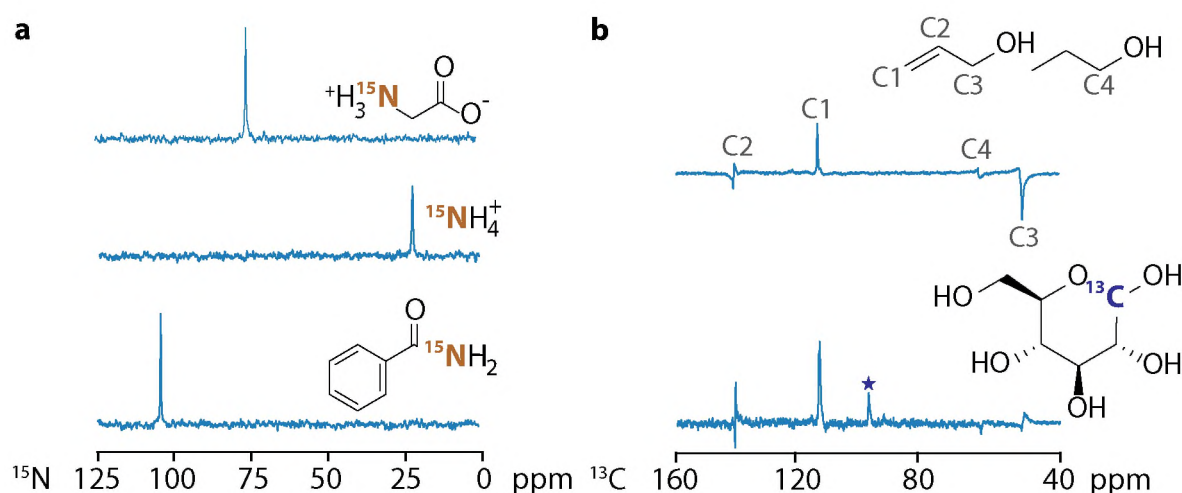


Figure 6.5: (a) Refocused  ${}^1\text{H}$ - ${}^{15}\text{N}$  INEPT NMR spectra of hyperpolarized  ${}^{15}\text{N}$ -glycine,  ${}^{15}\text{N}$ -ammonium, and  ${}^{15}\text{N}$ -benzamide by PHIP-X. (b) Refocused  ${}^1\text{H}$ - ${}^{13}\text{C}$  INEPT NMR spectrum of hyperpolarized products: allyl alcohol and propanol (above) and refocused  ${}^1\text{H}$ - ${}^{13}\text{C}$  INEPT NMR spectrum of D-[1- ${}^{13}\text{C}$ ]-glucose hyperpolarized via PHIP-X. The peaks at  $\approx 100$  ppm denoted with star originates from D-[1- ${}^{13}\text{C}$ ]-glucose.

(Fig. 6.5a). It is pertinent to note that the polarization levels could be improved by performing experiments in the optimum bubbling field, but even more by shortening the transfer time.

As the last result for this section, the investigations of PHIP-X hyperpolarized D-[1- ${}^{13}\text{C}$ ]-glucose are reported to show the feasibility of the use  ${}^1\text{H}$ - ${}^{13}\text{C}$  INEPT pulse sequence which allows straightforward spectral interpretation for hyperpolarized target compound unlike crowded  ${}^1\text{H}$ -NMR spectrum with numerous peaks originating from hyperpolarized protons of transfer and target compounds. For this purpose, the parahydrogen gas was bubbled at the Earth's field into a sample containing 60 mM D-[1- ${}^{13}\text{C}$ ]-glucose, 0.5 M propargyl alcohol and 5 mM Rh(I)-catalyst dissolved in the DMSO and acetone mixtures. Immediately after bubbling, the NMR tube was transferred into  ${}^1\text{H}$ - ${}^{13}\text{C}$  NMR spectrometer to acquire the refocused  ${}^1\text{H}$ - ${}^{13}\text{C}$  INEPT NMR spectrum of hyperpolarized D-[1- ${}^{13}\text{C}$ ]-glucose. In addition, to be able to assign the peaks, arising from the naturally abundant  ${}^{13}\text{C}$  isotope in hyperpolarized allyl alcohol and propanol, the PHIP solution consisting of the chemicals listed above, except for D-[1- ${}^{13}\text{C}$ ]-glucose, was measured in the same way. In Fig. 6.5b, the spectrum above is the result of the experiment with the mentioned solution without D-[1- ${}^{13}\text{C}$ ]-glucose, while the spectrum below is a consequence

of experiments with the sample containing D-[1- $^{13}\text{C}$ ]-glucose. The peak associated with hyperpolarized D-[1- $^{13}\text{C}$ ]-glucose arose at about 100 ppm with the 0.024% polarization level while the other peaks originate from hyperpolarized allyl alcohol and propanol.

Other proton-exchange-based hyperpolarization method (SABRE-RELAY) was also used to hyperpolarize [ $^{13}\text{C}$ ,  $^{15}\text{N}_2$ ]-urea and [ $^{13}\text{C}$ ]-urea. It has allowed achieving two orders of magnitude signal enhancement compared to thermally measured at 9.4 T [56]. Moreover, SABRE-RELAY hyperpolarization of [ $^{13}\text{C}$ ]-glucose has been reported to a lesser extent. Despite a lower polarization level compared to the results presented here, SABRE-RELAY has the advantage of allowing rehyperpolarization and signal accumulation without consuming transfer and target agents, thereby improving the signal-to-noise ratio [109]. On the other hand, we also reported that by increasing the amount of transfer agent, PHIP-X can be repeatable without the consumption of the target molecule.

## 6.3 PHIP-X in ZULF NMR

In this section, the first-ever implementation of the PHIP-X method into ZULF NMR spectroscopy is reported along with the results of optimization experiments.

### 6.3.1 Material and Methods

All chemicals exploited in the presented research were supplied from Sigma-Aldrich and used without further purification as stated above. For zero-field NMR using PHIP, solutions with 1 M propargyl alcohol, 7 mM Rh(I)-catalyst ( $[\text{Rh}(\text{dppb})(\text{COD})]\text{BF}_4$ ) were prepared in acetone. For PHIP/PHIP-X experiments at ultralow fields, solutions with 0.5 M propargyl alcohol, 7 mM Rh(I)-catalyst, and various amounts of distilled water were prepared in acetone. For each experiment, 500  $\mu\text{L}$  of sample was transferred to an NMR tube of 14 inch in length, which was made by welding two standard NMR tubes.

ZULF NMR experiments were conducted using the setup described in Chapter 5. The home-built parahydrogen generator and the bubbling system, which are already introduced in Chapter 5, were used to expose the prepared sample to partially para-

enriched hydrogen gas. Before each experiment, the NMR tube was attached to the bubbling system and the sample was purged with nitrogen gas, as previously described. After placing the NMR tube inside the shield, an experimental sequence was initiated through LabView software (for details see Sec. 5.2).

For PHIP experiments with zero-field NMR detection, after bubbling parahydrogen through the sample for about 20 s in the zero-field regime (inside the shield), a DC pulse was applied along the sensitive axis with a pulse angle of  $\pi/2$ , optimized for the  $^{13}\text{C}$ - $^1\text{H}$  spin system, i.e.,  $\pi/2 = \theta_H - \theta_C$ , where  $\theta_{H,C}$  are the rotation angles for  $^1\text{H}$  and  $^{13}\text{C}$  [46]. Then data acquisition was started (Fig. 6.6a). In PHIP/PHIP-X measurements at ultralow field, at first, a given field  $B_H$  was applied (0.1-3 mT) by the piercing solenoid during the bubbling. The solenoid was then switched off adiabatically and a  $\pi/2$ -proton DC pulse was applied to generate proton precession under the ultralow detection field,  $\approx 820$  nT (Fig. 6.7a). Additionally, a sample of 150  $\mu\text{L}$  distilled water was measured in an ultralow field after thermal prepolarization using a 1.4 T Halbach magnet to draw a comparison between thermal prepolarization and the PHIP/PHIP-X technique (for experimental details on thermal prepolarization see Sec. 3.2.2).

Post-processing and data analysis were conducted using Python code (see Sec. 3.2.3 for details). Simulations of ZULF NMR spectra were performed using the SpinDynamica package for Mathematica [4, 19]. Homonuclear  $^1\text{H}$ - $^1\text{H}$   $J$ -coupling constants used in the simulations were taken from the literature [45], while heteronuclear  $^{13}\text{C}$ - $^1\text{H}$  coupling constants were calculated based on the results presented in the previous section.

### 6.3.2 Results and Discussion

In this section, the implementation of the PHIP-X technique into ZULF NMR is discussed with the preliminary results. As mentioned in the previous section, PHIP-X consists of two main stages: hyperpolarization of the transfer molecule and polarization transfer to the target molecule. Therefore, first, hyperpolarization of the transfer molecule was investigated under ZULF conditions. As demonstrated in Figs. 6.6a and b, a solution of 1 M propargyl alcohol and 7 mM Rh(I)-catalyst in acetone was bubbled with parahydrogen

for 20 s in zero field, and, following a DC pulse, a zero field spectrum of hyperpolarized allyl alcohol was measured. This experimental sequence was repeated until no visible peaks were observed in the spectrum. Each recorded zero-field spectrum is presented in Fig. 6.6c. We observed three groups of peaks in the first eight transients, while the first transient showed a lower signal-to-noise ratio, which might be a result of either bubbling with remaining nitrogen in the system instead of parahydrogen for the first a few seconds of the experiments, hence delayed catalyst activation, or residual impurities (e.g., oxygen) causing fast relaxation. In the ninth transient spectrum, no peaks are observed due to the consumption of all substrates, except for the peak up to 10 Hz, corresponding to flicker noise.

For spectral interpretation, the isotopomers of the product compound, allyl alcohol, must be considered in detail. As the natural abundance of  $^{13}\text{C}$  nuclei is 1.1%, we examine zero-field spectra of  $[1-^{13}\text{C}_1]$ -,  $[2-^{13}\text{C}_1]$ - and  $[3-^{13}\text{C}_1]$ -allyl alcohols (Fig. 6.6d). Since the probability of existing a  $^{13}\text{C}$ - $^{13}\text{C}$  fragment within a molecule is about 0.0121% ( $1.1\% \times 1.1\%$ ), other isotopologues of allyl alcohol are negligible. As described previously, the main peaks in the zero-field spectra originate from the heteronuclear  $J$ -couplings such as the  $^{13}\text{C}$ - $^1\text{H}$  coupling, and they are split with the influence of the homonuclear couplings, e.g., between  $^1\text{H}$  nuclei. Based on that, we can easily interpret the spectrum as follows: a group of peaks at 140-165 Hz (orange shading in Fig. 6.6c) corresponds to the one-bond coupling of a parahydrogen-derived hydrogen to the  $^{13}\text{C}$  nucleus in  $[2-^{13}\text{C}_1]$ -allyl alcohol ( $J_{\text{CH}}^1=156.2$  Hz), which is then split by the coupling to other protons. Another group of peaks (215-245 Hz, marked in blue in Fig. 6.6c) arises mainly from the one-bond coupling between  $^{13}\text{C}$  nucleus and two magnetically inequivalent  $^1\text{H}$  nuclei in  $[3-^{13}\text{C}_1]$ -allyl alcohol ( $J_{\text{CH}}^1=156.3$  Hz,  $J_{\text{CH}}^1=158.2$  Hz). If we disregard the magnetic inequality of these two  $^1\text{H}$  nuclei for a while, thus we assume the dominant spin system as  $\text{XA}_2$  instead of  $\text{XAA}'$ , a line at frequency  $3/2J_{\text{CH}}^1$  (roughly 234 Hz) is expected. Also, it is inevitable that the line is split with other weaker interactions that form multiplets. In this way, we can explain the group of peaks that appears in the 215-245 Hz range in the zero-field spectrum. The low-frequency peaks, i.e., peaks up to about 30 Hz (green shading in Fig. 6.6c) are

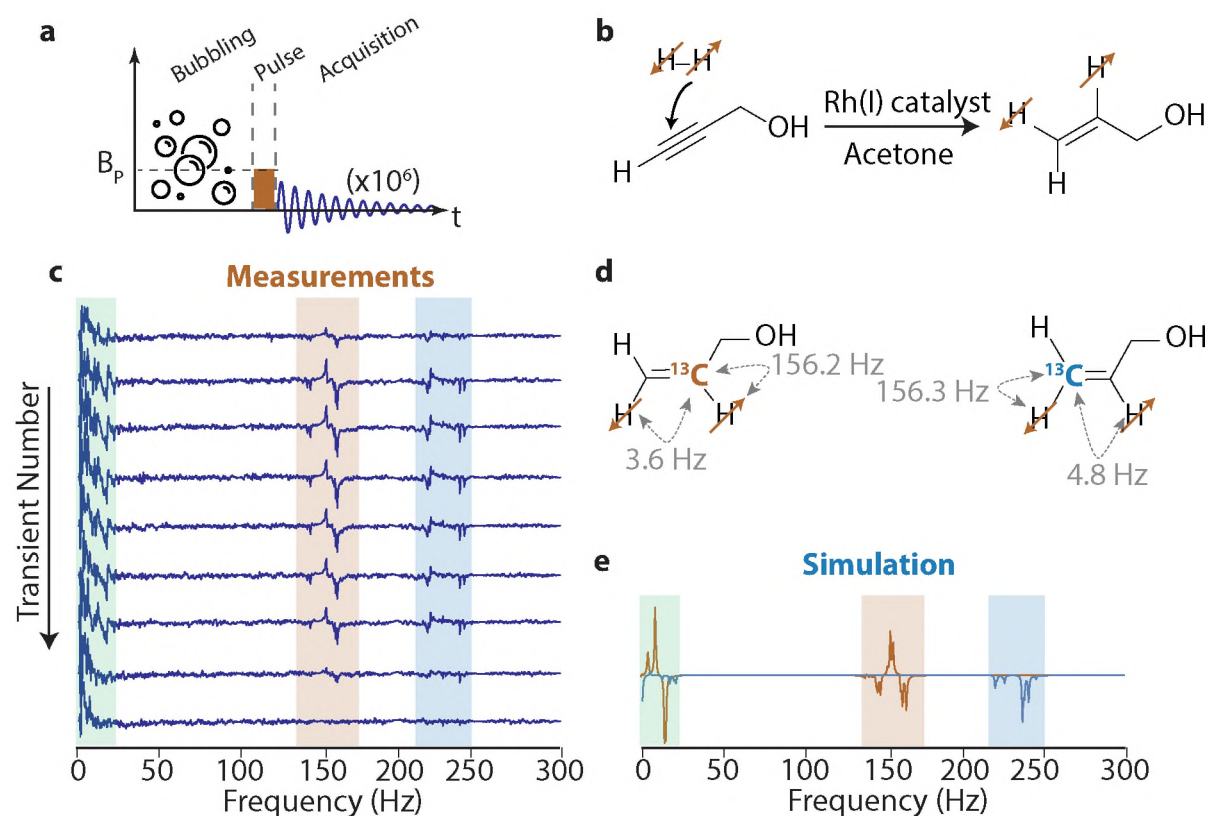


Figure 6.6: (a) Experimental sequence for the zero-field NMR experiment using PHIP. The sequence is divided into three stages: parahydrogen bubbling through a solution in the zero field, a DC magnetic-field pulse, and data acquisition. (b) Scheme of parahydrogenation reaction of propargyl alcohol to form allyl alcohol. (c) Experimental spectra of hyperpolarized allyl alcohol for many transients. (d) Structural formula of  $[2\text{-}^{13}\text{C}_1]$ -allyl alcohol and  $[3\text{-}^{13}\text{C}_1]$ -allyl alcohol with the heteronuclear  $J$ -coupling between parahydrogen-derived hydrogen and  $^{13}\text{C}$  nucleus used to generate the simulated spectra. Peaks in the zero-field spectra form three groups highlighted with green, orange, and blue shading (for further discussion, see main text). (e) Simulated spectra of  $[2\text{-}^{13}\text{C}_1]$ -allyl alcohol (orange solid line) and  $[3\text{-}^{13}\text{C}_1]$ -allyl alcohol (blue solid line).

results of the weak  $J$ -coupling interactions in  $[2\text{-}^{13}\text{C}_1]\text{-}$ , and  $[3\text{-}^{13}\text{C}_1]\text{-allyl}$  alcohol, as well as,  $[1\text{-}^{13}\text{C}_1]\text{-allyl}$  alcohol. Thus, due to many overlapping peaks in this small region of the spectrum, a complex spectral pattern is observed. Additionally, flicker noise hinders the analysis of the low-frequency region in a spectrum. However, apart from our interpretation of spectral patterns, the zero-field spectra of  $[2\text{-}^{13}\text{C}_1]\text{-allyl}$  alcohol and  $[3\text{-}^{13}\text{C}_1]\text{-allyl}$  alcohol were simulated (see Sec. 6.3.1) and a good agreement between the experimental and simulated spectra was achieved (as demonstrated in Figs. 6.6c and e).

As the next step, polarization transfer from the transfer agent, hyperpolarized allyl alcohol to a target molecule, water was investigated. A solution of 0.5 M propargyl alcohol, 7 mM Rh(I)-catalyst in acetone was bubbled with parahydrogen for 20 s at 3 mT magnetic field. Subsequently, the bubbling field was adiabatically switched off, and following a DC pulse, an ultra-low field spectrum of hyperpolarized allyl alcohol was acquired where the detection field was  $\approx 820$  nT (Fig. 6.7a). In the spectrum, the peak with high amplitude at about 35 Hz originates from the precession of protons uncoupled to  $^{13}\text{C}$ , while the peaks around this peak in 25-45 Hz range with a lower amplitude are results of  $^{13}\text{C}\text{-}^1\text{H}$  couplings in isotopomers of hyperpolarized allyl alcohol. The polarization level in the target molecule after the PHIP-X process strongly depends on the transfer agent polarization and also the distribution of the polarization across all protons in the molecule, especially labile proton in -OH group.

Since the peak at 35 Hz represents protons uncoupled to  $^{13}\text{C}$  including labile proton which exchanges between transfer agent and target molecule (e.g. water), we examine the influence of water content in sample on the intensity of this peak. Samples of 0.5 M propargyl alcohol and 7 mM Rh(I)-catalyst in acetone with various concentrations of water (0-5 M) were prepared for this study. Parahydrogen gas was bubbled through each sample for 20 s under 1 mT field. Subsequent a DC pulse, an ULF spectrum was recorded. For each sample the sequence repeated three times. Averaged peak amplitudes with standard error values are presented in Fig. 6.7c. A significant signal enhancement was observed in the spectrum of the sample with 0.5 M water and propargyl alcohol, supporting the argument that the most efficient polarization transfer occurs when the transfer

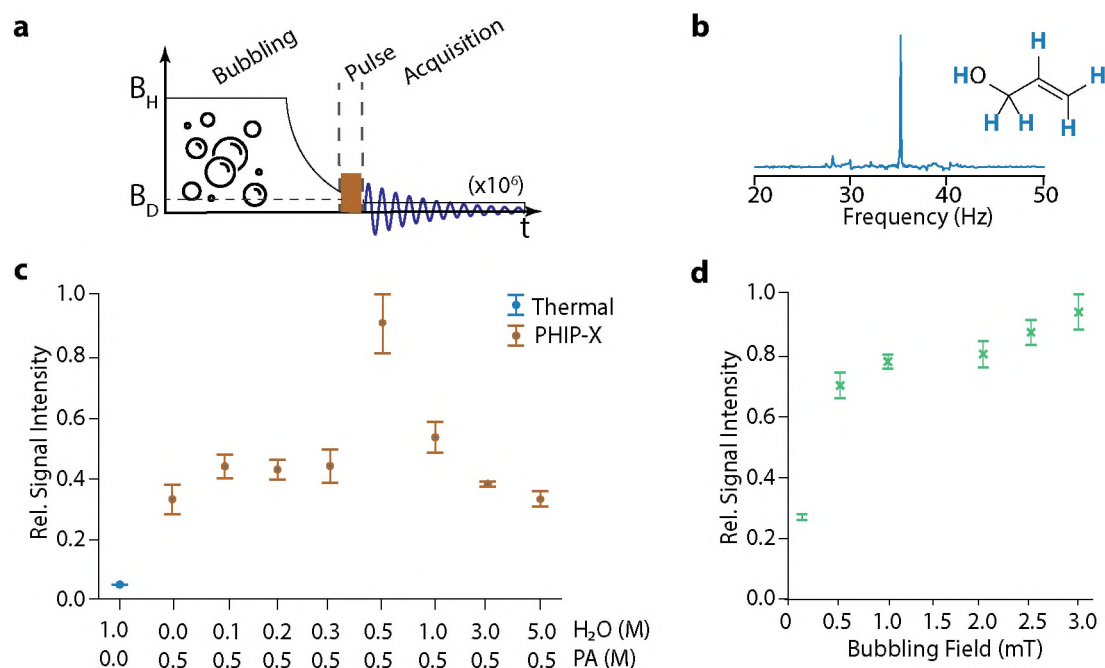


Figure 6.7: (a) Experimental sequence for the ULF NMR experiment using PHIP and PHIP-X. The sequence is divided into three steps: parahydrogen bubbling through a solution in ultralow field, a DC pulse, and data acquisition.  $B_H$  is the magnetic field during parahydrogen bubbling, while the detection field is denoted by  $B_D$ . (b) Ultralow-field NMR spectrum of hyperpolarized allyl alcohol. The main peak at 35 Hz arises from the precession of protons uncoupled to the  $^{13}\text{C}$  nucleus, while the peaks around the main line with a lower amplitude are results of  $^{13}\text{C}$ - $^1\text{H}$  couplings in isotopomers of allyl alcohol. (c) Comparison of signal amplitude at 35 Hz, originating from protons uncoupled to  $^{13}\text{C}$  in allyl alcohol (0.5 M), and various concentration of water (0–5 M) hyperpolarized via PHIP/PHIP-X (orange data points). For a comparison, relative signal intensity in the ULF NMR spectrum of thermally-prepolarized water protons is presented as a blue data point. Data is presented as mean  $\pm$  standard error. (d) The influence of the  $B_H$  (0.1–3 mT) field on the amplitude of peak associated with protons uncoupled to  $^{13}\text{C}$ . Data is presented as mean  $\pm$  standard error.

and target molecule are present at the same concentration in a sample. When samples contained higher amount of water, the signal intensity drops considerably. The reason might be inefficient polarization transfer as a result of too rapid proton exchange, and increased proton pool to polarize despite limited available parahydrogen (spin-dilution), besides the reduced catalyst activity in aqueous media [117, 118]. This limitation comes into prominence, particularly if PHIP-X is harnessed for a study with samples in aqueous media such as cell cultures, urine, blood, etc. Furthermore, in Fig. 6.7c, a result of measurement after thermal prepolarization using a 1.4 T magnet was denoted as a blue point demonstrating the achievement of two orders of magnitude signal enhancement for water protons via PHIP-X.

Lastly, the impact of bubbling field on signal enhancement by PHIP/PHIP-X was studied using ULF NMR. Samples of 0.5 M propargyl alcohol, 7 mM Rh(I)-catalyst, and 0.5 M water were prepared. Each sample was supplied with parahydrogen for 6 s at a given magnetic field (0.1-3 mT). The experimental sequence demonstrated in Fig. 6.7a repeated six times for each sample. Averaged peak amplitudes with standard error values are presented in Fig. 6.7d. When the bubbling field increases from 0.1 mT to 3 mT, the signal intensity grows gradually. This outcome is also congruent with the results of the high-field NMR study presented in the previous section. Especially, the elimination of the need for a sample transfer to apply desired ultralow field during the parahydrogenation reaction makes ZULF NMR an appealing method for PHIP-X. Even though we demonstrate the feasibility of the use of the PHIP-X technique for ZULF NMR spectroscopy, this subject requires further study towards the sensitive detection of a wide range of biomolecules.

## 6.4 Conclusion

In this study, we employed a newly investigated hyperpolarization, called PHIP-X, to hyperpolarize biologically relevant materials such as urea, amino acids, ammonium, and glucose. Owing to the reported optimization studies, we achieved a 17,100-fold enhancement of the urea signal through PHIP-X compared to the thermal signal measured at

1 T. We also showed that the use of a higher concentration of the transfer agent enables re-hyperpolarization of the target molecule with no change in its identity, thus yielding the acquisition of accumulated signals with a higher signal-to-noise ratio. In using hyperpolarized biomolecules as MRI probes for various diseases, the leading challenge is the non-biocompatible components of hyperpolarized samples: the toxic solvent, the catalyst, and, in the case of PHIP-X, the transfer agent, all of which must be removed from solution [45, 119, 120]. On the other hand, novel approaches to purify the sample solution after hyperpolarization using parahydrogen hold great promise for *in vivo* applications [121].

We have also introduced the PHIP-X technique for ZULF NMR spectroscopy, enabling the increase in the efficiency of the hyperpolarization of the transfer agent, allyl alcohol, and the target molecule, water. This study showed the feasibility of developing the ZULF NMR spectrometer combined with the PHIP-X technique as a highly specific, sensitive, compact, and inexpensive sensor for the analysis of (bio)chemicals.

# Chapter 7

## Zero- and Ultralow-Field NMR Relaxometry

The material presented in this chapter is being prepared for publication:

**S. Alcicek**, P. Put, A. Kubrak, F. Alcicek, J. Dybas, D. Barskiy, S. Pustelny, "Zero and Ultralow Field NMR Relaxometry for Chemical and Biological Analysis", In preparation.

### 7.1 Motivation and Overview

NMR relaxometry is a method that enables the characterization of physical and dynamical properties of samples by measuring nuclear-spin relaxation times. Measurements of longitudinal and transverse relaxation times, denoted as  $T_1$  and  $T_2$ , respectively, provide valuable information. For example, the  $T_2$  variation, originating from the change in the blood proteome, could be an indicator for inflammation, insulin resistance [122].

Recently, ultralow-field NMR relaxometry became possible [123]. This is due to the application of non-inductive sensors, which are overly sensitive to low-frequency signals (up to 1 kHz) [5]. As already mentioned many times, among the sensors, atomic magnetometers are the most promising candidates due to their high sensitivity, considerable bandwidth, low price, small size, and non-cryogenic operation [6, 7].

In this chapter, the investigation of NMR relaxometry of chemical and biological samples at zero and ultralow fields is presented. Initially, the effect of dissolved paramagnetic oxygen on relaxation processes is examined for trimethyl-phosphate, and  $^{13}\text{C}$ -formic acid which have long-lived heteronuclear singlet states [17] and numerous other states. This is done by the analysis of ZULF NMR line positions that are determined by the  $^1\text{H}$ - $^{31}\text{P}$  and  $^1\text{H}$ - $^{13}\text{C}$   $J$ -couplings. Next, different concentrations of  $\text{CuSO}_4$  solutions (0.2-1 mM), which are of particular interest for quantitative imaging studies using phantom (to mimic bio-

logical samples), are used to study the influence of paramagnetic agents on water-proton relaxation under ULFs (10-500  $\mu$ T). Finally,  $T_1$  and  $T_2$  relaxation magnetic-field profiles of human whole-blood and blood plasma are investigated at ULFs to demonstrate the potential of the ZULF NMR relaxometry method for analysis of biological samples.

## 7.2 Material and Methods

### 7.2.1 Sample Preparation

Trimethyl phosphate (CAS# 512-56-1) and  $^{13}\text{C}$ -formic acid (CAS# 1633-56-3) were purchased from Sigma-Aldrich. For each compound, four neat liquid samples with a 0.2-ml volume were prepared by transferring them into standard 5 mm NMR tubes. To investigate the effect of oxygen on relaxation times in each compound, one sample was sealed without being degassed. The other three samples were degassed one, two, and three times using the freeze-pump-thaw method with the use of a two-stage vacuum pump, enabling pumping out the pressure to a level of  $10^{-6}$  mbar. After the degassing, the tubes were flame-sealed under vacuum.

Copper sulfate ( $\text{CuSO}_4$ ) (CAS# 7758-98-7) was also purchased from Sigma-Aldrich. The  $\text{CuSO}_4$  solutions with various concentrations were prepared by dissolving the compound in distilled water. For each concentration, 0.2 ml of solution were placed inside two standard 5 mm NMR tubes. One of the tubes was sealed without degassing, the other tube was degassed twice using the freeze-pump-thaw method and then flame-sealed. The pressure on the frozen liquid after the second cycle was already  $<10^{-4}$  mbar.

Human whole-blood samples from healthy adults (male volunteers) were collected in tubes containing heparin as an anticoagulant (volume ratio: 9:1) on the day of the experiment (blood came from the Regional Blood Transfusion Centre, Kraków, Poland). The volunteer donors had not taken any medicines for the two weeks prior to the withdrawal. Informed consent was given by each volunteer prior to the blood withdrawal and the study was in accordance with the principles outlined in the World Medical Association (WMA) Declaration of Helsinki, as well as Bioethical Commission of the Jagiellonian University.

300  $\mu\text{L}$  of whole blood were kept for the measurements. The remaining whole blood was subjected to centrifugation (acceleration of 800 g and run time: 15 min at room temperature, with soft stop) to separate the plasma from the rest of the blood components. Plasma was collected and placed in Eppendorf tubes for further sample preparation procedures and measurements. 0.2 ml of whole blood and plasma were transferred to NMR tubes and flame-sealed without degassing. The samples were kept at 4°C to reduce the metabolism until measurement and they were measured up to an hour after blood withdrawal.

### 7.2.2 Experimental Technique

The ZULF NMR setups are described in Chapters 3 and 5 are used for zero-field and ultra-low field relaxation measurements, respectively. For this research, 1.4 T permanent magnet was placed on top of the mu-metal shield in the experimental setup with commercial magnetometers, after removing the vapor condenser (Fig. 5.3).

For the relaxation study using zero-field NMR, the NMR samples, prepolarized for about 20 s inside the permanent magnet, were mechanically shuttled to the zero-field region through a guiding field (10  $\mu\text{T}$ ) applied by the solenoid (Fig. 7.1a). The NMR sample reached the detection region in roughly 300 ms. Since sample transfer caused vibration noises that disrupted data acquisition, the generation of the zero-field NMR signal was suspended for another 700 ms, so that the shortest total time between the end of polarization and the beginning of the acquisition was 1 s. Varying this waiting time in the ultra-low field from 1 s to 15 s in zero-field NMR experiments, the longitudinal relaxation time constants ( $T_1$ ) for formic acid and trimethyl phosphate solutions with different dissolved oxygen concentrations were calculated. After the given storage time, the guiding field was switched off suddenly, and then an optimal transverse pulse was applied to generate oscillating magnetization with a maximum amplitude. For the  $^1\text{H}$ - $^{13}\text{C}$  spin system, the optimal pulse-induced rotation angle is  $4\pi$  for  $^1\text{H}$ , while in the  $^1\text{H}$ - $^{31}\text{P}$  system, the maximum amplitude is achieved with  $5\pi$  pulse angle for the  $^1\text{H}$  spin (see further discussion in Chapters 2 and 3).

For the investigation of the water-proton relaxation times in the  $\text{CuSO}_4$  solutions, whole blood, and plasma, ULF NMR spectroscopy was used. After thermal prepolarization for 15 s in 1.4 T permanent magnet, the NMR sample was transferred into the zero-field region in a minimum 500 ms period. Note the stepper motor in this experimental setup allowed us to perform faster sample transfer compared to the other setup in which zero-field measurements were conducted. When the sample reached the detection region, it was stored in various fields ( $10\text{--}510\ \mu\text{T}$ ) at the given time ( $0.5\text{--}6.05\ \text{s}$ ) to obtain the  $T_1$  relaxation profile. Then the guiding field was switched off while a detection field of  $\approx 800\ \text{nT}$ , corresponding to 34 Hz of precession of  $^1\text{H}$ , was applied along the transverse axis (Fig. 7.1b). The detection field was chosen in such a way that the proton-precession signal appeared in a convenient region of the spectrum, i.e., far from 50 Hz electrical noise and low-frequency flicker noise. It should be noted that further increase of the solenoid storage field could introduce inhomogeneities, which may affect relaxation.

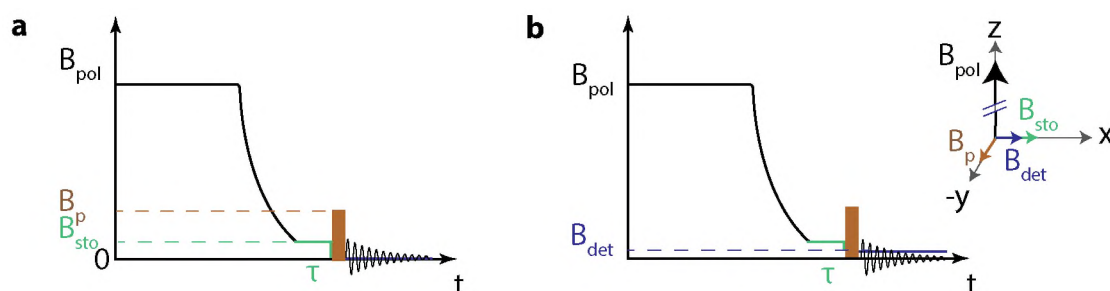


Figure 7.1: Experimental sequence for zero- (a) and ultralow-field (b) NMR relaxometry. The magnetic field, experienced by the sample during the experiment, is plotted. The initial period of thermal prepolarization in the strong field  $B_{\text{pol}}$  is followed by a non-adiabatic transfer to ZULF. Subsequent to transfer, the sample is kept under the storage field  $B_{\text{sto}}$  for a given time  $\tau$ . Then the DC pulse of the magnetic field  $B_{\text{p}}$  is applied to generate oscillating magnetization detectable by the magnetometer. By varying  $\tau$ , the relaxation profile of nuclei in ULFs can be determined. The main difference between (a) and (b) sequences is the application of the detection field  $B_{\text{det}}$  to obtain the ULF NMR spectrum (see main text).

In the  $T_1$  studies, each data point was obtained after averaging 4-16 transients and is presented with a standard deviation as an error bar. The transverse relaxation times  $T_2$  were calculated from the linewidths of the peaks in spectra. Full maximum half-width (FWHM) values were derived by fitting NMR spectra to the Lorentzian function (see Sec. 2.1.5). Standard errors on the fitting parameters are denoted with error bars. Data

processing and data analysis was performed using Python as described in Sec. 3.2.3.

## 7.3 Results and Discussion

### 7.3.1 Effect of Oxygen on Zero-field NMR

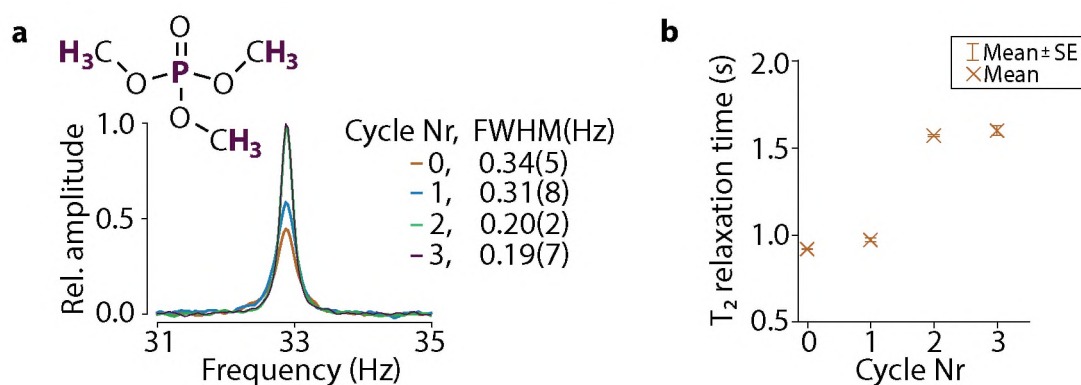


Figure 7.2: (a) Zero-field NMR resonance (33 Hz) measured in the  $J$ -spectra of the trimethyl phosphate samples prepared with a different number of the freeze-pump-thaw cycles (0, 1, 2, and 3). (b) Measured transverse relaxation time  $T_2$  of trimethyl phosphate versus the number of the freeze-pump-thaw cycles performed during sample preparation.

Paramagnetic nature of oxygen molecules, originating from unpaired electrons, influence the nuclear spin relaxation process [124]. This effect can be demonstrated using ZULF NMR relaxometry by observing zero-field NMR spectra and using the measurements to extract information about the longitudinal and transverse relaxation constants. To avoid fast spin relaxation of the molecules, caused by dissolved oxygen in NMR solutions, ZULF NMR samples are usually prepared using the freeze-thaw-pump technique. The technique allows to degas the samples, thereby affecting the lifetime of spin polarization, but the significance of this procedure on ZULF NMR has never been demonstrated. In order to study the effect, first, we examined the trimethyl phosphate solutions after application of the preparation procedure that involved a different number of freeze-thaw-pump cycles. The reduced amount of oxygen dissolved in trimethyl phosphate allows slower relaxation and hence quantitative measurements of spin relaxation.

The detailed discussion of the trimethyl phosphate zero-field spectra can be found in Chapter 3. Here, we only study the peak at about 33 Hz, arising from the  $^1\text{H}$ - $^{31}\text{P}$

three-bond  $J$ -coupling. The analysis allows us to extract the peak linewidth, and hence gain information about relaxation.

As demonstrated in Fig. 7.2a, a substantial increase in the amplitude of the 33 Hz peak and a decrease in its linewidth were observed up to two freeze-pump-thaw cycles. This change can be result of longer  $T_1$  and  $T_2$  relaxation process. The derived transverse relaxation times  $T_2$  are presented in Fig. 7.2b. The results show that no significant differences in the signal intensity and linewidth were observed by increasing the number of cycles above two (compare the results obtained with 2 and 3 cycles). Most likely, after two cycles of vacuuming, an oxygen level in the solution is small (potentially negligible) in terms of its contribution to relaxation. Thus no improvement in the signal with the third cycle of vacuuming was achieved. Alternatively, even though the vacuum pump is eligible to pump out the pressure over the frozen liquid to about  $10^{-6}$  mbar, it might not be sufficient to remove remaining dissolved oxygen any further after 2 cycles.

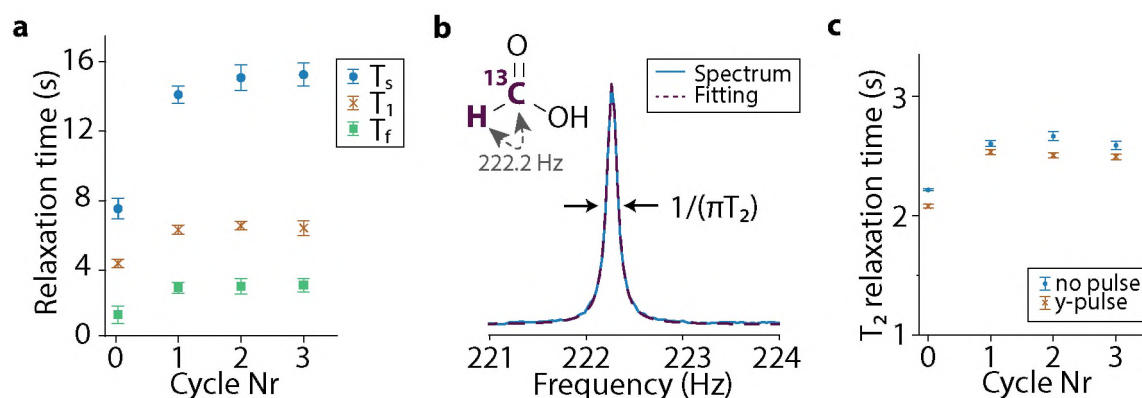


Figure 7.3: (a) Relaxation times  $T_1$ ,  $T_f$ , and  $T_s$  of  $^{13}\text{C}$ -formic acid versus the number of the freeze-pump-thaw cycles performed during sample preparation. (b) As an example, a part of the experimental zero-field spectrum of  $^{13}\text{C}$ -formic acid (solid blue line) containing the 222.2 Hz peak, originating from the  $^1\text{H}$ - $^{13}\text{C}$  coupling. The fitting of the peak to the Lorentzian function is presented as a dashed line. (c) Transverse relaxation time  $T_2$  extracted from experimental signal induced by a non-adiabatic transition to the zero field with/without  $y$ -pulse.

As the next step, we investigated the influence of paramagnetic oxygen on long-lived spin-singlet states, which are relatively protected from the source of relaxation. Such a state has been demonstrated in high-field NMR for homonuclear spin-1/2 pairs (e.g., parahydrogen), where two coupled spins are magnetically equivalent, and they compose

a spin-0 system (singlet state) and a spin-1 system (triplet state). The triplet states are symmetric and the singlet is antisymmetric with respect to the interchange of the spin labels. Since the main relaxation mechanism, the dipolar coupling, is symmetric with respect to the spin exchange of particles, it is unlikely to cause a symmetry-breaking singlet-triplet transition, thus dipole-dipole coupling cannot contribute to the relaxation of the singlet state [125]. Moreover, at zero-field, strong coupled heteronuclear spin-1/2 pairs can be considered as singlet-triplet states as well. Therefore, heteronuclear long-lived singlet states were previously reported for  $^{13}\text{C}$ -formic acid and  $^{13}\text{C}_1$ -benzene under the zero-field regime [17]. The zero-field spectrum of  $^{13}\text{C}$ -formic acid consists of a peak at about 222.2 Hz equal to the strength of the one-bond  $^1\text{H}$ - $^{13}\text{C}$  coupling while the proton in the hydroxyl group can be neglected due to fast exchange. As discussed in Sec. 2.2 in detail, after thermal prepolarization using the permanent magnet, followed by the non-adiabatic transfer to the ZULF region, the guiding field (so-called storage field) was switched off suddenly to generate coherences between the zero-field states (e.g., singlet and triplet). In this case, the relaxation was separated in two processes characterized by so-called slow relaxation (singlet lifetime) with  $T_s$  time constant and fast relaxation determined by the relaxation time  $T_f$ . The transition between the singlet  $|S_0\rangle$  and triplet states ( $|T_{+1}\rangle$ ,  $|T_0\rangle$ , and  $|T_{-1}\rangle$ ) causes slow decay and indicates the lifetime of the singlet state. On the other hand, the building the equilibrium among triplet states results in fast relaxation by reducing the population difference between  $|S_0\rangle$  and  $|T_0\rangle$ . These constants can be derived from bi-exponential decay of zero-field NMR signal (see Sec. 7.2.2 for details).

Alternatively, after a sudden switch of the guiding field, a transverse DC pulse ( $y$ -pulse) can be applied to manipulate the polarization in the singlet and triplet manifolds (Fig. 2.11). In this case, since a signal intensity is proportional to a population difference between the  $|T_{+1}\rangle$  and  $|T_{-1}\rangle$  states (Sec. 2.2), the lifetime of this difference is described by the  $T_1$  value, similar to that used in the high-field NMR description. This constant can be easily derived from an exponential decay of the zero-field signal (see Sec. 7.2.2 for details).

In this study, we examine the variation of the  $T_s$ ,  $T_f$ , and  $T_1$  constants measured in  $^{13}\text{C}$ -formic acid solutions prepared with different times of freeze-pump-thaw cycles (Fig. 7.3a). In addition, the linewidth of the peak at zero-field NMR is used to extract information about the transverse relaxation time,  $T_2$  (Fig. 7.3b). The calculated  $T_2$  constants are reported for two described experimental schemes (with/without the transverse  $y$ -pulse after the sudden switch off) in Fig. 7.3c. While the increase in all relaxation terms is observed up to 2 cycle of vacuuming due to efficient removal of oxygen from the sample, there is no significant contribution of the third free-thaw-vacuum cycle. To explain this, the discussion presented above can be refined. It should be mentioned that the highest relaxation constants derived in this study is lower than the values reported in Ref. [17]. The reason is that even though there is no peak in the zero-field spectrum of  $^{13}\text{C}$ -formic acid that originates from the exchangeable proton in the hydroxyl group, the contribution of the proton-exchange-mediated relaxation mechanism is noticed with the shortened relaxation time. Increasing the proton-exchange rate enables elimination of this relaxation mechanism. However, contrary to the previous study where water and acetonitrile were mixed to induce chemical exchange, in our study, we used neat  $^{13}\text{C}$ -formic acid. Nevertheless, besides presenting the influence of oxygen on the long-lived singlet state, we report the singlet lifetime in neat  $^{13}\text{C}$ -formic acid as about 16 s. It should be also noted that the experiments with a non-adiabatic transfer results in narrower zero-field peaks compared to the measurements with an additional transfers pulse which is an indicator of a longer  $T_2$  value. On the other hand, as discussed in Sec. 2.2, application of the transverse pulse increased the signal intensity.

As shown in this part of the work, a paramagnetic oxygen molecule has an impact on zero-field NMR, therefore dissolved oxygen level could be quantified through ZULF NMR relaxometry studies to detect paramagnetic gas impurities [126, 127].

### 7.3.2 Effect of Paramagnetic $\text{CuSO}_4$ on Ultralow Field NMR

In order to demonstrate the feasibility of the ZULF NMR relaxometry for the analysis of biological samples, paramagnetic copper sulfate ( $\text{CuSO}_4$ ), which is the widely utilized

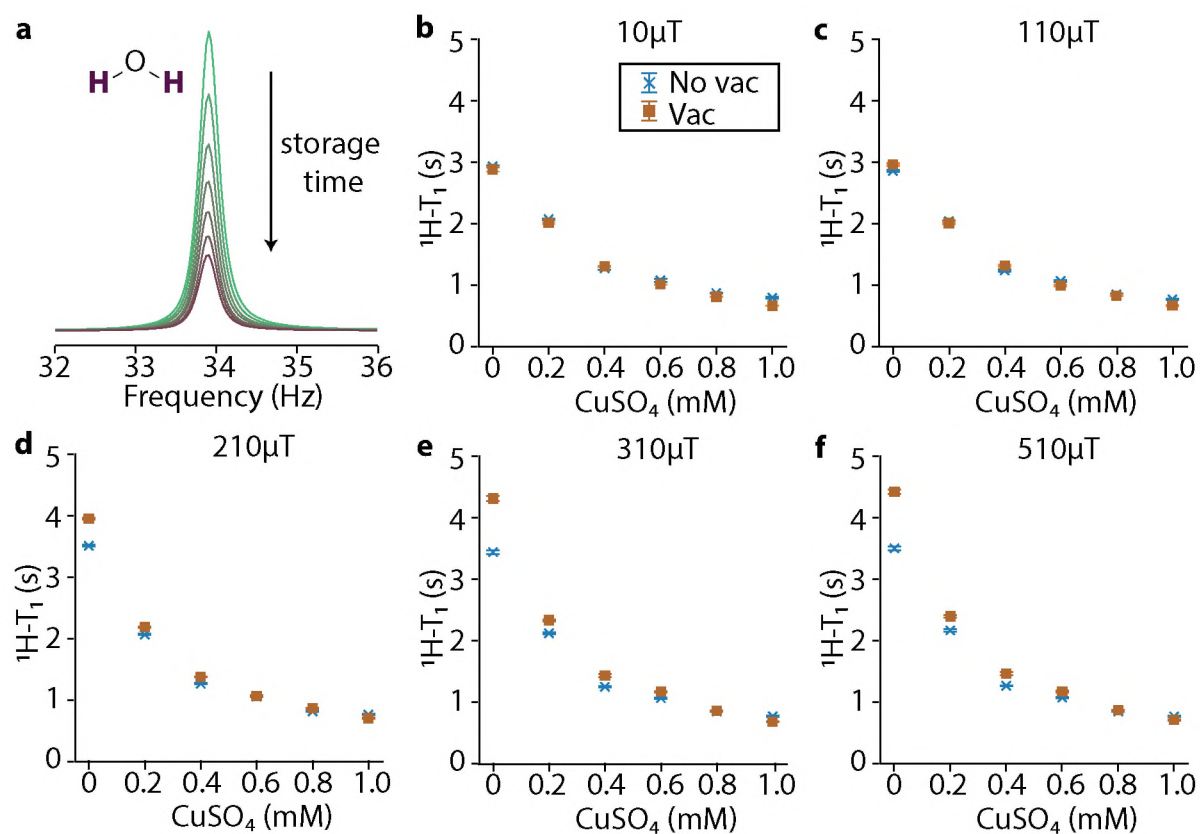


Figure 7.4: (a) Ultralow-field experimental spectra, consisting of a peak at about 34 Hz, versus the storage time. (b-f) The variation in  $T_1$  relaxation time constant of water protons at various ULFs (10-510  $\mu\text{T}$ ) with respect to the different  $\text{CuSO}_4$  concentration (mM) in a solution prepared with/without 2 cycles of freeze-pump-thaw (denoted as Vac and No vac, respectively) prior to the measurement.

for quantitative imaging studies of “phantoms” by mimicking samples with short relaxation times, was used [128]. Water-proton precession was observed using the ULF NMR spectroscopy to gather information about the  $T_1$  and  $T_2$  relaxation processes which are accelerated by paramagnetic ions in solution (Figs. 7.4a and 7.5a). As demonstrated in Fig. 7.4b-f, when the  $\text{CuSO}_4$  concentration in solution rose, the expected decreasing trend in the proton  $T_1$  relaxation time was observed. On the other hand, the gradual rise of the storage field from  $10\mu\text{T}$  to  $510\mu\text{T}$  results in the steady increase of the  $T_1$  relaxation time. It was also observed that oxygen has a great impact on the relaxation process in the case of higher storage fields and lower  $\text{CuSO}_4$  concentration. On the other hand, no significant influence of dissolved oxygen on  $T_1$  relaxation was observed when the  $\text{CuSO}_4$  concentration was increased and/or the storage field was decreased. This could be a result of the negligible contribution of dissolved oxygen to the  $T_1$  relaxation process, compared to the contribution of the high concentrate  $\text{CuSO}_4$ . Also, since the  $T_1$  relaxation time is shorter for lower storage fields, the effect of oxygen might become indistinguishable. Furthermore, to test the detection limit of ZULF NMR relaxometry, even higher concentrations of  $\text{CuSO}_4$  were measured. The proton  $T_1$  constants at  $10\mu\text{T}$  in 1.5 and 2 mM  $\text{CuSO}_4$  solutions were derived as  $0.382\pm0.05$  and  $0.364\pm0.07$ , and it should be also mentioned that the proton peak on the ULF NMR spectrum was visible evidently even after a single transient in spite of quick relaxation.

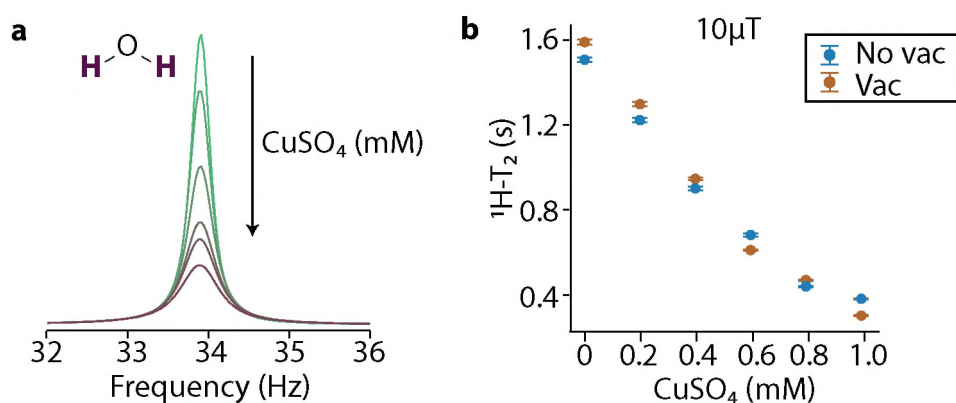


Figure 7.5: (a) As an example, a serial experimental ultralow-field spectra consisting of a peak at about 34 Hz versus various  $\text{CuSO}_4$  concentration (mM). (b)  $T_2$  relaxation time constants of water protons versus different  $\text{CuSO}_4$  concentration (mM) in solution prepared with/without 2 cycle of freeze-pump-thaw (denoted as Vac and No vac, respectively).

In Fig. 7.5a, the line-broadening, induced by paramagnetic  $\text{CuSO}_4$ , is demonstrated. As expected, the derived  $T_2$  time is gradually decrease by increase of an amount of paramagnetic species in solution. According to general theory, the relaxation rates ( $1/T_1$  and  $1/T_2$ ) are proportional to the concentration of the paramagnetic species and can be calculated by summing paramagnetic and diamagnetic relaxation rates [129]. As shown here, the results agree well with this general statement. Additionally, it is worth underlining that despite the fast relaxation of protons ( $T_1$  and  $T_2$  on the order of 300 ms) induced by paramagnetic ions, the peak originating from water protons in the ULF NMR spectrum can be visible evidently allowing us to perform a relaxation study. This phantom study has paved the way for the first-ever ULF NMR relaxometry study with human blood using an optical magnetometer.

### 7.3.3 Ultralow-Field Relaxometry of Whole Blood and Plasma

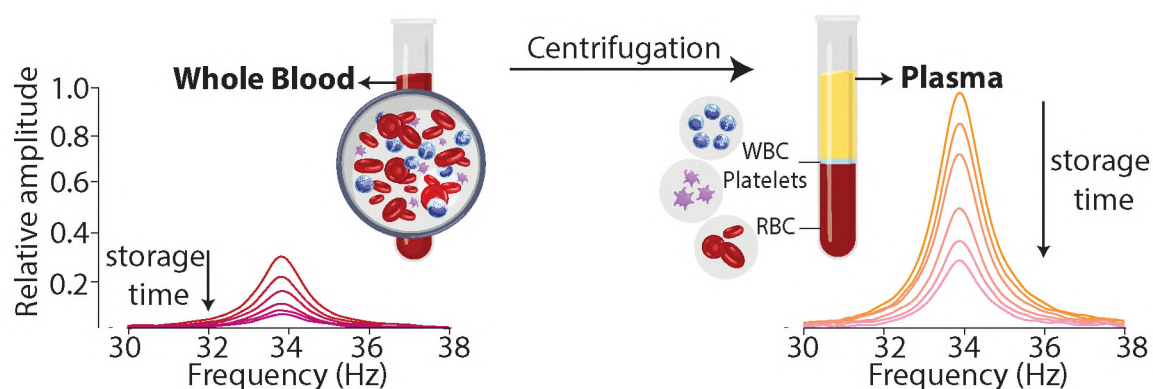


Figure 7.6: First-ever  $^1\text{H}$  ULF NMR signal of human whole-blood (left) and blood plasma (right) detected by optical magnetometer. The signals were measured with the 10  $\mu\text{T}$  field by increasing the storage time.

Blood is an attractive biological specimen for NMR relaxometry studies, which holds great promise in diagnoses and prognosis of metabolic disorders (e.g., insulin resistance and dyslipidemia), inflammation (candidiasis, malaria) and hemostatic disorders. This is achieved by probing the relaxation rate of protons [130–133]. The blood with all its components (white and red blood cells, platelets, and plasma) intact, that has been collected using a tube containing an anticoagulant solution, is called whole blood. On the other hand, plasma (45% of whole-blood) is the cell-free supernatant obtained by centrifuging

blood. The high water content of whole blood and plasma allows us to observe a ULF NMR proton signal. The relaxation properties of water can provide valuable information about blood proteomes, since water protons form hydrogen bonds with proteins, lipoproteins, and metabolites in the blood, influencing the relaxation process. While the contribution of macromolecules (proteins and lipoproteins) to the relaxation process is considerable, the influence of small metabolites (e.g., glucose and amino acids) on the process can remain negligible due to their fast molecular motion, causing inefficient dipolar relaxation [133, 134]. While the variation in the molecular weight profile of blood proteins also affects the relaxation rate, in general, a linear correlation between protein concentration and relaxation rate is expected due to the slower rotational and diffusional mobility of macromolecules (see Chapter 2 for detailed discussion) [135].

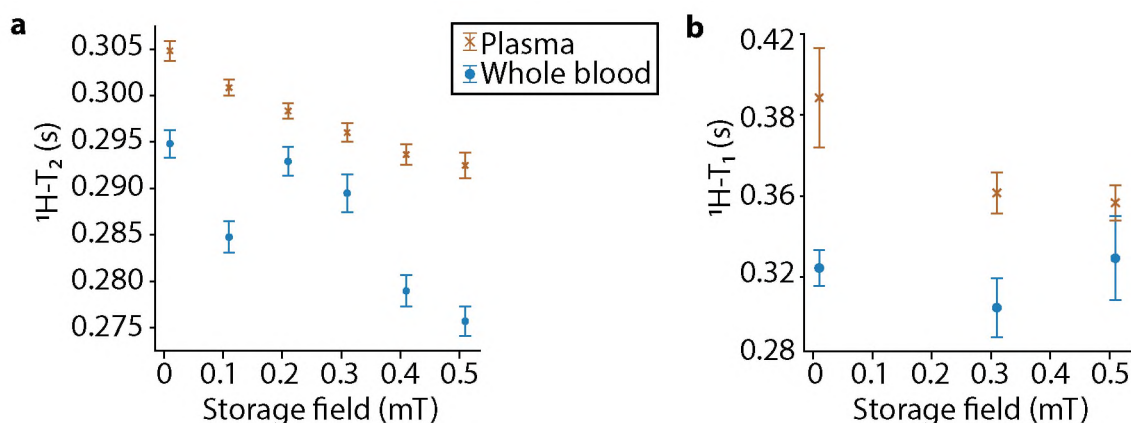


Figure 7.7:  $T_1$  and  $T_2$  relaxation magnetic-field profiles of human whole-blood and blood-plasma measured at ultralow fields (10-510  $\mu\text{T}$ ).

In the last part of the study, whole blood and blood plasma were used to demonstrate the applicability of ULF NMR relaxometry to biological samples (despite their short relaxation). As presented in Fig. 7.6, the  $^1\text{H}$  ULF NMR signal intensity was higher in plasma samples compared to whole-blood samples due to a higher water content in plasma and hence its slower relaxation [136]. The  $T_1$  and  $T_2$  relaxation times of protons at 10  $\mu\text{T}$  were determined as 0.30(4) s - 0.29(5) s; 0.3(9) s - 0.30(4) s for whole-blood and plasma, respectively. Additionally,  $T_1$  and  $T_2$  relaxation magnetic-field profiles of human whole-blood and blood plasma at ULFs was obtained to determine the limitation of the method (Fig. 7.7). The  $T_1$  and  $T_2$  relaxation times of protons in whole-blood samples

was varied with the change in a storage field from 10  $\mu$ T to 510  $\mu$ T without a specific pattern. The reason might be the affects of residual cellular metabolism, which may change the protein content. On the other hand, since there is no cellular component in plasma, the metabolites can remain stable over time [137]. Therefore, plasma and serum are preferable biofluids to study. The  $T_1$  and  $T_2$  relaxation times of protons in plasma decrease with the variation of the storage field from 10  $\mu$ T to 510  $\mu$ T. Besides this inverse relation between the relaxation times and the storage field does not agreed with the literature, it can be explained by the heating effect of an optical magnetometer. In these measurements, the storage field is increased step by step without the change in the sample. Since a sample can reach about 40°C around the optical magnetometer as mentioned in Chapter 2, the repeated measurements might cause an increase in the temperature of the sample over time and that might cause fast relaxation in ULFs [138]. To overcome this limitation, active cooling can be implemented into the setup to maintain temperature stability [123]. It should be also mentioned that the  $T_2$  times are consistently lower than  $T_1$  values because of the additional contribution of low-frequency fluctuations such as slow rotational motions, chemical exchange [8].

## 7.4 Conclusion

In summary, in this study, ZULF NMR relaxometry was employed to collect information on the molecular environment in solutions and biofluids. The influence of paramagnetic oxygen on ZULF NMR spectroscopy was demonstrated by monitoring  $^{31}\text{P}$ - $^1\text{H}$  and  $^{13}\text{C}$ - $^1\text{H}$  couplings. Further, the strong dependence of the lifetime of the heteronuclear singlet state on dissolved oxygen in solution was presented, leading to future research on oxygen level determination with this technique. The  $T_1$  and  $T_2$  relaxation magnetic-field profile, studied with phantoms consisting of paramagnetic  $\text{CuSO}_4$ , agreed well with general relaxation theory as a proof of the reliability of the method. Even though the measured relaxation times of protons are on the order of 300 ms, sensitive optical magnetometers enabled us to detect ULF NMR signals from human whole blood and blood plasma. Our

work demonstrates the applicability of ZULF NMR relaxometry by employing optical magnetometers as a portable, robust, inexpensive, and sensitive tool in chemical and biological analysis.

# Chapter 8

## Summary and Outlook

In this dissertation, the potential use of zero- and ultralow-field nuclear magnetic resonance (ZULF NMR) spectroscopy in many areas of modern science were examined. This was achieved by the development of the technique and its combination with other experimental methods. This technique was next used for the analysis of molecular/biomolecular structures by elucidating their molecular environment based on  $J$ -couplings. This allowed us to measure high-resolution spectra of the molecules and hence gain molecular-specific information about them. In this way, the great capabilities of the technique were demonstrated.

In order to provide good foundations for the presented experimental research, the dissertation started with the introduction of the basics of conventional NMR, and then ZULF NMR was discussed. This approach leads to comparing the techniques, and building intuitions based on the well-known principles of conventional NMR. Subsequently, promising parahydrogen-based hyperpolarization methods, used to solve the inherent sensitivity problem of NMR spectroscopy, were examined for conventional and ZULF NMR.

In Chapter 3, the first-ever theoretical and experimental zero-field NMR studies on a wide and diverse class of chemicals, organophosphorus compounds, were presented. These compounds are particularly attractive for ZULF NMR due to the existence of 100% abundant  $^{31}\text{P}$  (spin-1/2) isotope in the molecules. While reporting distinguishability of the molecules of similar structures, the strongest  $J$ -coupling ( $> 700$  Hz) ever measured in ZULF NMR was demonstrated. This research clearly revealed the capabilities of ZULF NMR in (bio-)chemical analysis of naturally abundant phosphorus compounds, which can be attractive for cell-metabolism studies. A particular example of such research

would be the assessment of energy metabolism that could be performed via monitoring of important phosphorylated metabolites and evaluating tumor differentiation by analysis of phospholipids. Although it is demonstrated that the spectra of coupled ten-spin systems were interpretable, the analysis of more complex systems could be facilitated with the use of additional tools (e.g., dedicated software), which is the subject of an ongoing project in Kraków.

As an alternative method, complex spin systems modified under the chemical-exchange process were investigated in Chapter 4. This approach enables simplifying observed spectral patterns. In this way, the spectral-complexity problem was addressed for several key biomolecules involved in protein metabolism (e.g., urea and amino acids). While monitoring the influence of solvent ( $\text{H}_2\text{O}/\text{D}_2\text{O}$  mixtures, DMSO, acidic/neutral/basic aqueous solutions) on zero-field spectra of the investigated molecules, a straightforward interpretation of  $^1\text{H}/\text{D}$  isotopologues of urea, was demonstrated by analyzing isolated spin subsystems. This study has shown the potential application of zero-field NMR for future *in-vivo/in-vitro* investigations of complex biomolecules in biofluids (such as blood and urine) without the need for water-signal suppression. However, it should not be disregarded that zero-field NMR studies via thermal prepolarization are often performed with highly concentrated (2-8 M) solutions because of the low sensitivity issue which must be addressed.

In this dissertation, to overcome the low-sensitivity limitation of ZULF NMR spectroscopy, parahydrogen-based hyperpolarization techniques were employed. In Chapter 5, *in-situ* SABRE method was studied and the ability of its application to perform multiple/long-term experiments for natural abundance  $^{15}\text{N}$ -pyridine derivatives was demonstrated. The investigated compounds are of particular interest in drug development because of their antimicrobial, antiviral, or anticancer properties. The first-ever  $J$ -spectra of SABRE hyperpolarized biomolecule, nicotinamide (a vitamin  $\text{B}_3$  amide and a potential contrast agent for imaging), and many other hyperpolarized  $^{15}\text{N}$ -pyridine derivatives were reported. With this research, the extension of capabilities of ZULF NMR spectroscopy as a highly specific, sensitive, compact, and inexpensive technique for

chemical and biochemical analysis was presented.

Since one of the goals of the presented research was to develop ZULF NMR spectroscopy as a widely applicable tool, a novel, yet appealing hyperpolarization method, called PHIP-X was studied. The technique is based on the indirect transfer of polarization from parahydrogen to the molecules of interest through a proton-exchange process, which makes the technique applicable for a broad range of molecules. With this advantage, the optimization of the PHIP-X technique was demonstrated using a benchtop NMR/robotic-arm setup for biomolecules and achieved a substantial (17,100-fold) enhancement of the urea signal (Chapter 6). The application of the technique was also shown for many other molecules, including glycine, ammonium, benzamide, and glucose. Finally, the PHIP-X technique was combined with ZULF NMR obtaining promising results on hyperpolarization of a transfer agent and successive polarization transfer to water protons. The use of such hyperpolarized biomolecules as MRI probes for various diseases is an appealing research subject, particularly with new developments on removing non-biocompatible components of hyperpolarized samples [45, 119–121]

Finally, in Chapter 7, another technique for (bio-)chemical analysis, namely ZULF NMR relaxometry was introduced. This technique allowed to gain information on a molecular environment in chemical solutions and biofluids, by investigating longitudinal and transverse relaxation processes in the molecules. Owing to this technique, the strong dependence of the lifetime of the heteronuclear singlet state on dissolved oxygen in solutions was reported, leading to future research on oxygen-level determination. The relaxation magnetic-field profiles of phantoms consisting of paramagnetic  $\text{CuSO}_4$ , which mimic biological samples, were determined and discussed with general relaxation theories. The prime result of these investigations was the first-ever human whole-blood and blood-plasma ultralow-field NMR signals detection using an optical magnetometer. This led to the study of magnetic-field relaxation process in blood, paving way for further investigations into biofluids towards ZULF MRI.

In conclusion, this dissertation has presented the effort on the development of ZULF NMR spectroscopy as a portable, robust, inexpensive, specific, and sensitive analytical

## SUMMARY AND OUTLOOK

---

method with many interesting applications in chemical and biological analysis to be developed in the future.

# Appendix A

## A.1 $J$ -Coupling Constants

Table A.1: The  $J$ -coupling constants of neat  $^{15}\text{N}$ -pyridine and  $^{15}\text{N}$ -pyridine in methanol solution [53].

| $J$ coupling | Neat $^{15}\text{N}$ -pyridine (Hz) | $^{15}\text{N}$ -pyridine in methanol (Hz) |
|--------------|-------------------------------------|--------------------------------------------|
| $J_{12}$     | -10.93                              | -10.14                                     |
| $J_{13}$     | -1.47                               | -1.56                                      |
| $J_{14}$     | 0.27                                | 0.18                                       |
| $J_{23}$     | 4.88                                | 4.87                                       |
| $J_{24}$     | 1.83                                | 1.85                                       |
| $J_{25}$     | 0.97                                | 1.01                                       |
| $J_{26}$     | -0.12                               | -0.15                                      |
| $J_{34}$     | 7.62                                | 7.65                                       |
| $J_{35}$     | 1.38                                | 1.35                                       |
| $J_{36}$     | 0.97                                | 1.01                                       |
| $J_{45}$     | 7.62                                | 7.65                                       |
| $J_{46}$     | 1.83                                | 1.85                                       |
| $J_{56}$     | 4.88                                | 4.87                                       |

Table A.2: The  $J$ -coupling constants of  $^{15}\text{N}$ -nicotinamide and  $^{15}\text{N}$ -3,5 dichloropyridine in methanol [139], [140].

| $J$ coupling | $^{15}\text{N}$ -nicotinamide (Hz) | $^{15}\text{N}$ -3,5 dichloropyridine (Hz) |
|--------------|------------------------------------|--------------------------------------------|
| $J_{12}$     | -10                                | -10                                        |
| $J_{13}$     | -                                  | -                                          |
| $J_{14}$     | -                                  | -                                          |
| $J_{15}$     | 1.7                                | -                                          |
| $J_{16}$     | -10.3                              | -10                                        |
| $J_{23}$     | -                                  | -                                          |
| $J_{24}$     | 2.3                                | -2.1                                       |
| $J_{25}$     | 0.8                                | -                                          |
| $J_{26}$     | -                                  | -2.1                                       |
| $J_{34}$     | -                                  | -                                          |
| $J_{35}$     | -                                  | -                                          |
| $J_{36}$     | -                                  | -                                          |
| $J_{45}$     | 8.0                                | -                                          |
| $J_{46}$     | 1.6                                | -                                          |
| $J_{56}$     | 4.9                                | -                                          |

Table A.3: The  $J$ -coupling constants of  $^{15}\text{N}$ -3-methoxypyridine and  $^{15}\text{N}$ -4-methoxypyridine in methanol [141].

| $J$ coupling | $^{15}\text{N}$ -3-methoxypyridine (Hz) | $^{15}\text{N}$ -4-methoxypyridine (Hz) |
|--------------|-----------------------------------------|-----------------------------------------|
| $J_{12}$     | -10                                     | -10.6                                   |
| $J_{13}$     | -                                       | -1                                      |
| $J_{14}$     | -1.4                                    | -                                       |
| $J_{15}$     | -                                       | -1                                      |
| $J_{16}$     | -10                                     | -10.6                                   |
| $J_{23}$     | -                                       | 5.75                                    |
| $J_{24}$     | 3                                       | -                                       |
| $J_{25}$     | 0.7                                     | 0.6                                     |
| $J_{26}$     | -0.35                                   | -0.2                                    |
| $J_{34}$     | -                                       | -                                       |
| $J_{35}$     | -                                       | 2.6                                     |
| $J_{36}$     | -                                       | 0.6                                     |
| $J_{45}$     | 8.65                                    | -                                       |
| $J_{46}$     | 1.4                                     | -                                       |
| $J_{56}$     | 4.75                                    | 5.75                                    |

Table A.4: The  $J$ -coupling constants of  $^{15}\text{N}$ -3-methylpyridine and  $^{15}\text{N}$ -4-methylpyridine in methanol [140].

| $J$ coupling | $^{15}\text{N}$ -3-methylpyridine (Hz) | $^{15}\text{N}$ -4-methylpyridine (Hz) |
|--------------|----------------------------------------|----------------------------------------|
| $J_{12}$     | -11.2                                  | -10.1                                  |
| $J_{13}$     | -                                      | -1.85                                  |
| $J_{14}$     | -0.4                                   | -                                      |
| $J_{15}$     | -                                      | -1.85                                  |
| $J_{16}$     | 11                                     | -10.1                                  |
| $J_{23}$     | -                                      | 5.2                                    |
| $J_{24}$     | 2.2                                    | -                                      |
| $J_{25}$     | 0.3                                    | 0.8                                    |
| $J_{26}$     | -0.35                                  | 0.4                                    |
| $J_{34}$     | 0.7                                    | 0.7                                    |
| $J_{35}$     | -                                      | 1.7                                    |
| $J_{36}$     | -                                      | 0.8                                    |
| $J_{45}$     | 7.8                                    | 0.7                                    |
| $J_{46}$     | 2.2                                    | -                                      |
| $J_{56}$     | 4.4                                    | 5.2                                    |

## A.2 Zero-field NMR Spectral Analysis via First-order Perturbation Theory

Table A.5: The observable transitions for  $^{13}\text{C}$ -methanol in ultralow field.

| Transition | $F$           | $m_F$                                | $\nu$                         |
|------------|---------------|--------------------------------------|-------------------------------|
| $\nu_1$    | $1 \mapsto 1$ | $0 \mapsto \pm 1$                    | $B_x(\gamma_H + \gamma_C)/2$  |
| $\nu_2$    | $2 \mapsto 2$ | $+1 \mapsto 0, +2; -1 \mapsto 0, -2$ | $B_x(3\gamma_H + \gamma_C)/4$ |
| $\nu_3$    | $1 \mapsto 1$ | $0 \mapsto \pm 1$                    | $B_x(5\gamma_H - \gamma_C)/4$ |
| $\nu_4$    | $0 \mapsto 1$ | $0 \mapsto -1$                       | $J_{CH} - \nu_1$              |
| $\nu_5$    | $0 \mapsto 1$ | $0 \mapsto +1$                       | $J_{CH} + \nu_1$              |
| $\nu_6$    | $1 \mapsto 2$ | $+1 \mapsto 0$                       | $2J_{CH} - \nu_3$             |
| $\nu_7$    | $1 \mapsto 2$ | $0 \mapsto -1$                       | $2J_{CH} - \nu_2$             |
| $\nu_8$    | $1 \mapsto 2$ | $-1 \mapsto -2$                      | $2J_{CH} - 2\nu_2 + \nu_3$    |
| $\nu_9$    | $1 \mapsto 2$ | $+1 \mapsto +2$                      | $2J_{CH} + 2\nu_2 - \nu_3$    |
| $\nu_{10}$ | $1 \mapsto 2$ | $0 \mapsto +1$                       | $2J_{CH} + \nu_2$             |
| $\nu_{11}$ | $1 \mapsto 2$ | $-1 \mapsto 0$                       | $2J_{CH} + \nu_3$             |

---

### A.3 Calculation of Optimal Delays for the INEPT Sequence

The simulations were performed for the spin density matrix where  $^1\text{H}$  nuclei were in a singlet state and other nuclei were non-polarized. Nondiagonal elements of the density matrix expressed in the high field eigenbasis were extracted considering the coherence damping because of averaging over parahydrogenation time. The corresponding matrix was subjected to the basic/ refocused INEPT pulse sequence (i.e., a series of rotations and free-evolution spin-echo delays). Optimum spin-echo delays were estimated by calculating transverse magnetization of  $^{15}\text{N}$ / $^{13}\text{C}$  nuclei (Table A.6).

Table A.6: Spin-echo delays used for the INEPT pulse sequence in the detection of each molecule of interest.

| Target compound                          | Transfer delay (ms) | Refocus delay (ms) |
|------------------------------------------|---------------------|--------------------|
| $[^{13}\text{C}, ^{15}\text{N}_2]$ -urea | 2.8                 | 1.4                |
| $[^{15}\text{N}_2]$ -urea                | 2.8                 | 1.4                |
| $[^{15}\text{N}]$ -glycin                | 2.7                 | 1.35               |
| $[^{15}\text{N}]$ -benzamide             | 2.8                 | 1.4                |
| $[^{15}\text{N}]$ -ammonium nitrate      | 3.3                 | 1.                 |
| D- $[1\text{-}^{13}\text{C}]$ -glucose   | 1.5                 | 1.5                |

### A.4 Conventional NMR Spectral Analysis for Kinetic Study of PHIP reaction

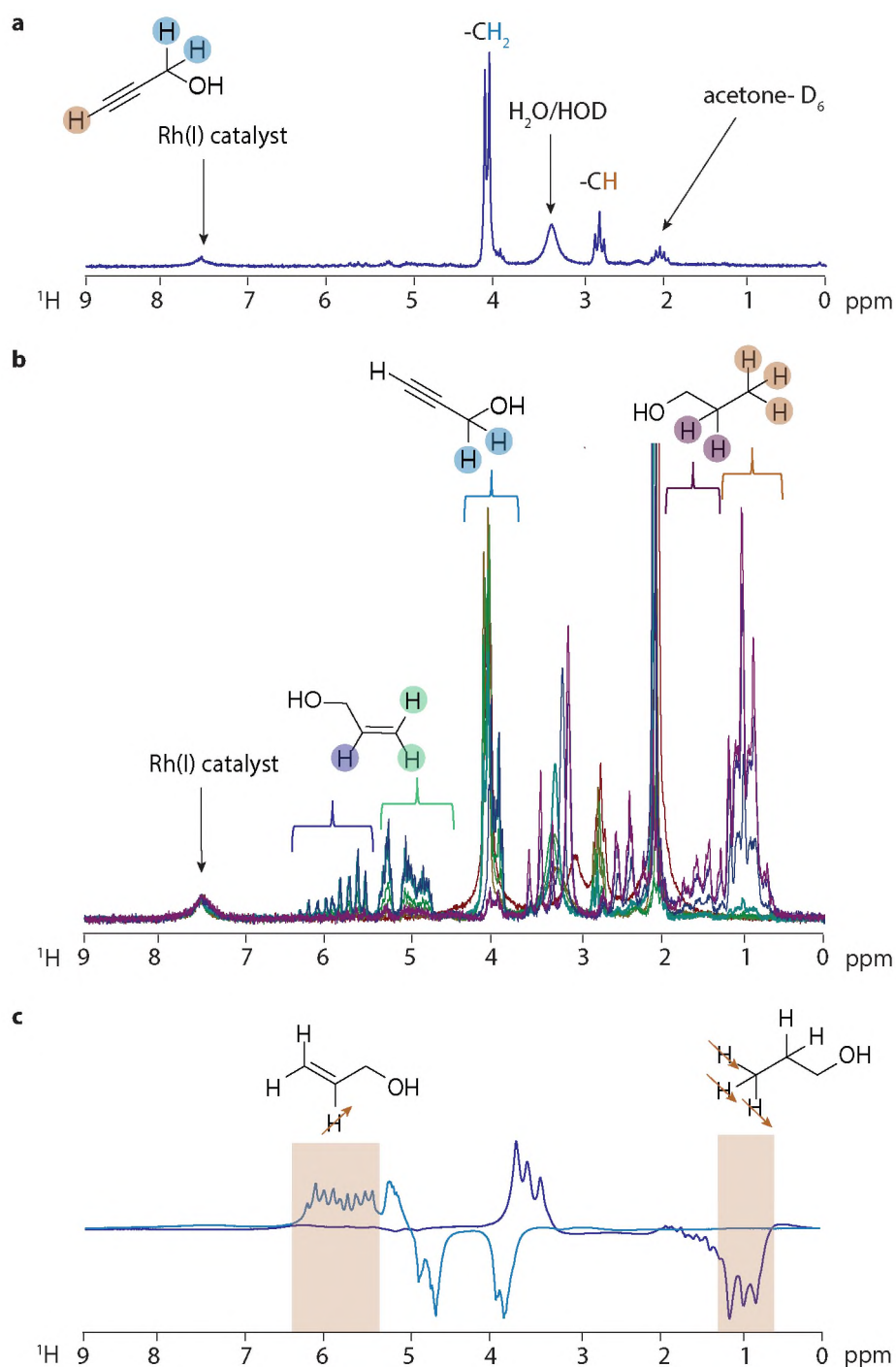


Figure A.1: (a)  $^1\text{H}$  NMR spectrum of the initial solution (propargyl alcohol, Rh(I)-catalyst in acetone- $\text{D}_6$ ) was recorded. Specifically, 5  $\mu\text{L}$  of  $\text{D}_2\text{O}$  was added into the solution to prevent overlapping as shown in Ref [45]. (b) Stacked  $^1\text{H}$  NMR thermal spectra of seven samples (from initial solution) after different times of hydrogen exposure (5-60 s). The peaks used in data analysis was shown explicitly with the structural formula of corresponding molecule (for details see Chapter 6). (c)  $^1\text{H}$  NMR spectra of hyperpolarized products (i.e. allyl alcohol and propanol) after ALTADENA PHIP. The peaks used in data analysis was shown again with the structural formula of corresponding molecule.

---

## A.5 Calibration of the Magnetic Field Using Gaussmeter

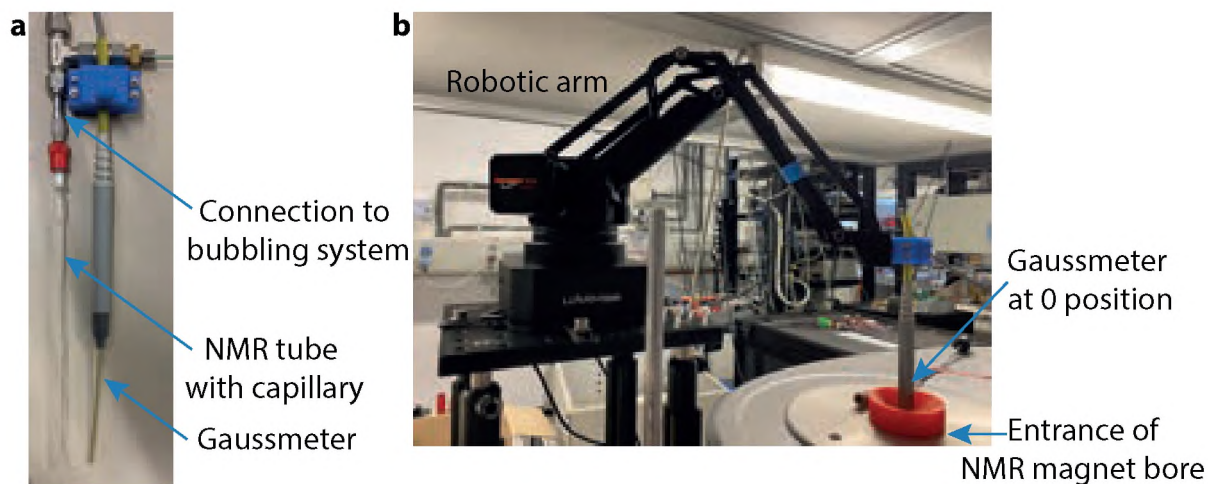


Figure A.2: (a) Photograph of a gaussmeter used for calibration of the benchtop NMR fringe magnetic field to which the NMR sample is subjected in the course of transfer by means of a robotic arm. (b) Photograph of the gaussmeter placed at the '0' position based on robot units (see Chapter 6). One unit change in the robot unit corresponds to one mm of displacement in the tube's position.

# References

- <sup>1</sup>J. W. Blanchard and D. Budker, “Zero- to Ultralow-field NMR”, eMagRes **5**, 1395–1410 (2016).
- <sup>2</sup>J. W. Blanchard, M. P. Ledbetter, T. Theis, M. C. Butler, D. Budker, and A. Pines, “High-Resolution Zero-Field NMR J-Spectroscopy of Aromatic Compounds”, Journal of the American Chemical Society **135**, 3607–3612 (2013).
- <sup>3</sup>A. Wilzewski, S. Afach, J. W. Blanchard, and D. Budker, “Method for Measurement of Spin-Spin Couplings with sub-mHz Precision Using Zero- to Ultralow-Field Nuclear Magnetic Resonance”, Journal of Magnetic Resonance **284**, 66–72 (2017).
- <sup>4</sup>D. Burueva, J. Eills, J. Blanchard, A. Garcon, R. Picazo-Frutos, K. Kovtunov, I. Koptug, and D. Budker, “Chemical Reaction Monitoring using Zero-Field Nuclear Magnetic Resonance Enables Study of Heterogeneous Samples in Metal Containers”, Angewandte Chemie International Edition **59**, 17026–17032 (2020).
- <sup>5</sup>M. C. D. Tayler, T. Theis, T. F. Sjolander, J. W. Blanchard, A. Kentner, S. Pustelny, A. Pines, and D. Budker, “Invited Review Article: Instrumentation for nuclear magnetic resonance in zero and ultralow magnetic field”, Review of Scientific Instruments **88**, 091101 (2017).
- <sup>6</sup>J. Blanchard, W. Teng, J. Eills, Y. hu, and D. Budker, “Zero- to Ultralow-Field Nuclear Magnetic Resonance J-Spectroscopy with Commercial Atomic Magnetometers”, Journal of Magnetic Resonance **314**, 106723 (2020).
- <sup>7</sup>P. Put, S. Pustelny, D. Budker, E. Druga, T. F. Sjolander, A. Pines, and D. A. Barskiy, “Zero- to Ultralow-Field NMR Spectroscopy of Small Biomolecules”, Analytical Chemistry **93**, 3226–3232 (2021).
- <sup>8</sup>M. Levitt, *Spin dynamics: basics of nuclear magnetic resonance, Second Edition* ( John Wiley & Sons, 2008).
- <sup>9</sup>R. R. Ernst, G. Bodenhausen, and A. Wokaun, *Principles of nuclear magnetic resonance in one and two dimensions* (Clarendon Press, Oxford, 1987).
- <sup>10</sup>J. Keeler, *Understanding NMR Spectroscopy, Second Edition* ( John Wiley & Sons, 2010).
- <sup>11</sup>R. Kimmich, “Chapter 1 Principle, Purpose and Pitfalls of Field-cycling NMR Relaxometry”, in *Field-cycling NMR Relaxometry: Instrumentation, Model Theories and Applications* (The Royal Society of Chemistry, 2019), pp. 1–41.
- <sup>12</sup>N. Bloembergen, E. M. Purcell, and R. V. Pound, “Relaxation Effects in Nuclear Magnetic Resonance Absorption”, Phys. Rev. **73**, 679–712 (1948).

## REFERENCES

---

- <sup>13</sup>M. Čuperlović-Culf, “3 - Experimental methodology”, in *NMR Metabolomics in Cancer Research*, edited by M. Cuperlovic-Culf, Woodhead Publishing Series in Biomedicine (Woodhead Publishing, 2013), pp. 139–213.
- <sup>14</sup>D. P. Goldenberg, “The product operator formalism: A physical and graphical interpretation”, *Concepts in Magnetic Resonance Part A* **36A**, 49–83 (2010).
- <sup>15</sup>G. A. Morris and R. Freeman, “Enhancement of nuclear magnetic resonance signals by polarization transfer”, *Journal of the American Chemical Society* **101**, 760–762 (1979).
- <sup>16</sup>J. W. Blanchard, D. Budker, and A. Trabesinger, “Lower than low: Perspectives on zero- to ultralow-field nuclear magnetic resonance”, *Journal of Magnetic Resonance* **323**, 106886 (2021).
- <sup>17</sup>M. Emondts, M. Ledbetter, S. Pustelny, T. Theis, B. Patton, J. Blanchard, M. Butler, D. Budker, and A. Pines, “Long-Lived Heteronuclear Spin-Singlet States in Liquids at a Zero Magnetic field”, *Physical review letters* **112**, 077601 (2014).
- <sup>18</sup>P. Put, “Ultra-Low and Truly Zero-Field Nuclear Magnetic Resonance”, PhD thesis (Jagiellonian University, Krakow, Poland, 2022).
- <sup>19</sup>C. Bengs and M. H. Levitt, “SpinDynamica: Symbolic and numerical magnetic resonance in a Mathematica environment”, *Magnetic Resonance in Chemistry* **56**, 374–414 (2018).
- <sup>20</sup>M. Jiang, J. Bian, Q. Li, Z. Wu, H. Su, M. Xu, Y. Wang, X. Wang, and X. Peng, “Zero- to ultralow-field nuclear magnetic resonance and its applications”, *Fundamental Research* **1**, 68–84 (2021).
- <sup>21</sup>T. Theis, J. W. Blanchard, M. C. Butler, M. P. Ledbetter, D. Budker, and A. Pines, “Chemical analysis using J-coupling multiplets in zero-field NMR”, *Chemical Physics Letters* **580**, 160–165 (2013).
- <sup>22</sup>M. C. Butler, M. P. Ledbetter, T. Theis, J. W. Blanchard, D. Budker, and A. Pines, “Multiplets at zero magnetic field: The geometry of zero-field NMR”, *The Journal of Chemical Physics* **138**, 184202 (2013).
- <sup>23</sup>M. Ledbetter, C. Crawford, A. Pines, D. Wemmer, S. Knappe, J. Kitching, and D. Budker, “Optical detection of NMR J-spectra at zero magnetic field”, *Journal of Magnetic Resonance* **199**, 25–29 (2009).
- <sup>24</sup>P. Bour, M. Budesinski, V. Spirko, J. Kapitan, J. Sebestik, and V. Sychrovsky, “A Complete Set of NMR Chemical Shifts and Spin-Spin Coupling Constants for l-Alanyl-l-alanine Zwitterion and Analysis of Its Conformational Behavior”, *Journal of the American Chemical Society* **127**, 17079–17089 (2005).
- <sup>25</sup>C. Taglang, D. E. Korenchan, C. von Morze, J. Yu, C. Najac, S. Wang, J. E. Blecha, S. Subramaniam, R. Bok, H. F. VanBrocklin, D. B. Vigneron, S. M. Ronen, R. Sriram,

- J. Kurhanewicz, D. M. Wilson, and R. R. Flavell, “Late-stage deuteration of  $^{13}\text{C}$ -enriched substrates for T1 prolongation in hyperpolarized  $^{13}\text{C}$  MRI”, *Chem. Commun.* **54**, 5233–5236 (2018).
- <sup>26</sup>S. Afach, *Spintrum*, <https://git.afach.de/samerafach/Spintrum>, (accessed: 15.10.2020).
- <sup>27</sup>M. P. Ledbetter, T. Theis, J. W. Blanchard, H. Ring, P. Ganssle, S. Appelt, B. Blümich, A. Pines, and D. Budker, “Near-Zero-Field Nuclear Magnetic Resonance”, *Phys. Rev. Lett.* **107**, 107601 (2011).
- <sup>28</sup>N. Iriguchi, “The power sensitivity of magnetic resonance experiments”, *Journal of Applied Physics* **73**, 2956–2957 (1993).
- <sup>29</sup>D. P. Weitekamp, A. Bielecki, D. Zax, K. Zilm, and A. Pines, “Zero-Field Nuclear Magnetic Resonance”, *Phys. Rev. Lett.* **50**, 1807–1810 (1983).
- <sup>30</sup>J. Clarke, M. Hatridge, and M. Mölle, “SQUID-Detected Magnetic Resonance Imaging in Microtesla Fields”, *Annual Review of Biomedical Engineering* **9**, 389–413 (2007).
- <sup>31</sup>R. H. Kraus, M. A. Espy, P. E. Magnelind, and P. L. Volegov, “Fundamental Principles of NMR and MRI at ULF”, in *Ultra-Low Field Nuclear Magnetic Resonance A New MRI Regime* (Oxford University Press, Oxford, UK, May 2014).
- <sup>32</sup>M. Mölle, S.-I. Han, W. R. Myers, S.-K. Lee, N. Kelso, M. Hatridge, A. Pines, and J. Clarke, “SQUID-detected microtesla MRI in the presence of metal”, *Journal of Magnetic Resonance* **179**, 146–151 (2006).
- <sup>33</sup>K. Fang, V. M. Acosta, C. Santori, Z. Huang, K. M. Itoh, H. Watanabe, S. Shikata, and R. G. Beausoleil, “High-Sensitivity Magnetometry Based on Quantum Beats in Diamond Nitrogen-Vacancy Centers”, *Phys. Rev. Lett.* **110**, 130802 (2013).
- <sup>34</sup>S. DeVience, L. Pham, I. Lovchinsky, A. Sushkov, N. Bar-Gill, C. Belthangady, F. Casola, M. Corbett, H. Zhang, M. Lukin, H. Park, A. Yacoby, and R. Walsworth, “Nanoscale NMR Spectroscopy and Imaging of Multiple Nuclear Species”, *Nature nanotechnology* **10**, 129–134 (2014).
- <sup>35</sup>D. Budker and M. Romalis, “Optical magnetometry”, *Nature Phys* **3**, 227–234 (2007).
- <sup>36</sup>W. Happer and H. Tang, “Spin-Exchange Shift and Narrowing of Magnetic Resonance Lines in Optically Pumped Alkali Vapors”, *Phys. Rev. Lett.* **31**, 273–276 (1973).
- <sup>37</sup>M. Limes, E. Foley, T. Kornack, S. Caliga, S. McBride, A. Braun, W. Lee, V. Lucivero, and M. Romalis, “Portable Magnetometry for Detection of Biomagnetism in Ambient Environments”, *Phys. Rev. Applied* **14**, 011002 (2020).
- <sup>38</sup>W. G. Clark, “Pulsed Nuclear Resonance Apparatus”, *Review of Scientific Instruments* **35**, 316–333 (1964).

## REFERENCES

---

- <sup>39</sup>R. A. Green, R. W. Adams, S. B. Duckett, R. E. Mewis, D. C. Williamson, and G. G. Green, “The theory and practice of hyperpolarization in magnetic resonance using parahydrogen”, *Progress in Nuclear Magnetic Resonance Spectroscopy* **67**, 1–48 (2012).
- <sup>40</sup>J. Natterer and J. Bargon, “Parahydrogen induced polarization”, *Progress in Nuclear Magnetic Resonance Spectroscopy* **31**, 293–315 (1997).
- <sup>41</sup>L. Buljubasich, M. B. Franzoni, and K. Münnemann, “parahydrogen Induced Polarization by Homogeneous Catalysis: Theory and Applications”, in *Hyperpolarization Methods in NMR Spectroscopy*, edited by L. T. Kuhn (Springer, Berlin, Heidelberg, 2013), pp. 33–74.
- <sup>42</sup>T. Sugimoto and K. Fukutani, “Electric-field-induced nuclear-spin flips mediated by enhanced spin–orbit coupling”, *Nature Physics* **7**, 307–310 (2011).
- <sup>43</sup>L. Pauling and E. B. Wilson, *Introduction to quantum mechanics, with applications to chemistry* (Dover, New York, United States, 1935).
- <sup>44</sup>D. Canet, C. Aroulanda, P. Mutzenhardt, S. Aime, R. Gobetto, and F. Reineri, “Parahydrogen enrichment and hyperpolarization”, *Concepts in Magnetic Resonance Part A* **28A**, 321–330 (2006).
- <sup>45</sup>K. Them, F. Ellermann, A. N. Pravdivtsev, O. G. Salnikov, I. V. Skovpin, I. V. Kopytyug, R. Herges, and J.-B. Hövener, “Parahydrogen-induced polarization relayed via proton exchange”, *Journal of the American Chemical Society* **143**, 13694–13700 (2021).
- <sup>46</sup>T. Theis, P. Ganssle, G. Kervern, S. Knappe, J. Kitching, M. P. Ledbetter, D. Budker, and A. Pines, “Parahydrogen enhanced zero-field nuclear magnetic resonance”, *Nature Physics* **7**, 571–575 (2011).
- <sup>47</sup>M. C. Butler, G. Kervern, T. Theis, M. P. Ledbetter, P. J. Ganssle, J. W. Blanchard, D. Budker, and A. Pines, “Parahydrogen-induced polarization at zero magnetic field”, *Journal of Chemical Physics* **138**, 234201 (2013).
- <sup>48</sup>K. D. Atkinson, M. J. Cowley, P. I. P. Elliott, S. B. Duckett, G. G. R. Green, J. López-Serrano, and A. C. Whitwood, “Spontaneous Transfer of Parahydrogen Derived Spin Order to Pyridine at Low Magnetic Field”, *Journal of the American Chemical Society* **131**, 13362–13368 (2009).
- <sup>49</sup>P. J. Rayner and S. B. Duckett, “Signal Amplification by Reversible Exchange (SABRE): From Discovery to Diagnosis”, *Angewandte Chemie International Edition* **57**, 6742–6753 (2018).
- <sup>50</sup>M. J. Cowley, R. W. Adams, K. D. Atkinson, M. C. R. Cockett, S. B. Duckett, G. G. R. Green, J. A. B. Lohman, R. Kerssebaum, D. Kilgour, and R. E. Mewis, “Iridium N-Heterocyclic Carbene Complexes as Efficient Catalysts for Magnetization Transfer from para-Hydrogen”, *Journal of the American Chemical Society* **133**, 6134–6137 (2011).

## REFERENCES

---

- <sup>51</sup>T. Theis, M. L. Truong, A. M. Coffey, R. V. Shchepin, K. W. Waddell, F. Shi, B. M. Goodson, W. S. Warren, and E. Y. Chekmenev, "Microtesla SABRE Enables 10% Nitrogen-15 Nuclear Spin Polarization", *Journal of the American Chemical Society* **137**, 1404–1407 (2015).
- <sup>52</sup>J. W. Blanchard, B. Ripka, B. A. Suslick, D. Gelevski, T. Wu, K. Münnemann, D. A. Barskiy, and D. Budker, "Towards large-scale steady-state enhanced nuclear magnetization with in situ detection", *Magnetic Resonance in Chemistry* **59**, 1208–1215 (2021).
- <sup>53</sup>T. Theis, M. P. Ledbetter, G. Kervern, J. W. Blanchard, P. J. Ganssle, M. C. Butler, H. D. Shin, D. Budker, and A. Pines, "Zero-Field NMR Enhanced by Parahydrogen in Reversible Exchange", *Journal of the American Chemical Society* **134**, 3987–3990 (2012).
- <sup>54</sup>P. M. Richardson, R. O. John, A. J. Parrott, P. J. Rayner, W. Iali, A. Nordon, M. E. Halse, and S. B. Duckett, "Quantification of hyperpolarisation efficiency in SABRE and SABRE-Relay enhanced NMR spectroscopy", *Phys. Chem. Chem. Phys.* **20**, 26362–26371 (2018).
- <sup>55</sup>K. Kovtunov, E. Pokochueva, O. Salnikov, S. Cousin, D. Kurzbach, B. Vuichoud, S. Jannin, E. Chekmenev, B. Goodson, D. Barskiy, and I. Koptiyug, "Hyperpolarized NMR Spectroscopy: d-DNP, PHIP, and SABRE Techniques", *Chemistry, an Asian journal* **13**, 1857–1871 (2018).
- <sup>56</sup>W. Iali, P. J. Rayner, and S. B. Duckett, "Using parahydrogen to hyperpolarize amines, amides, carboxylic acids, alcohols, phosphates, and carbonates", *Science Advances* **4**, eaao6250 (2018).
- <sup>57</sup>E. Dyke, J. Eills, R. Picazo-Frutos, K. Sheberstov, Y. hu, D. Budker, and D. Barskiy, "Relayed Hyperpolarization for Zero-Field Nuclear Magnetic Resonance", *chemrxiv*, 10.26434/chemrxiv-2022-1njs9 (2022).
- <sup>58</sup>S. Alcicek, P. Put, V. Kontul, and S. Pustelny, "Zero-Field NMR J-Spectroscopy of Organophosphorus Compounds", *The Journal of Physical Chemistry Letters* **12**, 787–792 (2021).
- <sup>59</sup>A. Tajti and G. Keglevich, *The importance of organophosphorus compounds as biologically active agents: Novel Developments* (De Gruyter, 2018), pp. 53–65.
- <sup>60</sup>L. C. Thomas, *The identification of functional groups in organophosphorus compounds* (Academic Press, 1974).
- <sup>61</sup>D. Corbridge, *Phosphorus* (Boca Raton, CRC Press, 2013).
- <sup>62</sup>Y. Liu, Y. Gu, and X. Yu, "Assessing tissue metabolism by phosphorous-31 magnetic resonance spectroscopy and imaging: a methodology review", *Quantitative Imaging in Medicine and Surgery* **7**, 707–716 (2017).

## REFERENCES

---

- <sup>63</sup>F. Cruz and S. Cerdán, “Cells Studied by NMR”, *Encyclopedia of Spectroscopy and Spectrometry*, 212–220 (2010).
- <sup>64</sup>J. S. Cohen, “Phospholipid and Energy Metabolism of Cancer Cells Monitored by <sup>31</sup>P Magnetic Resonance Spectroscopy: Possible Clinical Significance”, *Mayo Clinic Proceedings* **63**, 1199–1207 (1988).
- <sup>65</sup>B. D. Ross, O. Ben-Yoseph, and T. L. Chenevert, “In Vivo Magnetic Resonance Imaging and Spectroscopy: Application to Brain Tumors”, in, edited by H. Bachelard (Springer US, 1997), pp. 145–178.
- <sup>66</sup>D.-H. Ha, S. Choi, J. Oh, S. K. Yoon, M. Kang, and K.-U. Kim, “Application of <sup>31</sup>P MR spectroscopy to the brain tumors”, *Korean Journal of Radiology* **14**, 477–486 (2013).
- <sup>67</sup>I. Savukov and S. Seltzer, “Spin-exchange-relaxation-free (SERF) magnetometers”, *Optical Magnetometry*, 85–103 (2011).
- <sup>68</sup>J. Burg, “Maximum Entropy Spectral Analysis”, PhD thesis (Stanford University, CA, USA, 1975).
- <sup>69</sup>L. Chen, Z. Weng, L. Goh, and M. Garland, “An efficient algorithm for automatic phase correction of NMR spectra based on entropy minimization”, *Journal of Magnetic Resonance* **158**, 164–168 (2002).
- <sup>70</sup>V. Kontul, *ZULF-DSP*, <https://github.com/vlado48/ZULF-DSP.git>, (accessed: 05.06.2022).
- <sup>71</sup>J. C. Tebbby, *Handbook of Phosphorus-31 Nuclear Magnetic Resonance Data* (Boca Raton, CRC Press, 1991).
- <sup>72</sup>M. C. Tayler and L. F. Gladden, “Scalar relaxation of NMR transitions at ultralow magnetic field”, *Journal of Magnetic Resonance* **298**, 101–106 (2019).
- <sup>73</sup>R. K. Harris and E. M. McVicker, “Spin-lattice relaxation studies of organophosphorus compounds”, *J. Chem. Soc., Faraday Trans. 2* **72**, 2291–2297 (1976).
- <sup>74</sup>G. A. Gray, “Carbon-13 nuclear magnetic resonance of organophosphorus compounds. I. Diethyl phosphonates”, *Journal of the American Chemical Society* **93**, 2132–2140 (1971).
- <sup>75</sup>M. DeWolf, “The NMR spectra of dimethyl methyl phosphonate”, *Journal of Molecular Spectroscopy* **18**, 59–61 (1965).
- <sup>76</sup>M. Emondts, M. P. Ledbetter, S. Pustelny, T. Theis, B. Patton, J. W. Blanchard, M. C. Butler, D. Budker, and A. Pines, “Long-lived heteronuclear spin-singlet states in liquids at a zero magnetic field”, *Physical Review Letters* **112**, 077601 (2014).

## REFERENCES

---

- <sup>77</sup>D. Barskiy, M. Tayler, I. Marco-Rius, J. Kurhanewicz, D. Vigneron, S. Cikrikci, A. Aydogdu, M. Reh, A. Pravdivtsev, J.-B. Hövener, J. Blanchard, W. Teng, D. Budker, and A. Pines, “Zero-field nuclear magnetic resonance of chemically exchanging systems”, *Nature Communications* **10**, 3002 (2019).
- <sup>78</sup>S. Meier, P. Jensen, M. Karlsson, and M. Lerche, “Hyperpolarized NMR Probes for Biological Assays”, *Sensors* **14**, 1576–1597 (2014).
- <sup>79</sup>I. V. Linnik, P. J. Rayner, R. A. Stow, S. B. Duckett, and G. M. T. Cheetham, “Pharmacokinetics of the SABRE agent 4,6-d2-nicotinamide and also nicotinamide in rats following oral and intravenous administration”, *European Journal of Pharmaceutical Sciences* **135**, 32–37 (2019).
- <sup>80</sup>A. Nardi-Schreiber, A. Gamliel, T. Harris, G. Sapir, J. Sosna, J. Gomori, and R. Katz-Brull, “Biochemical phosphates observed using hyperpolarized  $^{31}\text{P}$  in physiological aqueous solutions”, *Nature Communications* **8**, 341 (2017).
- <sup>81</sup>V. Zhivonitko, I. Skovpin, and I. Koptug, “Strong  $^{31}\text{P}$  nuclear spin hyperpolarization produced via reversible chemical interaction with parahydrogen”, *Chemical Communications* **51**, 2506–2509 (2015).
- <sup>82</sup>M. Burns, P. Rayner, S. Duckett, G. Green, L. Highton, and R. Mewis, “Improving the Hyperpolarization of  $^{31}\text{P}$  Nuclei by Synthetic Design”, *Journal of Physical Chemistry B* **119**, 5020–5027 (2015).
- <sup>83</sup>I. M. Savukov, S. Seltzer, M. Romalis, and K. Sauer, “Tunable Atomic Magnetometer for Detection of Radio-Frequency Magnetic Fields”, *Physical review letters* **95**, 063004 (2005).
- <sup>84</sup>W. Chalupczak, R. Godun, S. Pustelny, and W. Gawlik, “Room temperature femtotesla radio-frequency atomic magnetometer”, *Applied Physics Letters* **100**, 242401 (2012).
- <sup>85</sup>G. Bloch, J. R. Chase, M. J. Avison, and R. G. Shulman, “In Vivo  $^{31}\text{P}$  NMR measurement of glucose-6-phosphate in the rat muscle after exercise”, *Magnetic Resonance in Medicine* **30**, 347–350 (1993).
- <sup>86</sup>C. M. Segebarth, D. F. Balériaux, D. L. Arnold, P. R. Luyten, and J. A. den Hollander, “MR image-guided P-31 MR spectroscopy in the evaluation of brain tumor treatment”, *Radiology* **165**, 215–219 (1987).
- <sup>87</sup>S. Alciçek, P. Put, D. Barskiy, V. Kontul, and S. Pustelny, “Zero-Field NMR of Urea: Spin-Topology Engineering by Chemical Exchange”, *The Journal of Physical Chemistry Letters* **12**, 10671–10676 (2021).
- <sup>88</sup>I. D. Weiner, W. E. Mitch, and J. M. Sands, “Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion”, *Clinical Journal of the American Society of Nephrology* **10**, 1444–1458 (2015).

## REFERENCES

---

- <sup>89</sup>A. J. Taylor and P. Vadgama, “Analytical Reviews in Clinical Biochemistry: The Estimation of Urea”, *Annals of Clinical Biochemistry* **29**, 245–264 (1992).
- <sup>90</sup>L. Liu, H. Mo, S. Wei, and D. Raftery, “Quantitative analysis of urea in human urine and serum by <sup>1</sup>H nuclear magnetic resonance”, *Analyst* **137**, 595–600 (2012).
- <sup>91</sup>C. Pundir, S. Jakhar, and V. Narwal, “Determination of urea with special emphasis on biosensors: A review”, *Biosensors and Bioelectronics* **123**, 36–50 (2019).
- <sup>92</sup>Z. J. Wang, M. A. Ohliger, P. E. Z. Larson, J. W. Gordon, R. A. Bok, J. Slater, J. E. Villanueva-Meyer, C. P. Hess, J. Kurhanewicz, and D. B. Vigneron, “Hyperpolarized <sup>13</sup>C MRI: State of the Art and Future Directions”, *Radiology* **291**, 273–284 (2019).
- <sup>93</sup>P. M. Nielsen, E. S. Szocska Hansen, T. S. Nørting, R. Nørregaard, L. Bonde Bertelsen, H. Stødkilde Jørgensen, and C. Laustsen, “Renal ischemia and reperfusion assessment with three-dimensional hyperpolarized <sup>13</sup>C,<sup>15</sup>N<sub>2</sub>-urea”, *Magnetic Resonance in Medicine* **76**, 1524–1530 (2016).
- <sup>94</sup>A. Z. Lau, J. J. Miller, M. D. Robson, and D. J. Tyler, “Cardiac perfusion imaging using hyperpolarized <sup>13</sup>C urea using flow sensitizing gradients”, *Magnetic Resonance in Medicine* **75**, 1474–1483 (2016).
- <sup>95</sup>P. Francis, S. Lewis, and K. Lim, “Analytical methodology for the determination of urea: Current practice and future trends”, *TrAC Trends in Analytical Chemistry* **21**, 389–400 (2002).
- <sup>96</sup>P. W. Kuchel, C. Naumann, B. E. Chapman, D. Shishmarev, P. Håkansson, G. Bacskey, and N. S. Hush, “NMR resonance splitting of urea in stretched hydrogels: Proton exchange and <sup>1</sup>H/<sup>2</sup>H isotopologues”, *Journal of Magnetic Resonance* **247**, 72–80 (2014).
- <sup>97</sup>O. Steinhof, E. J. Kibrik, G. Scherr, and H. Hasse, “Quantitative and qualitative <sup>1</sup>H, <sup>13</sup>C, and <sup>15</sup>N NMR spectroscopic investigation of the urea–formaldehyde resin synthesis”, *Magnetic Resonance in Chemistry* **52**, 138–162 (2014).
- <sup>98</sup>R. L. Vold, E. Daniel, and S. Chan, “Magnetic resonance measurements of proton exchange in aqueous urea”, *Journal of the American Chemical Society* **92**, 6771–6776 (1970).
- <sup>99</sup>P. Giraudeau, V. Silvestre, and S. Akoka, “Optimizing water suppression for quantitative NMR-based metabolomics: a tutorial review”, *Metabolomics* **11**, 1041–1055 (2015).
- <sup>100</sup>J. Bell, W. A. Gillespie, and D. B. Taylor, “The bearing of the dissociation constant of urea on its constitution”, *Transactions of the Faraday Society* **39**, 137–140 (1943).
- <sup>101</sup>I. Yavari and J. D. Roberts, “Nitrogen-15 nuclear magnetic resonance spectroscopy. NH proton exchange reactions of urea and substituted ureas”, *Organic Magnetic Resonance* **13**, 68–71 (1980).

## REFERENCES

---

- <sup>102</sup>C. L. Perrin, E. R. Johnston, C. P. Lollo, and P. A. Kobrin, “NMR studies of base-catalyzed proton exchange in amides”, *Journal of the American Chemical Society* **103**, 4691–4696 (1981).
- <sup>103</sup>I. M. Klotz and D. L. Hunston, “Proton exchange in aqueous urea solutions”, *The Journal of Physical Chemistry* **75**, 2123–2127 (1971).
- <sup>104</sup>E. Canet, D. Mammoli, P. Kadeřávek, P. Pelupessy, and G. Bodenhausen, “Kinetic isotope effects for fast deuterium and proton exchange rates”, *Physical Chemistry Chemical Physics* **18**, 10144–10151 (2016).
- <sup>105</sup>M. C. Tayler and L. F. Gladden, “Scalar relaxation of NMR transitions at ultralow magnetic field”, *Journal of Magnetic Resonance* **298**, 101–106 (2019).
- <sup>106</sup>D. Barskiy, J. Blanchard, M. Reh, T. Sjoelander, A. Pines, and D. Budker, “Zero-Field J-spectroscopy of Quadrupolar Nuclei”, *arXiv* **2011.05618** (2021).
- <sup>107</sup>S. Lunte, “AMINO ACIDS”, in *Encyclopedia of Analytical Science (Second Edition)*, edited by P. Worsfold, A. Townshend, and C. Poole, Second Edition (Elsevier, Oxford, 2005), pp. 61–69.
- <sup>108</sup>R. Gallagher, L. Pollard, A. Scott, S. Huguenin, S. Goodman, and Q. Sun, “Laboratory analysis of organic acids, 2018 update: A technical standard of the American College of Medical Genetics and Genomics (ACMG)”, *GENETICS in MEDICINE* **20**, 683–691 (2018).
- <sup>109</sup>A. N. Pravdivtsev, G. Buntkowsky, S. B. Duckett, I. V. Koptug, and J.-B. Hövener, “Parahydrogen-Induced Polarization of Amino Acids”, *Angewandte Chemie International Edition* **60**, 23496–23507.
- <sup>110</sup>E. Khan, “Pyridine Derivatives as Biologically Active Precursors; Organics and Selected Coordination Complexes”, *ChemistrySelect* **6**, 3041–3064 (2021).
- <sup>111</sup>R. V. Shchepin, D. A. Barskiy, D. M. Mikhaylov, and E. Y. Chekmenev, “Efficient Synthesis of Nicotinamide-1-<sup>15</sup>N for Ultrafast NMR Hyperpolarization Using Parahydrogen”, *Bioconjugate Chemistry* **27**, 878–882 (2016).
- <sup>112</sup>A. Svyatova, I. V. Skovpin, N. V. Chukanov, K. V. Kovtunov, E. Y. Chekmenev, A. N. Pravdivtsev, J.-B. Hövener, and I. V. Koptug, “<sup>15</sup>N MRI of SLIC-SABRE Hyperpolarized <sup>15</sup>N-Labelled Pyridine and Nicotinamide”, *Chemistry – A European Journal* **25**, 8465–8470 (2019).
- <sup>113</sup>I. Linnik, P. Rayner, R. Stow, S. Duckett, and G. Cheetham, “Pharmacokinetics of the SABRE agent 4,6-d<sub>2</sub>-Nicotinamide and also Nicotinamide in rats following oral and intravenous administration”, *European Journal of Pharmaceutical Sciences* **135**, 32–37 (2019).

- <sup>114</sup>W. Jiang, L. Lumata, W. Chen, S. Zhang, Z. Kovacs, D. Sherry, and C. Khemtong, “Hyperpolarized  $^{15}\text{N}$ -pyridine Derivatives as pH-Sensitive MRI Agents”, *Scientific reports* **5**, 9104 (2015).
- <sup>115</sup>P. Rovedo, S. Knecht, T. Bäumlisberger, A. L. Cremer, S. B. Duckett, R. E. Mewis, G. G. R. Green, M. Burns, P. J. Rayner, D. Leibfritz, J. G. Korvink, J. Hennig, G. Pütz, D. von Elverfeldt, and J.-B. Hövener, “Molecular MRI in the Earth’s Magnetic Field Using Continuous Hyperpolarization of a Biomolecule in Water”, *The Journal of Physical Chemistry B* **120**, 5670–5677 (2016).
- <sup>116</sup>M. Fekete, C. Gibard, G. J. Dear, G. G. R. Green, A. J. J. Hooper, A. D. Roberts, F. Cisnetti, and S. B. Duckett, “Utilisation of water soluble iridium catalysts for signal amplification by reversible exchange”, *Dalton Trans.* **44**, 7870–7880 (2015).
- <sup>117</sup>P. J. Rayner, B. J. Tickner, W. Iali, M. Fekete, A. D. Robinson, and S. B. Duckett, “Relayed hyperpolarization from para-hydrogen improves the NMR detectability of alcohols”, *Chem. Sci.* **10**, 7709–7717 (2019).
- <sup>118</sup>J.-B. Hövener, A. N. Pravdivtsev, B. Kidd, C. R. Bowers, S. Glöggl, K. V. Kovtunov, M. Plaumann, R. Katz-Brull, K. Buckenmaier, A. Jerschow, F. Reineri, T. Theis, R. V. Shchepin, S. Wagner, P. Bhattacharya, N. M. Zacharias, and E. Y. Chekmenev, “Parahydrogen-Based Hyperpolarization for Biomedicine”, *Angewandte Chemie International Edition* **57**, 11140–11162 (2018).
- <sup>119</sup>S. Månsson, E. Johansson, P. Magnusson, C. M. Chai, G. Hansson, J. S. Petersson, F. Ståhlberg, and K. Golman, “ $^{13}\text{C}$  imaging-a new diagnostic platform”, *Eur Radiol* **16**, 57–67 (2006).
- <sup>120</sup>J. Kurhanewicz, D. B. Vigneron, K. Brindle, E. Y. Chekmenev, A. Comment, C. H. Cunningham, R. J. DeBerardinis, G. G. Green, M. O. Leach, S. S. Rajan, R. R. Rizi, B. D. Ross, W. S. Warren, and C. R. Malloy, “Analysis of Cancer Metabolism by Imaging Hyperpolarized Nuclei: Prospects for Translation to Clinical Research”, *Neoplasia* **13**, 81–97 (2011).
- <sup>121</sup>S. Knecht, J. W. Blanchard, D. Barskiy, E. Cavallari, L. Dagys, E. V. Dyke, M. Tsukanov, B. Bliemel, K. Münnemann, S. Aime, F. Reineri, M. H. Levitt, G. Buntkowsky, A. Pines, P. Blümli, D. Budker, and J. Eills, “Rapid hyperpolarization and purification of the metabolite fumarate in aqueous solution”, *Proceedings of the National Academy of Sciences* **118**, e2025383118 (2021).
- <sup>122</sup>D. P. Cistola and M. D. Robinson, “Compact NMR relaxometry of human blood and blood components”, *TrAC Trends in Analytical Chemistry* **83**, 53–64 (2016).
- <sup>123</sup>S. Bodenstedt, M. Mitchell, and M. Tayler, “Fast-field-cycling ultralow-field nuclear magnetic relaxation dispersion”, *Nature Communications* **12**, 4041 (2021).
- <sup>124</sup>G. B. Benedek and E. M. Purcell, “Nuclear Magnetic Resonance in Liquids under High Pressure”, *The Journal of Chemical Physics* **22**, 2003–2012 (1954).

## REFERENCES

---

- <sup>125</sup>M. H. Levitt, “Singlet Nuclear Magnetic Resonance”, *Annual Review of Physical Chemistry* **63**, 89–105 (2012).
- <sup>126</sup>K. Livo, M. Prasad, and T. Graham, “Quantification of dissolved O<sub>2</sub> in bulk aqueous solutions and porous media using NMR relaxometry”, *Scientific Reports* **11**, 1–9 (2021).
- <sup>127</sup>N. Nestle, T. Baumann, and R. Niessner, “Oxygen determination in oxygen-supersaturated drinking waters by NMR relaxometry”, *Water Research* **37**, 3361–3366 (2003).
- <sup>128</sup>L. Kjaer, C. Thomsen, O. Henriksen, P. Ring, M. Stubgaard, and E. J. Pedersen, “Evaluation of Relaxation Time Measurements by Magnetic Resonance Imaging”, *Acta Radiologica* **28**, 345–351 (1987).
- <sup>129</sup>N. Bloembergen, E. M. Purcell, and R. V. Pound, “Relaxation Effects in Nuclear Magnetic Resonance Absorption”, *Phys. Rev.* **73**, 679–712 (1948).
- <sup>130</sup>M. D. Robinson, S. Deodhar, I. Mishra, V. Patel, K. Gordon, R. Vintimilla, L. Johnson, S. O’Bryant, and D. P. Cistola, “Abstract 620: Water as a Universal Biosensor for Inflammation, Insulin Resistance and Dyslipidemia”, *Arteriosclerosis, Thrombosis, and Vascular Biology* **35**, A620–A620 (2015).
- <sup>131</sup>L. R. Skewis, T. Lebedeva, V. Papkov, E. C. Thayer, W. Masefski, A. Cuker, C. Nagaswami, R. I. Litvinov, M. A. Kowalska, L. Rauova, M. Poncz, J. W. Weisel, T. J. Lowery, and D. B. Cines, “T<sub>2</sub> Magnetic Resonance: A Diagnostic Platform for Studying Integrated Hemostasis in Whole Blood—Proof of Concept”, *Clinical Chemistry* **60**, 1174–1182 (2014).
- <sup>132</sup>L. A. Neely, M. Audeh, N. A. Phung, M. Min, A. Suchocki, D. Plourde, M. Blanco, V. Demas, L. R. Skewis, T. Anagnostou, J. J. Coleman, P. Wellman, E. Mylonakis, and T. J. Lowery, “T<sub>2</sub> Magnetic Resonance Enables Nanoparticle-Mediated Rapid Detection of Candidemia in Whole Blood”, *Science Translational Medicine* **5**, 182ra54 (2013).
- <sup>133</sup>D. P. Cistola and M. D. Robinson, “Compact NMR relaxometry of human blood and blood components”, *TrAC Trends in Analytical Chemistry* **83**, 53–64 (2016).
- <sup>134</sup>Z. Wang, S. Pisano, V. Ghini, P. Kadeřávek, M. Zachrdla, P. Pelupessy, M. Kazmierczak, T. Marquardsen, J.-M. Tyburn, G. Bouvignies, G. Parigi, C. Luchinat, and F. Ferrage, “Detection of Metabolite–Protein Interactions in Complex Biological Samples by High-Resolution Relaxometry: Toward Interactomics by NMR”, *Journal of the American Chemical Society* **143**, 9393–9404 (2021).
- <sup>135</sup>J. H. Schuhmacher, J. H. Clorius, W. Semmler, H. Hauser, E. R. Matys, W. Maier-Borst, and W. E. Hull, “NMR relaxation times T<sub>1</sub> and T<sub>2</sub> of water in plasma from patients with lung carcinoma: Correlation of T<sub>2</sub> with blood sedimentation rate”, *Magnetic Resonance in Medicine* **5**, 537–547 (1987).

## REFERENCES

---

- <sup>136</sup>Y. S. Kang, J. C. Gore, and I. M. Armitage, “Studies of factors affecting the design of NMR contrast agents: manganese in blood as a model system”, *Magnetic Resonance in Medicine* **1**, 396–409 (1984).
- <sup>137</sup>V. Ghini, D. Quaglio, C. Luchinat, and P. Turano, “NMR for sample quality assessment in metabolomics”, *New Biotechnology* **52**, 25–34 (2019).
- <sup>138</sup>P. T. Vesanen, K. C. Zevenhoven, J. O. Nieminen, J. Dabek, L. T. Parkkonen, and R. J. Ilmonemi, “Temperature dependence of relaxation times and temperature mapping in ultra-low-field MRI”, *Journal of Magnetic Resonance* **235**, 50–57 (2013).
- <sup>139</sup>R. V. Shchepin, D. A. Barskiy, D. M. Mikhaylov, and E. Y. Chekmenev, “Efficient Synthesis of Nicotinamide-1-<sup>15</sup>N for Ultrafast NMR Hyperpolarization Using Parahydrogen”, *Bioconjugate Chemistry* **27**, 878–882 (2016).
- <sup>140</sup>W. Brügel, “Die Kernresonanzspektren von Pyridin-Derivaten”, *Zeitschrift für Elektrochemie, Berichte der Bunsengesellschaft für physikalische Chemie* **66**, 159–177 (1962).
- <sup>141</sup>U. Vögeli and W. von Philipsborn, “<sup>13</sup>C and <sup>1</sup>H NMR spectroscopic studies on the structure of N-methyl-3-pyridone and 3-hydroxypyridine”, *Organic Magnetic Resonance* **5**, 551–559 (1973).