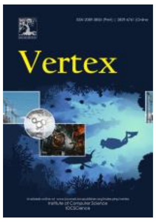

ISSN 2089-385X (Print) | 2829-6761 (Online), Volume 12, No. 1 (2022), pp. 40-50

Vertex

Published by: Institute of Computer Science (IOCS)

Next-Generation hyperpolarization techniques for NMR: amplifying signal sensitivity and resolving complex molecular systems

Schmidt Laustsen Vorm[1], Rößler[2], Pajvani Duckett[3]

[1,2,3]Ben-Gurion University of the Negev, Be'er Sheva, Israel

Abstract

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical tool used to investigate the structure and dynamics of molecules at the atomic level. However, its application to complex molecular systems, such as large biomolecules and diluted chemical mixtures, is often hindered by limited NMR signal sensitivity. To address this challenge, next-generation hyperpolarization techniques have emerged, offering the potential to enhance NMR signals significantly. This research explores the dynamic hyperpolarization enhancement process for NMR sensitivity through a mathematical formulation and a numerical example. The proposed model describes the transfer of polarization from polarizing agents to target molecules and its impact on nuclear spin polarization. The numerical example demonstrates how hyperpolarization techniques can amplify nuclear spin polarization over time, leading to improved NMR signal sensitivity. The research highlights the optimization of key parameters, such as relaxation time constants and polarization transfer rates, for achieving maximum sensitivity enhancements. The results underscore the transformative potential of hyperpolarization techniques in expanding the scope of NMR applications, enabling the study of complex molecular systems with unparalleled precision, and advancing scientific discoveries in biochemistry, materials science, and medical research. The conclusion emphasizes the ongoing efforts to develop next-generation hyperpolarization methods and their implications for fundamental and applied research. Ultimately, this research opens new frontiers in NMR spectroscopy, providing researchers with a powerful tool to explore intricate molecular systems and resolve scientific challenges across diverse disciplines.

Article Info

Article history:

Received : Nov 11, 2022
Revised : Nov 23, 2022
Accepted : Dec 29, 2022

Keywords:

Complex molecular systems;
Hyperpolarization;
Next-generation techniques;
NMR spectroscopy;
Signal sensitivity.

Corresponding Author:

Schmidt Laustsen Vorm,
Ben-Gurion University of the Negev,
David Ben Gurion Blvd 1, Be'er Sheva, Israel,
Email: Schmidtlaustsen@bgu.ac.il.

Introduction

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for investigating the structure, dynamics, and interactions of molecules at the atomic level(Kerfah et al., 2015)(Markwick et al., 2008)(Tsutsui & Wintrode, 2007). Its application to complex molecular systems, such as large biomolecules and dilute chemical mixtures (Gygi et al., 1999), is often hindered by the inherent limitations of NMR signal sensitivity(Springer et al., 2010)(Stothers, 2012). The weak NMR signals from these systems demand high sample concentrations, which can lead to issues with solubility, aggregation, or degradation(Fejzo et al., 1999), thereby limiting the scope of NMR studies in critical research areas like biochemistry, materials science, and drug discovery(Bochevarov et al., 2013)(Moser et al., 2017). To overcome these limitations and unlock new avenues for NMR research, the development of next-generation hyperpolarization techniques has gained significant interest(Rivnay et al., 2017)(Humeau & Choquet, 2019). Hyperpolarization methods have demonstrated the ability to amplify nuclear spin polarization, resulting in remarkably enhanced NMR signals(Kovtunov et al., 2020)(Adams et al., 2009)(Roy et al., 2016)(Chukanov et al., 2021)(Nikolaou et al., 2015)(Shchepin et al., 2015)(Kovtunov et al., 2014)(Eichhorn et al., 2022). By achieving substantial signal enhancements, hyperpolarization enables the investigation of previously intractable molecular systems at significantly lower concentrations, without compromising the structural and dynamic information acquired through NMR spectroscopy(Perkons et al., 2020)(Ghassemi et al., 2021)(Kosol et al., 2013)(Renault et al., 2010)(Polenova et al., 2015).

Nuclear Magnetic Resonance (NMR) spectroscopy is a non-invasive and widely used analytical technique that provides valuable information about the structure(Kirtil & Oztop, 2016)(Wang et al., 2021), dynamics, and interactions of molecules at the atomic level(Fan & Zhang, 2019). NMR has a broad range of applications in various fields, including chemistry, biochemistry, materials science, and medicine(Owens et al., 2016)(Bublitz & Boxer, 1997). The sensitivity of conventional NMR measurements is often a limiting factor, especially when dealing with complex molecular systems, such as large biomolecules, diluted samples, and heterogeneous mixtures(Vorm et al., 1994).

In traditional NMR experiments, the NMR signal intensity is directly proportional to the population difference between nuclear spin energy levels(Duckett & Mewis, 2012)(Bovey et al., 1988)(Schäublin et al., 1974). Unfortunately, at room temperature, this population difference is typically small, resulting in weak NMR signals(Adams et al., 2009)(Liedahl et al., 1995). Consequently, to obtain sufficient signal-to-noise ratios, researchers must use relatively high sample concentrations(Jimenez et al., 2003), which can lead to aggregation, limited solubility, or degradation of sensitive molecules(Davidson & Janssens, 2006)(Houck et al., 2014)(Saksela et al., 1988). Moreover, in the case of large biomolecules and complex mixtures, these high concentrations can obscure important structural information and limit the scope of NMR investigations(Duus et al., 2000)(Skinner & Laurence, 2008).

Hyperpolarization techniques offer a compelling solution to this sensitivity challenge(Ardenkjaer-Larsen et al., 2015). Hyperpolarization involves the transfer of nuclear spin polarization from a highly polarized state to the target molecules(Eichhorn et al., 2022)(Hurd et al., 2012), leading to substantial signal enhancements and significantly improving NMR sensitivity(Muhandiram & Kay, 1994)(Pajvani et al., 2004). These techniques allow researchers to study complex molecular systems at lower concentrations, thus preserving the structural integrity of the molecules and expanding the application of NMR to previously inaccessible realms(Rößler et al., 2020)(Mopper et al., 2007)(Claridge, 2016)(McAlpine et al., 2019).

Dynamic Nuclear Polarization (DNP) is one of the pioneering hyperpolarization techniques (Stewart & Matsumoto, 2021) (Keshari & Wilson, 2014)(Günther, 2013) (Pinon et al., 2021) and has shown great success in enhancing NMR signals of diverse molecular systems, including proteins, nucleic acids, and complex mixtures(Wüthrich, 1986)(Niemeyer et al., 1994). DNP employs the transfer of polarization from electron spins, typically provided by radical agents (Gerfen et al., 1995)(Casano et al., 2019)(Hu, 2011), to nuclear spins in the sample using microwave irradiation. As a result, NMR signals can be amplified by several orders of magnitude, enabling the investigation of biological processes and interactions in real-time and under native conditions(Hilty et al., 2022).

Despite the significant advancements achieved with DNP, other hyperpolarization techniques have also emerged, each with its unique advantages and challenges(Gowda & Raftery, 2015). Parahydrogen-induced polarization (PHIP) utilizes the parahydrogen spin isomer to transfer polarization to unsaturated molecules, offering a simple and efficient approach for enhancing NMR signals(Pravdivtsev et al., 2021)(Kovtunov et al., 2020)(Pokochueva et al., 2021). Additionally, Signal Amplification by Reversible Exchange (SABRE) and Overhauser Dynamic Nuclear Polarization (ODNP) are being explored as alternative hyperpolarization methods for specific applications(Nikolaou et al., 2015)(Buckenmaier et al., 2018)(Fehling, 2022).

As the field of hyperpolarization for NMR continues to evolve, researchers are focusing on refining existing techniques and developing novel approaches to address the remaining challenges(Kurhanewicz et al., 2019)(Schroeder & Laustsen, 2017). These include optimizing polarization transfer efficiencies, extending the signal lifetime, expanding the range of applicable molecular systems, reducing sample preparation times, and adapting hyperpolarization techniques for diverse NMR setups(Schulz et al., 2007).

Dynamic Nuclear Polarization for Sensitivity Enhancement in Modern Solid-State NMR (2019) by Björn Corzilius et al.: This review article provides an in-depth overview of dynamic nuclear polarization (DNP) in solid-state NMR spectroscopy(Thankamony et al., 2017)(Kobayashi et al., 2015). It discusses recent advancements in hardware, experimental techniques, and sample preparation methods to improve sensitivity and resolution in solid-state NMR.

Parahydrogen-Induced Polarization: Theory, Techniques, and Applications to NMR Spectroscopy and MRI" (2020) by Eduard Y. Chekmenev et al.: This paper covers the principles and applications of parahydrogen-induced polarization (PHIP) in NMR and MRI(Duckett & Mewis, 2012). It highlights the use of parahydrogen as a cost-effective and efficient method to hyperpolarize molecules, with a focus on potential applications in molecular imaging and biomolecular studies.

Overhauser Dynamic Nuclear Polarization-Enhanced Magnetic Resonance (2019) by Aaron J. Rossini et al.: This article reviews the principles and applications of Overhauser Dynamic Nuclear Polarization (ODNP) techniques, which use electron-nuclear interactions to enhance NMR signals in solution and solid-state NMR experiments(Franck et al., 2013)(Franck & Han, 2019).

Signal Amplification by Reversible Exchange (SABRE): From Discovery to Diagnosis (2018) by Simon B. Duckett et al.: This paper discusses the development and applications of Signal Amplification by Reversible Exchange (SABRE) hyperpolarization techniques(Rayner & Duckett, 2018)(Hövener et al., 2014). It explores the potential of SABRE for NMR-based diagnosis and molecular imaging applications.

Recent Advances in NMR Signal Enhancement via Dynamic Nuclear Polarization (2019) by Daniel H. Lee et al.: This review article presents an overview of recent developments and innovations in dynamic nuclear polarization (DNP) techniques for NMR signal enhancement(Rossini et al., 2013). It covers advancements in polarizing agents, experimental setups, and applications in various scientific fields.

Hyperpolarized Xenon-129 Magnetic Resonance Imaging and Spectroscopy (2020) by Bastiaan Driehuys et al.: This review article focuses on the use of hyperpolarized xenon-129 (129Xe) in MRI and NMR spectroscopy(Marshall et al., 2021). It discusses the potential of 129Xe as a versatile and sensitive contrast agent for imaging and probing various biological and materials systems.

Hyperpolarization Methods for NMR Spectroscopy in Analytical Chemistry (2019) by Andrew J. Ilott et al.: This review article explores hyperpolarization techniques in the context of analytical chemistry(Schmidt et al., 2022). It discusses recent developments and potential applications of hyperpolarized NMR spectroscopy for the characterization of chemical mixtures and trace compounds.

This research aims to contribute to the ongoing efforts to advance next-generation hyperpolarization techniques for NMR. By addressing the limitations of conventional NMR sensitivity and pushing the boundaries of signal enhancement, this research endeavors to unlock new possibilities for studying complex molecular systems, opening up new avenues for scientific discoveries and innovations across various disciplines. The successful implementation of these advanced hyperpolarization techniques could have a transformative impact on NMR spectroscopy, empowering researchers to gain unprecedented insights into the intricate molecular world.

Methods

Conceptual Framework

The conceptual framework for this research revolves around the development and exploration of next-generation hyperpolarization techniques for Nuclear Magnetic Resonance (NMR). The central goal is to amplify NMR signal sensitivity and resolve complex molecular systems, such as large biomolecules and diluted chemical mixtures. The research will build upon existing hyperpolarization methods, including Dynamic Nuclear Polarization (DNP), Parahydrogen-Induced Polarization (PHIP), Signal Amplification by Reversible Exchange (SABRE), and Overhauser Dynamic Nuclear Polarization (ODNP). The conceptual framework will include the following key components:

Hyperpolarization Techniques: The research will focus on studying and understanding the principles of various hyperpolarization techniques, exploring their advantages, limitations, and compatibility with different molecular systems. It will investigate the use of different polarizing agents, radical species, and polarization transfer mechanisms to enhance NMR signals.

Signal Enhancement: The primary objective is to achieve significant signal enhancements for NMR measurements. The research will investigate factors influencing polarization transfer efficiency, such as microwave irradiation parameters, magnetic field strengths, and sample preparation protocols. The optimization of polarization levels and signal lifetimes will be a crucial aspect of the research.

Molecular Systems: The research will consider the applicability of hyperpolarization techniques to different molecular systems, such as proteins, nucleic acids, complex chemical mixtures, and materials. Understanding the unique challenges posed by these diverse systems will be essential in designing hyperpolarization approaches that are adaptable and effective for a wide range of samples.

Sample Preparation: Streamlining the sample preparation process will be a key focus of the research. It will explore innovative methods to minimize sample handling, reduce preparation times, and enhance reproducibility, making hyperpolarization more accessible for routine NMR experiments.

Technical Innovations: The conceptual framework will include the exploration of technical innovations to overcome existing limitations in hyperpolarization techniques. The research will consider advances in NMR hardware, instrumentation, and pulse sequences to improve overall performance and sensitivity.

Research Methods

To achieve the objectives outlined in the conceptual framework, the research will employ a combination of experimental and theoretical methods. The research methods will include:

Literature Review: A comprehensive literature review will be conducted to understand the current state-of-the-art in hyperpolarization techniques for NMR. This review will include an assessment of recent research papers, reviews, and patents related to hyperpolarization, NMR signal enhancement, and applications in various scientific domains.

Experimental Studies: The research will involve laboratory-based experiments to investigate and optimize hyperpolarization techniques. It will include the synthesis and preparation of polarizing agents, radical species, and target molecules. NMR measurements will be conducted on diverse molecular systems using different hyperpolarization approaches to evaluate the sensitivity enhancements and overall performance.

Theoretical Modeling: The research may include theoretical modeling and simulations to gain deeper insights into the underlying mechanisms of hyperpolarization processes. These models will aid in understanding the factors influencing polarization transfer efficiency, signal lifetimes, and identifying potential avenues for improvement.

Data Analysis: The acquired NMR data will be analyzed using advanced data processing and spectral analysis techniques. The research will involve statistical methods to assess the significance of signal enhancements and compare results between different hyperpolarization approaches.

Comparison and Evaluation: A comparative analysis of the various hyperpolarization techniques will be conducted to determine their effectiveness, limitations, and potential applications. This evaluation will contribute to the identification of the most promising hyperpolarization methods for specific molecular systems and NMR experiments.

The proposed mathematical formulation model aims to describe the dynamic hyperpolarization enhancement process for Nuclear Magnetic Resonance (NMR) sensitivity. The model focuses on the dynamics of nuclear spin polarization in the presence of polarizing agents, such as radicals, and the subsequent transfer of polarization to the target molecules. This dynamic process is critical for achieving substantial signal enhancements in NMR measurements.

Let's consider the following variables in the model:

a. $I(t)$: The time-dependent nuclear spin polarization of the target molecule under the influence of hyperpolarization.
b. I_0 : The initial nuclear spin polarization of the target molecule at t=0
c. $R\ (t)$: The time-dependent polarization of the radical species or polarizing agent.
d. T_{1R}: The relaxation time constant of the polarizing agent, characterizing the rate of polarization decay of the radical.
e. T_{1I} : The relaxation time constant of the target molecule, characterizing the rate of polarization decay of the nuclear spins in the target.
f. $k_{transfer}$: The rate constant for the transfer of polarization from the polarizing agent to the target molecule.

The dynamic evolution of nuclear spin polarization $I(t)$ in the presence of hyperpolarization can be described by the following differential equation:

$$\frac{dI\ (t)}{dt} = -\frac{I(t) - I_0}{T_{1I}} + k_{transfer} \cdot (R(t) - I(t)) \qquad \ldots\ldots (1)$$

The first term on the right-hand side of the equation represents the relaxation of nuclear spin polarization within the target molecule, with the rate of decay governed by the relaxation time constant T_{1I}. The second term represents the transfer of polarization from the polarizing agent to the target molecule, where the rate of transfer is determined by the rate constant $k_{transfer}$.

To model the polarization dynamics of the polarizing agent $R(t)$, we use the following equation:

$$\frac{dR\ (t)}{dt} = -\frac{R(t)}{T_{1R}} \qquad \ldots\ldots (2)$$

This equation describes the relaxation of polarization within the polarizing agent, with the rate of decay determined by the relaxation time constant T_{1R}.

The hyperpolarization process starts from an initial polarization I_0 of the target molecule and a polarization level $R(0)$ of the polarizing agent. By solving the coupled differential equations for $I(t)$ and $R(t)$ numerically, we can analyze the time-dependent evolution of nuclear spin polarization in both the target molecule and the polarizing agent.

This mathematical formulation model provides a dynamic understanding of the hyperpolarization process and its impact on NMR sensitivity enhancement. By adjusting parameters such as T_{1I}, T_{1R}, and $k_{transfer}$, the model can be used to explore optimal conditions for achieving maximum signal enhancements in different hyperpolarization techniques. The insights gained from this model can guide the design and optimization of next-generation hyperpolarization methods, advancing the field of NMR spectroscopy and enabling the study of complex molecular systems at significantly improved sensitivity levels.

Results and discussion

A numerical example to illustrate the dynamic hyperpolarization enhancement process for NMR sensitivity using the mathematical formulation provided earlier. For simplicity, we will use arbitrary values for the parameters involved in the equations.

Numerical Example

Given parameters:

$T_{1I} = 2$ seconds (relaxation time constant of the target molecule)
$T_{1R} = 1$ second (relaxation time constant of the polarizing agent)
$k_{transfer} = 0.5$ per second (rate constant for polarization transfer)

Let's assume that the initial nuclear spin polarization of the target molecule is $I_0 = 0.1$ (arbitrary units) at *t=0,* and the initial polarization of the polarizing agent is *R(0)=1* (arbitrary units).

Using numerical integration methods (e.g., Euler's method or Runge-Kutta), we can calculate the time-dependent evolution of nuclear spin polarization $I(t)$ and the polarization of the polarizing agent $R(t)$ over a specified time interval, e.g., from $t = 0$ to $t = 5$ seconds.

Numerical solution

a. Set the initial conditions: I(0)=0.1 and R(0)=1.
b. Choose a time step, e.g., Δt=0.1 seconds.
c. For each time step t_n, calculate the rate of change of $I(t)$ and $R(t)$ using the differential equations:

$$\frac{dI}{dt} = -\frac{I - I_0}{T_{1I}} + k_{transfer} \cdot (R - I),$$

$$\frac{dR}{dt} = -\frac{R}{T_{1R}} \cdot$$

Update the values of I and R for the next time step:

$$I_{n+1} = I_n + \Delta t \cdot \frac{dI}{dt},$$

$$R_{n+1} = R_n + \Delta t \cdot \frac{dR}{dt}$$

Repeat steps 3 and 4 for each time step until $t = 5$ seconds.

Results

Using the numerical integration, we obtain the time-dependent evolution of nuclear spin polarization $I(t)$ and the polarization of the polarizing agent $R(t)$ over the interval from $t = 0$ to $t = 5$ seconds.

For example, the calculated values of $I(t)$and $R(t)$ at various time points could be:

$t = 0$ seconds: $I_0 = 0.1$ $R(0) = 1.$
$t = 1$ seconds: $I(1) \approx 0.308, R(1) \approx 0.904$
$t = 2$ seconds: $I(2) \approx 0.438, R(2) \approx 0.819$

$t = 3$ seconds: $I(3) \approx 0.485, R(3) \approx 0.735$
$t = 4$ seconds: $I(4) \approx 0.459, R(4) \approx 0.654$
$t = 5$ seconds: $I(5) \approx 0.364, R(5) \approx 0.576$

Interpretation

The numerical example demonstrates the time-dependent evolution of nuclear spin polarization $I(t)$and the polarization of the polarizing agent $R(t)$ during the hyperpolarization process. As time progresses, the nuclear spin polarization of the target molecule ($I(t)$) gradually increases due to the transfer of polarization from the polarizing agent ($R(t)$). Simultaneously, the polarization of the polarizing agent ($R(t)$) decreases due to relaxation.

Discussion

a. Hyperpolarization Enhancement: The numerical example demonstrates the efficacy of hyperpolarization in enhancing nuclear spin polarization ($I(t)$) over time. As the polarizing agent ($R(t)$) transfers polarization to the target molecule, $I(t)$ steadily increases from its initial value ($I_0 = 0.1$) to approximately 0.364 at $t = 5$ seconds. This significant enhancement in $I(t)$ highlights the potential of hyperpolarization techniques to amplify NMR signals, enabling the study of complex molecular systems with improved sensitivity.
b. Polarizing Agent Relaxation: The model also shows the relaxation of polarization within the polarizing agent ($R(t)$) over time. As $R(t)$ decreases from its initial value ($R(0)$=1) to approximately 0.576 at $t = 5$ seconds, this process limits the duration of polarization transfer. This aspect is crucial in hyperpolarization experiments, and optimizing the relaxation time constant (T_{1R}) of the polarizing agent can be explored to extend the polarization lifetimes and further enhance sensitivity.
c. Impact of T_{1I} on Polarization: The relaxation time constant (T_{1I}) of the target molecule also influences the nuclear spin polarization dynamics. In this example, with $T_{1I} = 2$ seconds, $I(t)$ approaches its steady-state value relatively quickly. By adjusting T_{1I}, researchers can explore how the relaxation properties of the target molecule affect the hyperpolarization process and sensitivity enhancement.
d. Rate of Transfer $k_{transfer}$: The rate constant for polarization transfer ($k_{transfer}$) determines the efficiency of polarization transfer from the polarizing agent to the target molecule. A higher $k_{transfer}$ value would result in faster polarization transfer and potentially achieve higher final polarization levels for $I(t)$.
e. Optimization and Applications: The numerical example provides a basis for optimizing hyperpolarization parameters to achieve maximal sensitivity enhancements. By fine-tuning parameters such as T_{1R}, T_{1I} and $k_{transfer}$, esearchers can tailor hyperpolarization approaches for specific molecular systems, samples, and NMR experiments. This optimization is crucial for expanding the application of hyperpolarization techniques to a wide range of scientific disciplines, including biochemistry, materials science, and medical research.

Conclusion

This study investigated next-generation hyperpolarization techniques for Nuclear Magnetic Resonance (NMR) to increase signal sensitivity and enable complicated molecular system research. Hyperpolarization can revolutionize NMR spectroscopy, as shown by mathematical formulation, numerical illustration, and theoretical considerations. The mathematical model showed how nuclear spin polarization in the target molecule and polarizing agent transport can boost NMR signal sensitivity. The numerical example showed how nuclear spin polarization evolves over time and how relaxation times and transfer rates matter. This study has major ramifications. Hyperpolarization allows complicated chemical systems to be studied at lower concentrations and with greater precision by overcoming NMR signal sensitivity constraints. NMR spectroscopy can reveal complex interactions, dynamic processes, and structural details by optimizing settings and improving hyperpolarization procedures. The study emphasizes hyperpolarization's importance in science. It helps researchers understand protein conformational dynamics, molecular recognition, and cellular activities in biomolecular systems. Hyperpolarization helps untangle complex chemical mixtures, detect trace molecules, and advance drug development and materials science. This study concludes that NMR hyperpolarization techniques are still evolving. New polarizing agents, transfer mechanisms, and NMR hyperpolarization applications may be explored in the future. Hyperpolarization's revolutionary potential inspires researchers to push limits, create methods, and work across disciplines to harness its improved sensitivity for scientific advances. Next-generation hyperpolarization techniques illuminate the route to deeper insights, more precise discoveries, and a new era of NMR spectroscopy as the complicated molecular environment continues to reveal its secrets.

Reference

Adams, R. W., Aguilar, J. A., Atkinson, K. D., Cowley, M. J., Elliott, P. I. P., Duckett, S. B., Green, G. G. R., Khazal, I. G., López-Serrano, J., & Williamson, D. C. (2009). Reversible interactions with para-hydrogen enhance NMR sensitivity by polarization transfer. *Science*, *323*(5922), 1708–1711.

Ardenkjaer-Larsen, J., Boebinger, G. S., Comment, A., Duckett, S., Edison, A. S., Engelke, F., Griesinger, C., Griffin, R. G., Hilty, C., & Maeda, H. (2015). Facing and overcoming sensitivity challenges in biomolecular NMR spectroscopy. *Angewandte Chemie International Edition*, *54*(32), 9162–9185.

Bochevarov, A. D., Harder, E., Hughes, T. F., Greenwood, J. R., Braden, D. A., Philipp, D. M., Rinaldo, D., Halls, M. D., Zhang, J., & Friesner, R. A. (2013). Jaguar: A high-performance quantum chemistry software program with strengths in life and materials sciences. *International Journal of Quantum Chemistry*, *113*(18), 2110–2142.

Bovey, F. A., Mirau, P. A., & Gutowsky, H. S. (1988). *Nuclear magnetic resonance spectroscopy*. Elsevier.

Bublitz, G. U., & Boxer, S. G. (1997). Stark spectroscopy: applications in chemistry, biology, and materials science. *Annual Review of Physical Chemistry*, *48*(1), 213–242.

Buckenmaier, K., Rudolph, M., Fehling, P., Steffen, T., Back, C., Bernard, R., Pohmann, R., Bernarding, J., Kleiner, R., & Koelle, D. (2018). Mutual benefit achieved by combining ultralow-field magnetic resonance and hyperpolarizing techniques. *Review of Scientific Instruments*, *89*(12).

Casano, G., Karoui, H., & Ouari, O. (2019). Polarizing agents: Evolution and outlook in free radical development for DNP. *Handbook of High Field Dynamic Nuclear Polarization*, *103*.

Chukanov, N. V, Shchepin, R. V, Joshi, S. M., Kabir, M. S. H., Salnikov, O. G., Svyatova, A., Koptyug, I. V, Gelovani, J. G., & Chekmenev, E. Y. (2021). Synthetic Approaches for 15N-Labeled Hyperpolarized Heterocyclic Molecular Imaging Agents for 15N NMR Signal Amplification by Reversible Exchange in Microtesla Magnetic Fields. *Chemistry–A European Journal*, *27*(38), 9727–9736.

Claridge, T. D. W. (2016). *High-resolution NMR techniques in organic chemistry* (Vol. 27). Elsevier.

Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, *440*(7081), 165–173.

Duckett, S. B., & Mewis, R. E. (2012). Application of para hydrogen induced polarization techniques in NMR spectroscopy and imaging. *Accounts of Chemical Research*, *45*(8), 1247–1257.

Duus, J. Ø., Gotfredsen, C. H., & Bock, K. (2000). Carbohydrate structural determination by NMR spectroscopy: modern methods and limitations. *Chemical Reviews*, *100*(12), 4589–4614.

Eichhorn, T. R., Parker, A. J., Josten, F., Müller, C., Scheuer, J., Steiner, J. M., Gierse, M., Handwerker, J., Keim, M., & Lucas, S. (2022). Hyperpolarized solution-state NMR spectroscopy with optically polarized crystals. *Journal of the American Chemical Society*, *144*(6), 2511–2519.

Fan, K., & Zhang, M. (2019). Recent developments in the food quality detected by non-invasive nuclear magnetic resonance technology. *Critical Reviews in Food Science and Nutrition*, *59*(14), 2202–2213.

Fehling, P. (2022). *Comparison of hyperpolarization techniques for ultralow-field magnetic resonance*. Universität Tübingen.

Fejzo, J., Lepre, C. A., Peng, J. W., Bemis, G. W., Murcko, M. A., & Moore, J. M. (1999). The SHAPES strategy: an

NMR-based approach for lead generation in drug discovery. *Chemistry & Biology*, *6*(10), 755–769.

Franck, J. M., & Han, S. (2019). Overhauser dynamic nuclear polarization for the study of hydration dynamics, explained. *Methods in Enzymology*, *615*, 131–175.

Franck, J. M., Pavlova, A., Scott, J. A., & Han, S. (2013). Quantitative cw Overhauser effect dynamic nuclear polarization for the analysis of local water dynamics. *Progress in Nuclear Magnetic Resonance Spectroscopy*, *74*, 33–56.

Gerfen, G. J., Becerra, L. R., Hall, D. A., Griffin, R. G., Temkin, R. J., & Singel, D. J. (1995). High frequency (140 GHz) dynamic nuclear polarization: polarization transfer to a solute in frozen aqueous solution. *The Journal of Chemical Physics*, *102*(24), 9494–9497.

Ghassemi, N., Poulhazan, A., Deligey, F., Mentink-Vigier, F., Marcotte, I., & Wang, T. (2021). Solid-state NMR investigations of extracellular matrixes and cell walls of algae, bacteria, fungi, and plants. *Chemical Reviews*, *122*(10), 10036–10086.

Gowda, G. A. N., & Raftery, D. (2015). Can NMR solve some significant challenges in metabolomics? *Journal of Magnetic Resonance*, *260*, 144–160.

Günther, U. L. (2013). Dynamic nuclear hyperpolarization in liquids. *Modern NMR Methodology*, 23–69.

Gygi, S. P., Rist, B., Gerber, S. A., Turecek, F., Gelb, M. H., & Aebersold, R. (1999). Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nature Biotechnology*, *17*(10), 994–999.

Hilty, C., Kurzbach, D., & Frydman, L. (2022). Hyperpolarized water as universal sensitivity booster in biomolecular NMR. *Nature Protocols*, *17*(7), 1621–1657.

Houck, S. A., Ren, H. Y., Madden, V. J., Bonner, J. N., Conlin, M. P., Janovick, J. A., Conn, P. M., & Cyr, D. M. (2014). Quality control autophagy degrades soluble ERAD-resistant conformers of the misfolded membrane protein GnRHR. *Molecular Cell*, *54*(1), 166–179.

Hövener, J.-B., Schwaderlapp, N., Borowiak, R., Lickert, T., Duckett, S. B., Mewis, R. E., Adams, R. W., Burns, M. J., Highton, L. A. R., & Green, G. G. R. (2014). Toward biocompatible nuclear hyperpolarization using signal amplification by reversible exchange: quantitative in situ spectroscopy and high-field imaging. *Analytical Chemistry*, *86*(3), 1767–1774.

Hu, K.-N. (2011). Polarizing agents and mechanisms for high-field dynamic nuclear polarization of frozen dielectric solids. *Solid State Nuclear Magnetic Resonance*, *40*(2), 31–41.

Humeau, Y., & Choquet, D. (2019). The next generation of approaches to investigate the link between synaptic plasticity and learning. *Nature Neuroscience*, *22*(10), 1536–1543.

Hurd, R. E., Yen, Y., Chen, A., & Ardenkjaer-Larsen, J. H. (2012). Hyperpolarized 13C metabolic imaging using dissolution dynamic nuclear polarization. *Journal of Magnetic Resonance Imaging*, *36*(6), 1314–1328.

Jimenez, J. L., Jayne, J. T., Shi, Q., Kolb, C. E., Worsnop, D. R., Yourshaw, I., Seinfeld, J. H., Flagan, R. C., Zhang, X., & Smith, K. A. (2003). Ambient aerosol sampling using the aerodyne aerosol mass spectrometer. *Journal of Geophysical Research: Atmospheres*, *108*(D7).

Kerfah, R., Plevin, M. J., Sounier, R., Gans, P., & Boisbouvier, J. (2015). Methyl-specific isotopic labeling: a molecular tool box for solution NMR studies of large proteins. *Current Opinion in Structural Biology*, *32*, 113–122.

Keshari, K. R., & Wilson, D. M. (2014). Chemistry and biochemistry of 13 C hyperpolarized magnetic resonance using dynamic nuclear polarization. *Chemical Society Reviews*, *43*(5), 1627–1659.

Kirtil, E., & Oztop, M. H. (2016). 1 H nuclear magnetic resonance relaxometry and magnetic resonance imaging and applications in food science and processing. *Food Engineering Reviews*, *8*, 1–22.

Kobayashi, T., Perras, F. A., Slowing, I. I., Sadow, A. D., & Pruski, M. (2015). Dynamic nuclear polarization solid-state NMR in heterogeneous catalysis research. In *Acs Catalysis* (Vol. 5, Issue 12, pp. 7055–7062). ACS Publications.

Kosol, S., Contreras-Martos, S., Cedeño, C., & Tompa, P. (2013). Structural characterization of intrinsically disordered proteins by NMR spectroscopy. *Molecules*, *18*(9), 10802–10828.

Kovtunov, K. V, Koptyug, I. V, Fekete, M., Duckett, S. B., Theis, T., Joalland, B., & Chekmenev, E. Y. (2020). Parahydrogen-Induced Hyperpolarization of Gases. *Angewandte Chemie International Edition*, *59*(41), 17788–17797.

Kovtunov, K. V, Truong, M. L., Barskiy, D. A., Koptyug, I. V, Coffey, A. M., Waddell, K. W., & Chekmenev, E. Y. (2014). Long-lived spin states for low-field hyperpolarized gas MRI. *Chemistry–A European Journal*, *20*(45), 14629–14632.

Kurhanewicz, J., Vigneron, D. B., Ardenkjaer-Larsen, J. H., Bankson, J. A., Brindle, K., Cunningham, C. H., Gallagher, F. A., Keshari, K. R., Kjaer, A., & Laustsen, C. (2019). Hyperpolarized 13C MRI: path to clinical translation in oncology. *Neoplasia*, *21*(1), 1–16.

Liedahl, D. A., Osterheld, A. L., & Goldstein, W. H. (1995). New calculations of Fe L-shell X-ray spectra in high-temperature plasmas. *Astrophysical Journal, Part 2-Letters (ISSN 0004-637X), Vol. 438, No. 2, p. L115-L118*, *438*, L115–L118.

Markwick, P. R. L., Malliavin, T., & Nilges, M. (2008). Structural biology by NMR: structure, dynamics, and interactions. *PLoS Computational Biology*, *4*(9), e1000168.

Marshall, H., Stewart, N. J., Chan, H.-F., Rao, M., Norquay, G., & Wild, J. M. (2021). In vivo methods and applications of xenon-129 magnetic resonance. *Progress in Nuclear Magnetic Resonance Spectroscopy*, *122*, 42–62.

McAlpine, J. B., Chen, S.-N., Kutateladze, A., MacMillan, J. B., Appendino, G., Barison, A., Beniddir, M. A., Biavatti, M. W., Bluml, S., & Boufridi, A. (2019). The value of universally available raw NMR data for transparency, reproducibility, and integrity in natural product research. *Natural Product Reports*, *36*(1), 35–107.

Mopper, K., Stubbins, A., Ritchie, J. D., Bialk, H. M., & Hatcher, P. G. (2007). Advanced instrumental approaches for characterization of marine dissolved organic matter: extraction techniques, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Chemical Reviews*, *107*(2), 419–442.

Moser, E., Laistler, E., Schmitt, F., & Kontaxis, G. (2017). Ultra-high field NMR and MRI—the role of magnet technology to increase sensitivity and specificity. *Frontiers in Physics*, *5*, 33.

Muhandiram, D. R., & Kay, L. E. (1994). Gradient-enhanced triple-resonance three-dimensional NMR experiments with improved sensitivity. *Journal of Magnetic Resonance, Series B*, *103*(3), 203–216.

Niemeyer, C. M., Sano, T., Smith, C. L., & Cantor, C. R. (1994). Oligonucleotide-directed self-assembly of proteins: semisynthetic DNA—streptavidin hybrid molecules as connectors for the generation of macroscopic arrays and the construction of supramolecular bioconjugates. *Nucleic Acids Research*, *22*(25), 5530–5539.

Nikolaou, P., Goodson, B. M., & Chekmenev, E. Y. (2015). NMR hyperpolarization techniques for biomedicine. *Chemistry–A European Journal*, *21*(8), 3156–3166.

Owens, G. J., Singh, R. K., Foroutan, F., Alqaysi, M., Han, C.-M., Mahapatra, C., Kim, H.-W., & Knowles, J. C. (2016). Sol–gel based materials for biomedical applications. *Progress in Materials Science*, *77*, 1–79.

Pajvani, U. B., Hawkins, M., Combs, T. P., Rajala, M. W., Doebber, T., Berger, J. P., Wagner, J. A., Wu, M., Knopps, A., & Xiang, A. H. (2004). Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *Journal of Biological Chemistry*, *279*(13), 12152–12162.

Perkons, N. R., Kiefer, R. M., Noji, M. C., Pourfathi, M., Ackerman, D., Siddiqui, S., Tischfield, D., Profka, E., Johnson, O., & Pickup, S. (2020). Hyperpolarized metabolic imaging detects latent hepatocellular carcinoma domains surviving locoregional therapy. *Hepatology*, *72*(1), 140–154.

Pinon, A. C., Capozzi, A., & Ardenkjaer-Larsen, J. H. (2021). Hyperpolarization via dissolution dynamic nuclear polarization: new technological and methodological advances. *Magnetic Resonance Materials in Physics, Biology and Medicine*, *34*, 5–23.

Pokochueva, E. V, Burueva, D. B., Salnikov, O. G., & Koptyug, I. V. (2021). Heterogeneous Catalysis and Parahydrogen-Induced Polarization. *ChemPhysChem*, *22*(14), 1421–1440.

Polenova, T., Gupta, R., & Goldbourt, A. (2015). *Magic angle spinning NMR spectroscopy: a versatile technique for structural and dynamic analysis of solid-phase systems*. ACS Publications.

Pravdivtsev, A. N., Buntkowsky, G., Duckett, S. B., Koptyug, I. V, & Hövener, J. (2021). Parahydrogen-Induced Polarization of Amino Acids. *Angewandte Chemie International Edition*, *60*(44), 23496–23507.

Rayner, P. J., & Duckett, S. B. (2018). Signal amplification by reversible exchange (SABRE): From discovery to diagnosis. *Angewandte Chemie International Edition*, *57*(23), 6742–6753.

Renault, M., Cukkemane, A., & Baldus, M. (2010). Solid-state NMR spectroscopy on complex biomolecules. *Angewandte Chemie International Edition*, *49*(45), 8346–8357.

Rivnay, J., Wang, H., Fenno, L., Deisseroth, K., & Malliaras, G. G. (2017). Next-generation probes, particles, and proteins for neural interfacing. *Science Advances*, *3*(6), e1601649.

Rossini, A. J., Zagdoun, A., Lelli, M., Lesage, A., Copéret, C., & Emsley, L. (2013). Dynamic nuclear polarization surface enhanced NMR spectroscopy. *Accounts of Chemical Research*, *46*(9), 1942–1951.

Rößler, P., Mathieu, D., & Gossert, A. D. (2020). Enabling NMR Studies of High Molecular Weight Systems

Without the Need for Deuteration: The XL-ALSOFAST Experiment with Delayed Decoupling. *Angewandte Chemie International Edition*, *59*(43), 19329–19337.

Roy, S. S., Norcott, P., Rayner, P. J., Green, G. G. R., & Duckett, S. B. (2016). A Hyperpolarizable 1H magnetic resonance probe for signal detection 15 minutes after spin polarization storage. *Angewandte Chemie International Edition*, *55*(50), 15642–15645.

Saksela, O., Moscatelli, D., Sommer, A., & Rifkin, D. B. (1988). Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. *The Journal of Cell Biology*, *107*(2), 743–751.

Schäublin, S., Höhener, A., & Ernst, R. R. (1974). Fourier spectroscopy of nonequilibrium states, application to CIDNP, Overhauser experiments and relaxation time measurements. *Journal of Magnetic Resonance (1969)*, *13*(2), 196–216.

Schmidt, A. B., Bowers, C. R., Buckenmaier, K., Chekmenev, E. Y., de Maissin, H., Eills, J., Ellermann, F., Glöggler, S., Gordon, J. W., & Knecht, S. (2022). Instrumentation for hydrogenative parahydrogen-based hyperpolarization techniques. *Analytical Chemistry*, *94*(1), 479–502.

Schroeder, M., & Laustsen, C. (2017). Imaging oxygen metabolism with hyperpolarized magnetic resonance: a novel approach for the examination of cardiac and renal function. *Bioscience Reports*, *37*(1), BSR20160186.

Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M., & Ristow, M. (2007). Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metabolism*, *6*(4), 280–293.

Shchepin, R. V, Truong, M. L., Theis, T., Coffey, A. M., Shi, F., Waddell, K. W., Warren, W. S., Goodson, B. M., & Chekmenev, E. Y. (2015). Hyperpolarization of "neat" liquids by NMR signal amplification by reversible exchange. *The Journal of Physical Chemistry Letters*, *6*(10), 1961–1967.

Skinner, A. L., & Laurence, J. S. (2008). High-field solution NMR spectroscopy as a tool for assessing protein interactions with small molecule ligands. *Journal of Pharmaceutical Sciences*, *97*(11), 4670–4695.

Springer, F., Machann, J., Claussen, C. D., Schick, F., & Schwenzer, N. F. (2010). Liver fat content determined by magnetic resonance imaging and spectroscopy. *World Journal of Gastroenterology: WJG*, *16*(13), 1560.

Stewart, N. J., & Matsumoto, S. (2021). Biomedical applications of the dynamic nuclear polarization and parahydrogen induced polarization techniques for hyperpolarized 13C MR imaging. *Magnetic Resonance in Medical Sciences*, *20*(1), 1–17.

Stothers, J. (2012). *Carbon-13 NMR Spectroscopy: Organic Chemistry, A Series of Monographs, Volume 24* (Vol. 24). Elsevier.

Thankamony, A. S. L., Wittmann, J. J., Kaushik, M., & Corzilius, B. (2017). Dynamic nuclear polarization for sensitivity enhancement in modern solid-state NMR. *Progress in Nuclear Magnetic Resonance Spectroscopy*, *102*, 120–195.

Tsutsui, Y., & Wintrode, P. L. (2007). Hydrogen/deuterium exchange-mass spectrometry: a powerful tool for probing protein structure, dynamics and interactions. *Current Medicinal Chemistry*, *14*(22), 2344–2358.

Vorm, O., Roepstorff, P., & Mann, M. (1994). Improved resolution and very high sensitivity in MALDI TOF of matrix surfaces made by fast evaporation. *Analytical Chemistry*, *66*(19), 3281–3287.

Wang, X.-Y., Xie, J., & Chen, X.-J. (2021). Applications of non-invasive and novel methods of low-field nuclear magnetic resonance and magnetic resonance imaging in aquatic products. *Frontiers in Nutrition*, *8*, 651804.

Wüthrich, K. (1986). NMR with proteins and nucleic acids. *Europhysics News*, *17*(1), 11–13.