Helmholtz Young Investigator Group VH-NG-1432 – Hyperpolarized Magnetic Resonance

Final Report

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This report summarizes research results that have been obtained within the Helmholtz-Young Investigator Group VH-NG-1432.

Project duration: 6.5 years (6 years + cost neutral extension by 0.5 years)
Project start date: 1.3.2019
Project end date: 31.08.2025

Part I - Key Objectives and Outcomes

The key objective of the proposal was to realize the potential of rapid-transfer DNP (now known as bullet-DNP), which is a method to boost signals in Nuclear Magnetic Resonance (NMR) Spectroscopy and Magnetic Resonance Imaging (MRI). In bullet-DNP, a target substance is mixed with a radical in a glass-forming solvent. This solvent is then pipetted into a small plastic bucket (known as the bullet), frozen and inserted into a DNP polarizer, where polarization is transferred from the electron spin of the radical to nuclear spins on the target substance at a temperature of 1.5 K and a field of 3 – 7 Tesla. The bullet is then ejected from the polarizer, the sample is dissolved, and hyperpolarized liquid-state NMR spectra are recorded.

The key objectives defined in the proposal are:

- To develop rapid-transfer DNP into a method that can provide liquid hyperpolarized solutions at the target magnet with negligible polarization losses and minimal dilution.
- To maximize the amount of attainable spectral information through miniaturized, parallel acquisition and innovative pulse sequences.
- To demonstrate the power of the approach via application to benchmark samples such as pyruvate and ubiquitin.
- To apply the methodology to ongoing research in biochemistry and biology, and in particular to a study of the metabolome of zebrafish liver extracts across the circadian clock cycle, on a single specimen level.

The first three objectives have been achieved.

Regarding the first objective, hyperpolarized solutions can be provided up to every 20 min, with world-leading polarization levels. To the best of our knowledge, we are the only laboratory operating a fully automated bullet-DNP / dissolution-DNP system globally. We have reported the observation of ^{13}C-labelled amino acids and other molecules at nano-Molar to low micro-Molar concentrations, with excellent mass sensitivity. We are in the process of publishing results on hyperpolarized ^{1}H NMR, where we achieve the highest mass sensitivity, with signal enhancements of > 2,000 at only 20-fold dilution.

Regarding the second objective, we have been able to improve the spectral resolution 10-fold, through improved instrumentation (notably the use of back-pressure), and boost sensitivity further using pulse sequences that are based on the famous INEPT NMR sequence.

Polarization levels of > 30 % have been achieved on pyruvate. Notably we have also been able to hyperpolarize the CO carbons of proteins in prototype experiments.

Regarding the fourth objective, we have shifted the focus and applied the methodology towards ligand-binding, with a view towards hyperpolarized drug screening applications. Here it has been possible to detect ligand-binding in a single scan, using minute quantities of ligand and protein.

Part II – Detailed List of Research Outcomes

A detailed list of published research outcomes is given below.

Instrumentation

Following the setup of the laboratory at Karlsruhe Institute of Technology, we published the performance characteristics of a new bullet-DNP system in 2021. We were able to improve the resolution of hyperpolarized 13C spectra by more than an order of magnitude from 30 Hz to 2 Hz. The setup also achieved a higher degree of automation, with the possibility to automatically shoot hyperpolarized bullets from the polarizer into the liquid-state NMR magnet, and also to dispose of empty bullets automatically. [1]

Low-field Relaxation

During the transfer of the hyperpolarized bullet from the polarizer to the liquid-state NMR magnet, the sample invariably travels through regions of low-field. In order to minimize polarization losses due to relaxation, we planned to study relaxation losses at low-field using fast-field-cycling (FFC) relaxometry. In a cooperation with the gropu of John Owers-Bradley at the University of Nottingham, we recorded relaxation time constants at fields ranging from 5 mT to 2 T, and were able to model the observed phenomena using only a minimal set of parameters. Our work was published in two studies that describe relaxation phenomena of protons, and those of carbon, respectively. [2,3]

Rheo-NMR and Peptide binding

Although not anticipated during the writing of the proposal, our group was able to contribute to studies that (i) investigated anisotropic NMR parameters under shear, with control from within the NMR sequence, and (ii) validated the binding of peptides to a fuel cell membrane material.[4,5]

Carbonates

Several studies are related to carbonates. Compared to conventional D-DNP, the transfer of the hyperpolarized sample as a solid in bullet-DNP enables unique experiments. In a collaboration with the group of Dennis Kurzbach at the University of Vienna we observed so-called pre-nucleation species (PNS) that exist in the early stages of the formation of carbonate. In a cooperation with the group of Aharon Blank, then at Technion Haifa, we used carbonates as a "storage" medium for hyperpolarization. In these experiments, ^{13}C labelled target molecules were embedded in a partially ^{13}C-labelled carbonate matrix. The long T_1 of the carbonate carbons then acts as a "battery" for polarization, that replenishes the polarization of ^{13}C on the labelled target molecules, enabling the detection of hyperpolarized signals many minutes after ejection from the polarizer.[6–8]

Long-lived states

A challenge of using hyperpolarization is that – following ejection of the hyperpolarized sample from the polarizer – the polarization decays rapidly, typically with the T1 of the nucleus that is detected. Long-lived states are quantum states of pairs or larger groups of spins exhibit a relaxation time constant that is longer than T1. Additionally, these states are often populated

naturally as a consequence of the hyperpolarization step. In a collaboration with the group of Geoffrey Bodenhausen at ENS Paris, mostly funded by our joined ERC Synergy project HiSCORE, we were able to demonstrate the direct hyperpolarization of ^{1}H long-lived states in aliphatic chains.[9]

Sensitivity and Ligand-Binding

The detection of ligand-binding with the highest possible sensitivity has become a key research objective of our group. This requires that (i) the ligand can be detected with the highest sensitivity and (ii) a contrast mechanism to detect the binding of the ligand to a protein target. Focussing first on sensitivity, we developed a sensitization scheme for carbon-labelled molecules, with which we were able to detect pyruvate at nanomolar concentrations, and 13C-labelled amino acids at low micromolar concentrations, using 13C hyperpolarization, and an INEPT transfer to protons in the liquid, followed by proton detection. We then combined this scheme with low-field relaxation as contrast agent, and demonstrated the detection of ligand binding in a single scan, using ligand and protein concentrations of only 14 and 2 µM, respectively. [10,11]

Hyperpolarization and Sensitivity

Hyperpolarization can boost the sensitivity of NMR by orders of magnitude, but often the signal enhancements are bought at the expense of lower resolution, lower throughput, or high dilution. Whether hyperpolarization can be used with added value is often not a trivial question. We therefore wrote a detailed analysis of the interplay of these parameters. [12]

Non-secular resonances

Following the low-field relaxation measurements listed above, the University of Nottingham donated their unique fast-field-cycling (FFC) apparatus to our group. This enabled us to test a prediction by Boris Fine at the University of Leipzig, according to which resonances far off the nuclear Larmor frequency can drive spin dynamics, with potential applications for polarization transfer and structure elucidation. We designed an experiment to test this prediction using the FFC apparatus and successfully observed a non-secular resonance predicted by Fine.[13]

Dissolution-DNP Review

Finally, we published an extended review article, in which we describe the dissolution-DNP experiment in substantial detail. [14]

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List of Publications

1. Kouřil, K., Gramberg, M., Jurkutat, M., Kouřilová, H. & Meier, B. A Cryogen-Free, Semi-Automated Apparatus for Bullet-Dynamic Nuclear Polarization With Improved Resolution. *Magn. Reson.* **2**, 815–825 (2021).
2. Jurkutat, M. *et al.* Radical-Induced Low-Field ^{1}H Relaxation in Solid Pyruvic Acid Doped With Trityl-OX063. *J. Phys. Chem. Lett.* **13**, 10370–10376 (2022).
3. Kouřilová, H. *et al.* Radical-Induced Hetero-Nuclear Mixing and Low-Field ^{13}C Relaxation in Solid Pyruvic Acid. *Phys. Chem. Chem. Phys.* **24**, 28242–28249 (2022).
4. Hoffmann, F., Kouřil, K., Berger, S. T., Meier, B. & Luy, B. Rheo-Nmr At the Phase Transition of Liquid Crystalline Poly-γ-benzyl-l-glutamate: Phase Kinetics and a Valuable Tool for the Measurement of Residual Dipolar Couplings. *Macromolecules* **56**, 7782–7794 (2023).
5. Schmidt, D. *et al.* Selective Peptide Binders To the Perfluorinated Sulfonic Acid Ionomer Nafion. *Adv. Funct. Mater.* **34**, (2023).
6. Katz, I. *et al.* Long-Lived Enhanced Magnetization-A Practical Metabolic MRI Contrast Material. *Sci. Adv.* **10**, eado2483 (2024).
7. Turhan, E. *et al.* Biphasic NMR of Hyperpolarized Suspensions–real-Time Monitoring of Solute-To-Solid Conversion To Watch Materials Grow. *J. Phys. Chem. C* **127**, 19591–19598 (2023).
8. Turhan, E. *et al.* Short-Lived Calcium Carbonate Precursors Observed in Situ Via Bullet-Dynamic Nuclear Polarization. *Commun. Chem.* **7**, 210 (2024).

9. Razanahoera, A. *et al.* Hyperpolarization of Long-Lived States of Protons in Aliphatic Chains By Bullet Dynamic Nuclear Polarization, Revealed on the Fly By Spin-Lock-Induced Crossing. *J. Phys. Chem. Lett.* **15**, 9024–9029 (2024).
10. Narwal, P. *et al.* Bullet-DNP Enables NMR Spectroscopy of Pyruvate and Amino Acids At Nanomolar To Low Micromolar Concentrations. *Anal. Chem.* **96**, 14734–14740 (2024).
11. Narwal, P., Lorz, N., Minaei, M., Gossert, A. D. & Meier, B. Single-Scan Detection of Ligand-Binding Using Hyperpolarization and Low-Field Relaxation. *Commun. Chem.* **in press**, (2026).
12. Kouřil, K. & Meier, B. Hyperpolarization and Sensitivity in Nuclear Magnetic Resonance. *J. Magn. Reson. Open* **21**, 100172 (2024).
13. Jurkutat, M. *et al.* Nuclear Magnetic Resonance Far Off the Larmor Frequency: Nonsecular Resonances in CaF_2. *Phys. Rev. B* **112**, L06032 (2025).
14. Meier, B. The Dissolution-Dynamic Nuclear Polarization Experiment. *Prog. Nucl. Magn. Reson. Spectrosc.* **in press**, 101598 (2026).