Simulation of complex magnetokinetic processes

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This is a tutorial lecture describing how the fundamentals of spin dynamics change in the presence of linear and non-linear chemical kinetics, hydrodynamics, and diffusion. Examples are drawn from the simulation problems encountered in parahydrogen-induced polarisation (PHIP), chemically induced dynamic nuclear polarisation (CIDNP), and magnetic resonance imaging (MRI).

On the chemical kinetics side, linear processes (networks of first-order reactions) are easy to handle because the mathematics is compatible with the linear dynamics expected by the Liouville - von Neumann equation. Significant complications that appear when the kinetics becomes non-linear will be discussed in this lecture, with simulation examples programmed using *Spinach* library [1].

In the simulation of complex magnetic resonance processes subject to diffusion and stationary flow, the principal problem is impractically large matrices that result from taking Kronecker products of spin Liouvillians with three-dimensional spatial evolution generators [2]. The numerical complexity problem was recently solved [3]; this lecture will discuss the use of polyadic decompositions of spin and space evolution generators in the simulation of processes that feature simultaneous spin dynamics, chemical kinetics, as well as three-dimensional diffusion and flow.

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Developing analytical chemistry applications for parahydrogen hyperpolarization

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Parahydrogen hyperpolarization techniques increase NMR signals by several orders of magnitude. Applications' development has been focusing mainly on two directions: a) generating solutions of hyperpolarized single compounds for imaging purposes; and b) utilizing the signal enhancement to detect dilute analytes that occur below the LOD of normal NMR.

The Tessari group developed methodology for parahydrogen enhanced chemosensing of sub-μM analytes[1] and 2D resolution of the hyperpolarized spectra.[2] Herein we build on this work and demonstrate the first practical (bio)analytical application of parahydrogen hyperpolarization.

We developed a protocol for pharmacokinetics and followed the urinary elimination of nicotine and its metabolites during the onset and withdrawal from nicotine consumption and compared different routes of administration. Since nicotine metabolism has been thoroughly studied, we could compare our results to orthogonal analytical techniques to validate the pharmacokinetic data obtained by parahydrogen hyperpolarization. Sample preparation by solid phase extraction (SPE) and detection by hyperpolarized chemosensing allowed a limit of detection of 30 mM for the method and very good repeatability by using 50% enriched parahydrogen.

However, SPE can be seen as a drawback, since it imposes another layer of chemoselectivity and retains only a specific fraction of the biofluid. In order to allow a more broad-spectrum analysis, we have made efforts towards removing the SPE step. I will present our latest results on detecting endogenous metabolites that occur below the LOD of normal NMR with minimal urine sample manipulation before hyperpolarized analysis.

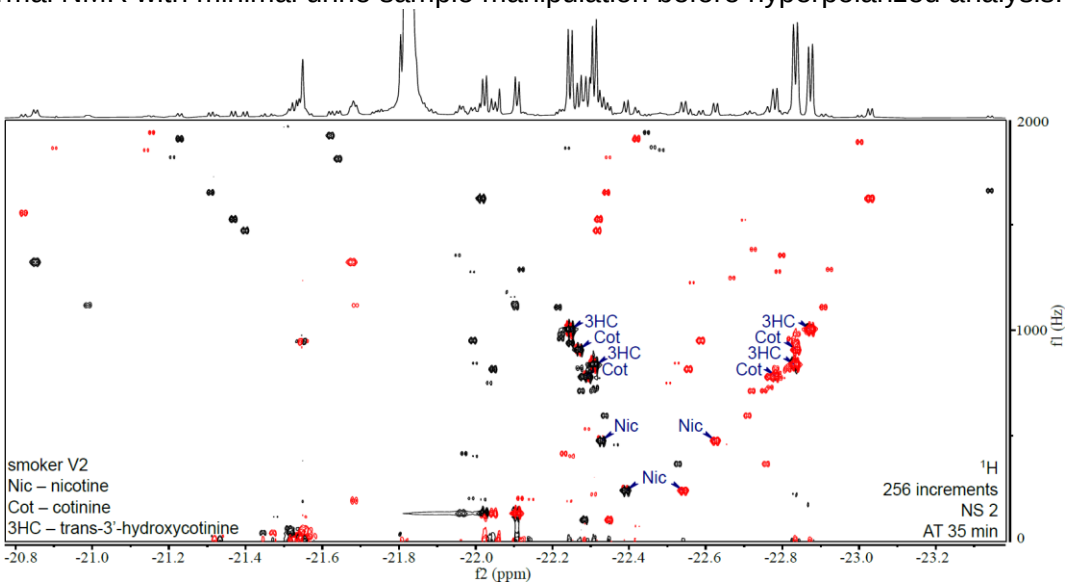

Figure 1. 2D plot of the hyperpolarized signals corresponding to nicotine and its metabolites in a sample prepared from tobacco smoker's urine

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Photochemically Induced Dynamic Nuclear Polarization of Heteronuclear Singlet Order

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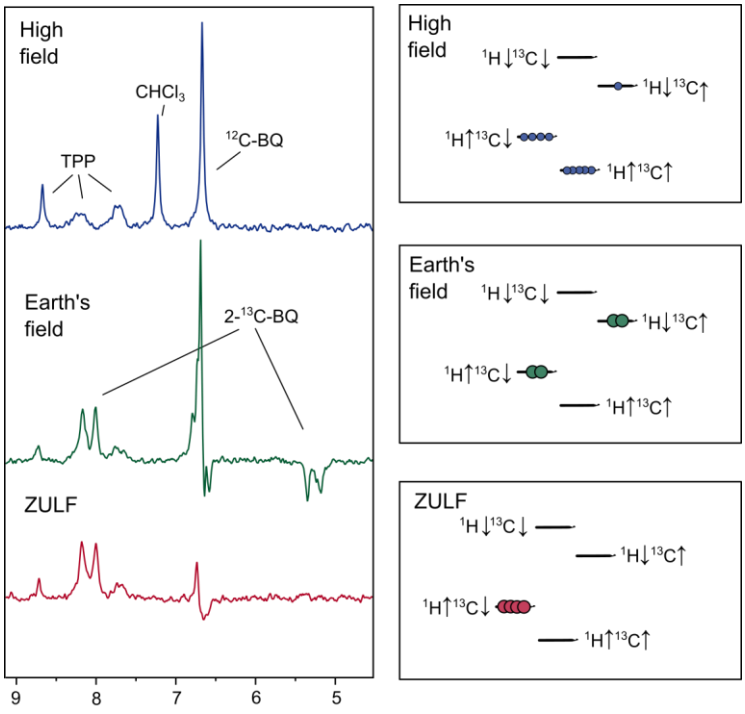

Figure 1. 60 MHz ^{1}H NMR spectra (left) and spin state populations (right) and for the three cases. (1) Thermal equilibrium case. (2) photo-CIDNP generated at 30 μT and detected at high field. (3) photo-CIDNP generated at 1 μT and detected at high field. Samples contain 0.5 mM TPP, 5 mM BQ diluted in degassed mixture of $CDCl_3$ (70%) and CH_3OH (30%).

Dynamic nuclear polarization (DNP), chemically induced dynamic nuclear polarization (CIDNP) and parahydrogen-induced polarization (PHIP) are the most general methods to hyperpolarize nuclear spins. Interestingly, the first observations of CIDNP were explained by a DNP-related mechanism [1], and the first observation of PHIP was explained by a CIDNP-related mechanism [2]. This chain of confusions reflects the complexity of the involved processes and hints that there are certain similarities between them.

In this work, we demonstrate photo-CIDNP experiments that have direct analogies with classical ALTADENA [3] and PASADENA [4] experiments. The demonstration was done for photo-CIDNP of tetraphenylporphyrin (TPP) and para-benzoquinone (BQ). It reveals a general situation that should be applicable to many systems known to exhibit photo-CIDNP. Heteronuclear spin states (e.g. between ^{1}H and ^{13}C spins) are explored. Analysis of the spin dynamics shows that the hyperpolarized states of the ^{1}H-^{13}C spin pairs can be characterized by two-spin order (Earth's field) and singlet order (zero- to ultralow-field, ZULF) [5]. Photo-CIDNP is a reversible process and in the studied system it was possible to repeat experiments for hundreds of times enabling measurements of the relaxation kinetics. The heteronuclear singlet state produced at ZULF had two times longer lifetime than the state hyperpolarized at the Earth's field. These experiments have an advantage that light irradiation can be performed efficiently outside of the NMR spectrometer. NMR signals were enhanced by more than 200 times as was observed in a benchtop NMR spectrometer (Figure 1) after shuttling the sample from the low-field region to the NMR spectrometer.

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Hyperpolarised (1-^{13}C) Fumarate on a Microfluidic Chip

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Nuclear magnetic resonance (NMR) spectroscopy is an ideal tool to follow chemical and biochemical processes in microfluidic lab-on-a-chip (LoC) devices due to its non-invasive nature and the ability to quantify metabolites. However, the main limitation remains its low sensitivity. One of the ways to address this issue is to use hyperpolarisation methods. Parahydrogen induced polarisation is an inexpensive way of polarising molecules, where polarisation is transferred from the singlet order of hydrogen gas to a target molecule via a chemical reaction.

In our recent work we produce a continuous stream of hyperpolarised (1-^{13}C) fumarate in a microfluidic device at 2.5 μL scale (fig. 1a) utilising singlet-to-magnetisation (S2M) pulse sequence. Besides pulse sequence efficiency, the polarisation yield of this method directly depends on the initial spin-order created by the chemical reaction. It is known that hydrogen molecules and the heavy metal catalyst form intermediate hydrate species where two hydrogen spins take non-equivalent positions.[1] Therefore, if the residence time in such complexes is long enough, nuclear singlet state will evolve to one of the triplet states (S-T mixing), decreasing the efficiency of S2M sequence.[2] It has been shown that applying a broadband pulse prior S2M (fig 1b) can circumvent S-T mixing but such effects are difficult to study in conventional NMR set ups because they require high degree of accuracy and reproducibility.[3-4] Additionally, since hyperpolarised species have a limited life time multiple samples have to prepared.

Our microfluidic technology allows to establish a constant stream of a hyperpolarised product providing stable and reproducible conditions. This allows us to study complex physical phenomena such as S-T mixing (fig 1c). We show that circumventing S-T mixing improves the polarisation yield 5-fold leading to 4% ^{1}H polarisation.

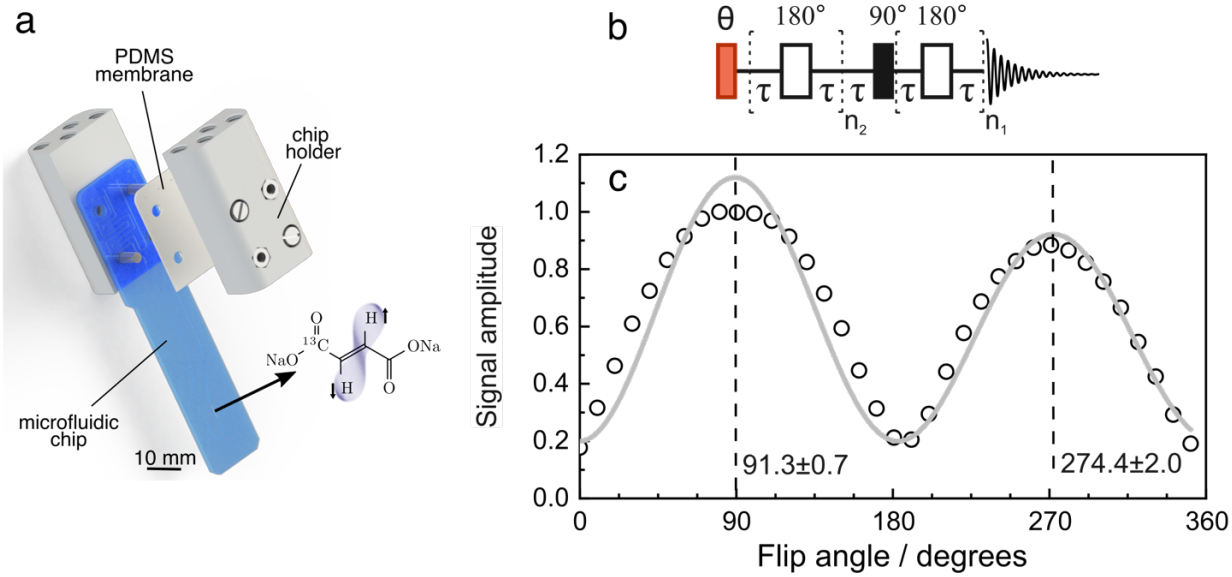

Figure 1: a) Microfluidic device. b) θ - S2M pulse sequence. c) Signal intensity of hyperpolarised (1-^{13}C) fumarate as a function of the pre-pulse angle.

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"Direct" ^{13}C Hyperpolarization of Oxalates by SABRE

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Over the last few years SABRE (Signal Amplification by Reversible Exchange) has significantly expanded its scope to hyperpolarize a larger class of substrates away from typical N-heterocyclic targets. Alongside the techniques of SABRE-Relay, co-ligand based strategies have beginning to show its potential in hyperpolarizing molecules of high biological interests that were earlier thought to be challenging by the standard SABRE methods.[1, 2] A landmark in SABRE research was achieved in 2019 when pyruvate was finally shown to hyperpolarize via SABRE by using appropriate co-ligands that provide required stabilisation of the active catalytic species.[3] As identified soon after, oxalate also contains similar α-ketoacid motif and were expected to be amenable to the same recipe. However, initial studies revealed that oxalates bind to the catalyst rather strongly forming a chelated compound and hindering the effect of SABRE.[4]

In this study we demonstrate a simple route to directly hyperpolarize oxalates via SABRE by synthesising pH-calibrated substrates, alleviating the issue of stable chelation of such compounds. Using 50% enriched para-hydrogen, we achieved up to 400-fold ^{13}C signal enhancement in sodium oxalates compared to thermal signal at 9.4 T. Further, based on the strong correlation of hyperpolarization effect with the pH level, we show a potential route to use these substrates as pH sensing agents. When an analogous of doubly labelled ^{13}C oxalate is used, 'direct' long-lived singlet hyperpolarization is achieved with an extended spin lifetime that may prove beneficial for their further applications.

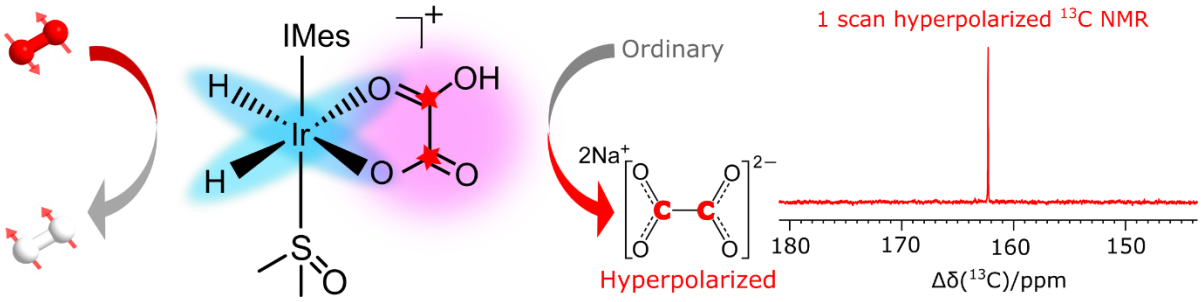

Figure 1: (Left) Schematic of SABRE mechanism of sodium oxalate in the presence of DMSO as a co-ligand. (Right) Hyperpolarized ^{13}C spectra of natural abundance sodium oxalate at SABRE-SHEATH condition.

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Para-Hydrogen HyperPolarized molecules in the landscape of the in vivo diagnostic tools: promises, pitfalls, perspectives

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Since the early days of the first PHIP experiments there was a great expectation that the outstanding properties of PHIP molecules could be exploited for improving the available diagnostic procedures and for tackling unmet diagnostic needs.
In principle several PHIP molecules own properties (long T1, biocompatibility, etc.) that make them potential systems to compete with currently used perfusion and angiographic imaging procedures.
Of course much attention is currently devoted to assess whether PHIP molecules can be employed to report on metabolic processes. The implementation of the PHIP-SAH approach paves the way to report on in vivo enzymatic activities previously accessible only to DNP hyperpolarized molecules.
In this presentation I'll survey the major issues that still hamper the clinical translation of PHIP-based in vivo diagnostics.

Toward Continuous-Flow Hyperpolarisation of Metabolites via Heterogenous Catalysis, Side-Arm-Hydrogenation, and Membrane Dissolution of Parahydrogen

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Side-arm hydrogenation (SAH) by homogeneous catalysis has extended the reach of the parahydrogen enhanced NMR technique to key metabolites such as pyruvate[1,2]. However, homogeneous hydrogenation requires rapid separation of the dissolved catalyst and purification of the hyperpolarised species with a purity sufficient for safe in-vivo use. An alternate approach is to employ heterogeneous hydrogenation in a continuous-flow reactor, where separation from the solid catalysts is straightforward. Using a TiO_2-nanorod supported Rh catalyst, we demonstrate continuous-flow parahydrogen enhanced NMR by heterogeneous hydrogenation of a model SAH precursor, propargyl acetate, at a flow rate of 1.5 mL/min. Parahydrogen gas was introduced into the flowing solution phase using a novel tube-in-tube membrane dissolution device based on previous designs[3,4]. Without much optimization, the ALTADENA[5] proton NMR signal enhancements of up to 297 (relative to the thermal equilibrium signals) at 9.4 Tesla were shown to be feasible on allyl-acetate at a continuous total yield of 33 %. The results are compared to those obtained with the standard batch-mode technique of parahydrogen bubbling through a suspension of the same catalyst.

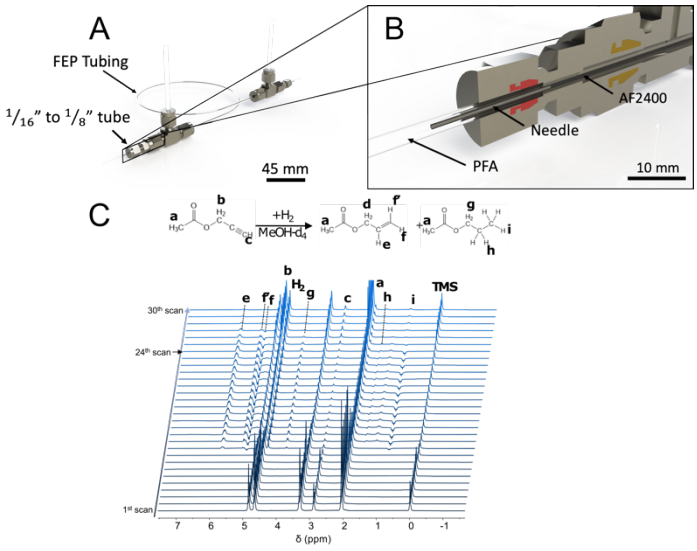

Figure 1: A: A CAD rendered image showing the tube-in-tube design. B: A Cut-out rendered image showing the sealing point of the tube-in-tube. C: Reaction scheme and 30 scan continuous flow experiment displaying the growing and decay of hyperpolarised signal.

References

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Acknowledgements

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65% Parahydrogen from a Liquid Nitrogen Cooled Generator

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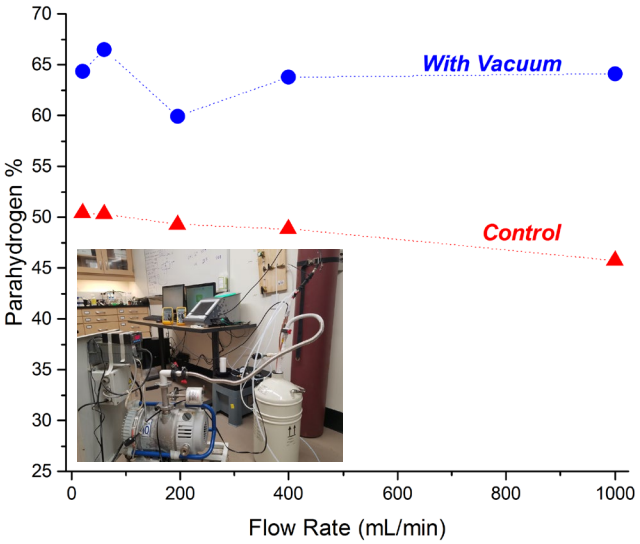

Figure 1: Comparing the improved pH_2 generation using vacuum-mediated boiling point supression of liquid nitrogen (blue) vs control experiments at 77 K (red). *Inset*: photograph of the pH_2 generator setup.

The isomeric enrichment of parahydrogen (pH_2) gas is readily accomplished by lowering the gas temperature in the presence of a catalyst. This is often pursued at two distinct temperatures: ~50% pH_2 is generated at liquid nitrogen temperatures (77 K), while nearly 100% pH_2 can be produced at liquid hydrogen temperatures (20 K). While the liquid nitrogen cooled generator is attractive due to the low cost of entry, there are benefits to having access to >50% pH_2 for NMR applications. In this work, we introduce a low-cost modification to an existing laboratory-constructed liquid nitrogen cooled pH_2 generator that provides ~65% pH_2. This modification takes advantage of vacuum-mediated boiling point suppression of liquid nitrogen[1], allowing the temperature of the liquid to be lowered from 77 K to around 63 K. The existing pH_2 generator consisted of a conversion coil (3 m of ¼" coiled copper tubing filled with FeO_2H catalyst), 10 L liquid nitrogen dewar, and flow controller. To this, we added an airtight plug with pass-throughs for the coil, vacuum inlet, and temperature sensors (one measured liquid nitrogen temperatures, the other measured pH_2 temperature). We monitored the change in temperatures and dewar pressure during pH_2 production over the course of 60 min, reaching ~63 K and ~16 torr, respectively. This allowed the generation of pH_2 fractions of 60-66% at gas flow rates from 20-1000 mL/min. We compare this to equivalent experiments that did not utilize the temperature-lowering effects of pressure reduction (Fig. 1); these controls generally maintained pH_2 fractions of ~50%. All results (experimental and control) generally agree with the theoretically expected values at these temperatures[2]. This low-cost modification to an existing pH_2 generator may be of interest to researchers beginning their foray into parahydrogen, as well as those at institutions with limited research funding and infrastructure.

Acknowledgements: We would like to thank Max Gemeinhardt and Boyd Goodson (SIUC), Eduard Chekmenev (Wayne State), and Sam Lofland (Rowan) for helpful conversations. This research was supported by Rowan University Seed Funding.

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Radiofrequency encoded Only Parahydrogen SpectroscopY

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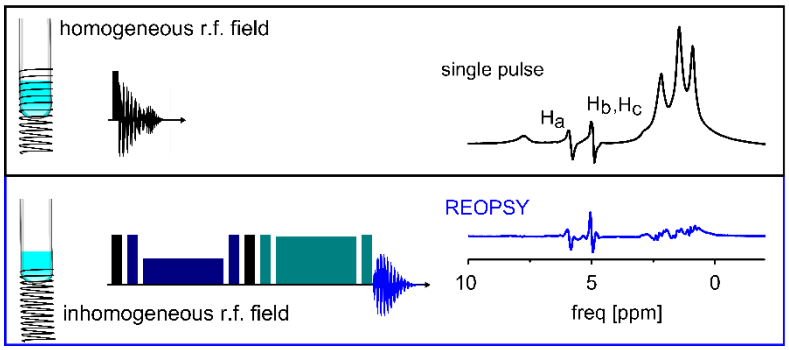

Hyperpolarized hexene (top right) thermal signals may be removed by applying a pulse sequence that takes advantage of the inhomogeneous r.f. field presented in the borders of the coil (bottom pannel).

Despite the large degree of polarization in PHIP experiments compared to the Boltzmann factor, the presence of a large amount of non-reacted molecules with thermal polarization is an important obstacle when dealing with diluted samples, poor reaction efficiency, or due to non-deuterated (or partially deuterated) solvents. A new pulse sequence aimed to filter out NMR signals coming from thermally polarized protons in PHIP experiments based on the OPSY [1] pulse sequence (Only Parahydrogen SpectroscopY) is presented. In analogy to OPSY, which removes thermal polarization by using a pair of magnetic field gradient pulses with an intensity ratio 1:2 and equal duration, the same effect can be achieved using inhomogeneous radiofrequency fields. As a proof of principle we used the varying magnetic field in the bore of a standard birdcage coil, where short r.f. pulses produce an average of 90° over a determined volume, whilst long excitations produce a dephasing over the sample volume. The spatial dependence of the radiofrequency field is used to control the Hamiltonian, which results in an effective suppression of thermal contributions in the NMR signal, while PHIP originated signals remain unmodified
A theoretical model for the Radiofrequency Encoded Only Parahydrogen SpectroscopY (REOPSY) sequence is presented along with an experimental implementation on a birdcage coil in a 7 T magnetic field. The control level achieved by this strategy allows the inclusion of a long train of refocusing pulses. Therefore, the new sequence can be combined with the parahydrogen discriminated PHIP (PhD-PHIP) [2] pulse sequence as a detection block to improve sensitivity and resolution in a single–scan experiment. Experiments with REOPSY and REOPSY+PhD-PHIP are presented in thermally and hyperpolarized samples.

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Next-Generation Equipment for Parahydrogen-Based Hyperpolarization Experiments

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Parahydrogen-based polarization (PHIP) methods enable enhancement of NMR signals by several orders of magnitude and have significant translational potential for next-generation MRI contrast agents. The effectiveness of PHIP experiments depends on the level of parahydrogen enrichment and the manipulation of reaction conditions such as temperature, magnetic field, sample shuttling and sample detection times. We present an integrated PHIP polarizer for utility in a wide variety of parahydrogen-based hyperpolarization experiments in microtesla magnetic fields. The device features automated degaussing, regulated parahydrogen flow and pressure control, non-magnetic variable temperature module and many others. We report on the recent advancement in automated equipment for parahydrogen enrichment to >98.5% at a production rate of 4 standard liters per minute. This is made possible by the use of 3/16 in. outside diameter copper spiral tubing filled with ~20g iron-oxide catalyst. Contact time of parahydrogen with the catalyst under cryogenic temperature is optimized and well regulated by the use of a pulsed injection system to yield production rate of 4 standard liters per minute.

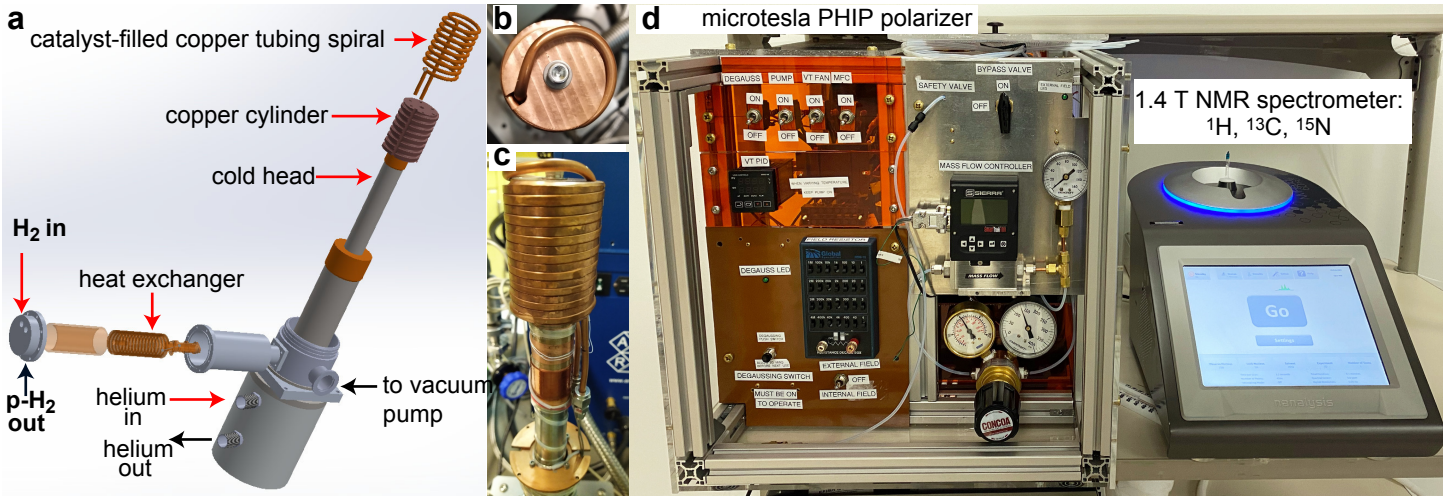

Figure 1. a) Annotated 3D rendering of cold head assembly of parahydrogen cold head; b) Top view of catalyst filled spiral with the top winding running along the length of copper core; c) Side view of catalyst filled spiral sitting on the cold head. The tubing employed in the heat exchanger is made of stainless steel; d) Integrated microtesla PHIP polarizer (mark 2.2) and a benchtop 1.4 T $^{1}H/^{13}C/^{15}N$ NMR spectrometer on a portable laboratory bench for PHIP experimentations.

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Challenges of *in vivo* hyperpolarized imaging-- and how parahydrogen can save us

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In vivo MRI using solution state hyperpolarized agents presents a variety of practical challenges, and these are even more more limiting when investigating metabolic products. Although metabolic pathways utilize thousands of molecules, metabolic *imaging* is limited to those with spin-lattice relaxation times exceeding the time needed to circulate from the venous system through the heart, then be taken up by the cells of interest, and finally achieve enzymatic conversion. Relaxation due to enzymatic or serum protein binding must be minimal, and the metabolite chemical shift must be distinguishable from that of the agent despite the relatively poor shims achievable *in vivo*.

Fortunately a few such molecules exist; most notably pyruvate, but even here there are concerns that physiologically relevant agent concentrations are too dilute to be imaged, while superphysiological concentrations can lead to intracellular acidification, redox imbalance, saturation of enzymatic activity, and rapid downregulation of the metabolic process being studied-- thereby reducing the sensitivity to disease or treatment that was originally sought.

There is currently no way to entirely avoid these difficulties with agents polarized using Dynamic Nuclear Polarization, as it is inherently a batch process which does not lend itself to extended signal averaging. Parahydrogen-induced polarization, on the other hand, can take place very rapidly and (in principle) in small enough batches to approximate a continuous-flow process. Thus, one can consider low-concentration, signal-averaged imaging schemes analogous to those we and others have employed using the long-T_1 hyperpolarized gases.

I will discuss our group's experience imaging bolus-injected hyperpolarized agents *in vivo*, results from long averages of dissolved HP ^{129}Xe *in vivo*, and our-- somewhat speculative-- thoughts about a parahydrogen-based system which could combine these approaches to allow for physiologically relevant metabolic imaging.

Studying Nuclear Spin Singlet Relaxation Mechanisms by NMR Spectroscopy and Molecular Dynamics

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Nuclear spins states have been shown to exceed spin-lattice relaxation times several fold, with impressive demonstrations of singlet lifetimes of more than an hour in organic molecules in solution. Over the years, several relaxation mechanisms have been identified, including dipolar coupling, chemical shift anisotropy, paramagnetic relaxation, spin rotation and spin-internal motion, and the scalar relaxation of the second kind. While in principle, many of the mechanisms are well understood, estimating their size can be difficult. Furthermore, multiple experimental examples have been found that decidedly defy expectations.

We present here work on directly estimating singlet relaxation mechanisms from molecular dynamics simulations. Prior work by Pär Håkansson is noted, which was limited to intramolecular mechanisms. Here we show calculations for intermolecular mechanisms and find good agreement with experiment. It is particularly surprising to see that such mechanisms as intermolecular coupling to ^{35}Cl and ^{37}Cl nuclear spins (of the chloroform solvent) could be rate limiting for singlet states. In addition, we also show work on ^{31}P spin singlets, and compare their lifetimes to those from molecular dynamics trajectories and ab initio calculations of chemical shift anisotropy tensors, which show good agreement.

Calculations of this sort may help in the design of particularly long-lived singlet states, or could be used to identify new probes for dynamics.

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