Memorial Talk for Konstantin L'vovich Ivanov

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Our esteemed colleague and good friend Konstantin ("Kostya") L'vovich Ivanov has become one of the first victims of the pandemic in our community. He passed away in a hospital in Novosibirsk on March 5, 2021. We shall deeply miss Kostya as an exceptional human being: he was a creative yet rigorous scientist, a generous and attentive friend, and a considerate and eminently civilized colleague. He was not only a great scientist, but also a good human being, always sincere, honest, and considerate. In addition, he was a great citizen of the scientific community; aside from his demanding job as the director of the International Tomography Center (ITC), Novosibirsk, he kept his research at a high level and organized a multitude of meetings, seminars and webinars. Kostya had built a strong relationship with Rob Kaptein when they worked together in Novosibirsk, with the support of a Russian "megagrant" that Kostya had helped to secure. Kostya started further initiatives, so that another megagrant was recently awarded to ITC with participation of Geoffrey Bodenhausen.

After his doctorate in 2002 under supervision of Renad Sagdeev and Nikita Lukzen, he entered in collaboration with the experimental groups at ITC (A. Yurkovskaya) and FU Berlin (H.M. Vieth) the scientific field of hyperpolarization, in which he soon developed into one of the leading players of the field by developing a series of important theoretical concepts which shaped the field and led to the development of new and more efficient experimental tools. The result of this scientific endeavor was not only his Second Doctorate (the Russian equivalent to the habilitation, known in German speaking countries) with his masterly thesis «Kinetics of multistage liquid phase processes involving particles with spin degrees of freedom», but also the establishment of several large scale international collaborations such as the EU COST action of hyperpolarization, where he headed the theoretical group, the EU DNP-design evaluation and many others and the realization of the roles of scalar interactions and Level Anti-Crossings in SABRE and related techniques. In 2016 Kostya was finally appointed to Professor of Physics and in 2018 he became the Director of the ITC.

With his never-ending enthusiasm and energy, he established and successfully conducted new joint projects with many groups ranging from T. Takui and K. Sato in Japan, over P. Madhu in India, G. Buntkowsky in Germany, G. Bodenhausen, Suisse, R. Kaptein in the Netherlands, D. Abergel and F. Ferrage in France to M. Levitt, UK, to name just a few of them. The most important of these collaborations was the megagrant from the Russian government, which enabled Kostya together with Rob Kaptein to renew the scientific infrastructure of the ITC and transform it into one of the leading places of hyperpolarization research in the world.

His scientific work was honored by several important prizes and awards, including a Fellowship of the Alexander von Humboldt Foundation (Germany) in 2008, the Medal of the European Academy of Science in 2010, a Fellowship of the Japanese Society for Promotion of Science in 2016 and the Laukien Prize for SABRE in 2020.

With all his enthusiasm in scientific collaboration and exchange, it is not surprising that Kostya was also the spiritual father and one of the four organizers of the "Intercontinental NMR Seminar", ICONS, which was after his untimely death renamed after him. This series of "Zoom-Seminars" and virtual conferences was his answer to the break-down of scientific communication and exchange caused by the COVID pandemic, the disease which finally extinguished Kostya's brilliant flame.

In my contribution I will look back on his scientific contributions with a particular focus on his recent achievements in parahydrogen related hyperpolarization as he himself described them in his commemorative speech for the Laukien Prize.

Parahydrogen-induced ^{13}C Hyperpolarizer Using A Flow Guide For Magnetic Field Cycling To Evoke ^{1}H-^{13}C Spin Order Transfer Toward Metabolic MRI.

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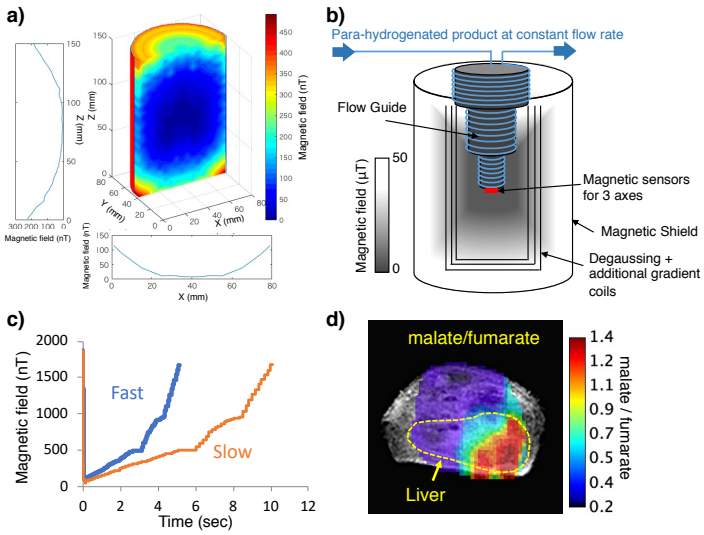

Fig.1 PHIP ^{13}C hyperpolarizer system using a flow guide. a) Magnetic field distribution in a zero-field chamber. b) Concept of the flow guide for MFC. c) Examples of estimated fast and slow MFCs profiles. d) An application of HP^{13}C MRI of hepatic cell death imaging by [1-^{13}C] fumarate in hepatitis mouse.

Objective: Pair-wise addition of parahydrogen, a singlet form of molecular hydrogen, to unsaturated precursors evokes hyperpolarization (HP) of the parahydrogen-derived two ^{1}H nuclear spins, known as parahydrogen induced polarization (PHIP). Following spin order transfer from the ^{1}H to surrounding ^{13}C nuclear spins by magnetic field cycling (MFC) results in substantial signal enhancement of magnetic resonance imaging (MRI) of ^{13}C-labeled metabolic tracers. Here, we report a development of an automated PHIP ^{13}C hyperpolarizer system using a flow guide for MFC process. **Methods:** The optimal MFC scheme for ^{1}H to ^{13}C spin order transfer was quantum mechanically simulated from the network of spin-spin coupling values of ^{13}C-labeled metabolic tracers[1]. The flow guide system was designed individually for each metabolic tracer based on the simulated MFC scheme and pre-measured magnetic field distribution in a zero-field chamber, and implemented so that the spin order of ^{1}H is efficiently transferred to ^{13}C during the para-hydrogenated tracer passes through the flow guide at a constant flow rate. **Results:** ^{13}C polarizations of 6% for [1-^{13}C] pyruvate and 12% for [1-^{13}C] fumarate were achieved at the time of NMR/MRI detection using the developed ^{13}C hyperpolarizer system, and necrotic cell death imaging by HP [1-^{13}C] fumarate in a hepatitis mouse was feasible by PHIP using a 1.5T MRI system[2]. **Conclusion:** The flow guide is a simple and efficient tool to automate the optimal MFC scheme required for spin order transfer to heteronucleus in PHIP and would be useful alternative of dissolution dynamic nuclear polarization to prepare hyperpolarized ^{13}C labeled tracers for metabolic MRI study.

REFERENCES: (1) Stewart, N.J., Matsumoto, S.; et al. *J. Magn. Reson*. 2018, 296, 85-92 (2) Stewart, N.J. , Matsumoto, S.; et al. *Chem. Commun.* 2021, 10(2), 915-23.

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Hyperpolarization of Spectator Molecules via PHIP-RASER

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Parahydrogen-Induced Polarization (PHIP) technique allows one to hyperpolarize various compounds via pairwise addition of parahydrogen (p-H_2) to corresponding unsaturated precursor. Consequently, wide array of compounds cannot be hyperpolarized by PHIP directly because the corresponding unsaturated precursor does not exist. In some cases this issue can be remedied by rapid chemical transformation of p-H_2 addition product to the molecule of interest[1]. Recently introduced PHIP-X technique enables hyperpolarization of wider range of compounds which contain exchangeable protons[2].

Here we report that homogeneous hydrogenation of vinyl acetate (VA) with p-H_2 in the Earth's magnetic field results in observation of PHIP-RASER[3,4] (Radio Amplification by Stimulated Emission of Radiation) effect at 7.05 T. When hydroquinone is present in the solution as a spectator along with VA, its NMR signal is enhanced by ~5 fold. Moreover, hyperpolarization was also observed for CH and CH_2 protons of the reactant vinyl acetate, ethyl acetate (EA) CH_3 group and o-H_2. The replacement of VA with 2-hydroxyethyl acrylate also provides the mentioned effects but with lower signal enhancements (SE). Wide range of other hydrogen bonding substrates may be hyperpolarized using this approach including methanol, ethanol, benzyl alcohol, 1,1,1,3,3,3-hexafluoropropanol, phenol and ethylene glycol. Moreover, such aprotic substrates as tetrahydrofuran, N,N-dimethylformamide, chloroform and benzene which are not expected to chemically interact with EA can also be hyperpolarized with the highest SE = 17 observed for benzene. The polarization of spectator molecules maximized ~15–60 s after placing the sample inside the NMR probe indicating that polarization transfer occurs at high magnetic field. Moreover, when the sample resides right above the NMR probe for 20 s until ethyl acetate hyperpolarization relaxes to the extent at which RASER is not induced, the spectator molecule is not hyperpolarized. The work is in progress to fully explain the observed effects.

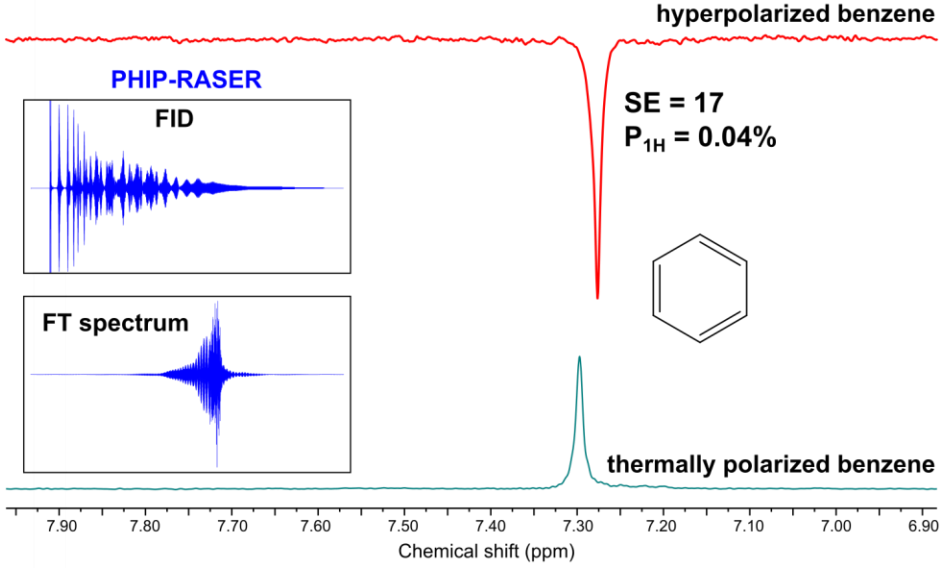

Figure 1. ^{1}H NMR spectra of hyperpolarized and thermally polarized benzene. Inset: FID and FT spectrum of PHIP-RASER.

REFERENCES: (1) Reineri, F.; et al. *Nat. Commun.* **2015**, *6*, 5858. (2) Them, K.; et al. **2020**, arXiv:2012.03626. (3) Pravdivtsev, A. N.; et al. *ChemPhysChem* **2020**, *21*, 667–672. (4) Joalland, B.; et al. *Angew. Chem. Int. Ed.* **2020**, *59*, 8654–8660.

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Singlet-triplet conversion in molecular hydrogen and its role in parahydrogen induced polarization

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An analysis of singlet-triplet conversion in molecular hydrogen dissolved in solution with organometallic complexes used in experiments with parahydrogen (the H_2 molecule in its nuclear singlet spin state) is reported. We demonstrate that such conversion is an efficient process, which gives rise to the formation of orthohydrogen (the H_2 molecule in its nuclear triplet spin state) and also strongly reduces the resulting NMR (nuclear magnetic resonance) signal enhancement, here of ^{15}N nuclei polarized at high fields using special NMR pulse sequences. We make use of a simple improvement of the traditional pulse sequences, utilizing a single pulse on the proton channel and giving rise to an additional strong increase of the signal. Furthermore, the analysis of the enhancement as a function of the length of such pulse allows one to estimate the actual population of the spin states of H_2. We are also able to demonstrate that the spin conversion process in H_2 is strongly affected by the presence of ^{15}N nuclei. This observation allows us to explain the dependence of the ^{15}N signal enhancement on the abundance of ^{15}N isotopes.

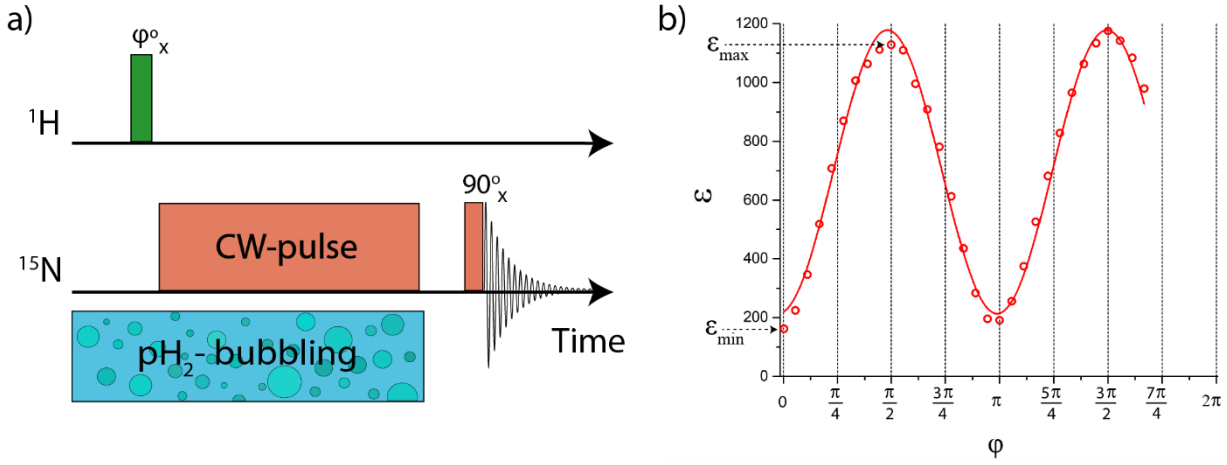

Figure 1. (a) Experimental protocol used to run high-field SABRE experiments, aimed at enhancing ^{15}N signals [1]. Additional pulse with the flip angle φ on the proton channel is performed in order to study singlet-triplet conversion in molecular hydrogen. **(b)** The dependence of ^{15}N signal enhancement on the proton magnetization flip angle, φ. Enhancement factor strongly depends on the flip angle φ, consequently the H_2 nuclear spin-state is no longer a pure singlet-state (in this case enhancement would not change its value, since the singlet state does not evolve under the influence of rotation operators) but rather a combination of S and T_0 states. The experiments were performed with ^{15}N-Py as a polarized substrate.

References: [1] S. Knecht, A. S. Kiryutin, A. V. Yurkovskaya and K. L. Ivanov, *Mol. Phys.*, 2018, 2018, 1-10.

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Heterogeneous ^{1}H and ^{13}C Parahydrogen-Induced Polarization of Acetate and Pyruvate Esters

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^{13}C magnetic resonance imaging of [1-^{13}C]carboxylates hyperpolarized by d-DNP allows one to visualize abnormal metabolism in tumors and other pathologies. In this context, PHIP technique represents a more affordable and high-throughput alternative. Many biologically relevant carboxylates cannot be hyperpolarized by PHIP directly due to the lack of corresponding unsaturated precursor. However, PHIP by means of side arm hydrogenation (PHIP-SAH) approach allows one to overcome this limitation[1]. In PHIP-SAH, parahydrogen is added to an unsaturated alcoholic moiety of the ester precursor in a pairwise manner. Subsequently polarization is transferred from parahydrogen-derived protons to ^{13}C nuclei and ester is hydrolyzed to form ^{13}C-hyperpolarized carboxylate.

While homogeneous hydrogenation catalysts provide higher polarizations in PHIP experiments than heterogeneous ones, the latter catalysts can be easily separated from hyperpolarized reaction product. Here we present systematic study of PHIP-SAH hyperpolarization of acetate and pyruvate esters with ethyl, propyl and allyl alcoholic moieties over heterogeneous Rh/TiO_2 catalysts in CD_3OD and D_2O[2]. Hyperpolarization of pyruvate esters using heterogeneous PHIP was demonstrated for the first time, though its efficiency was inferior to that of acetates in terms of both polarization levels and conversion of the reactants. Notably, hydrogenolysis of the C–O bond leading to formation of carboxylic acids and hydrocarbons occurred as a side process in case of both types of esters, though it was more prevalent for pyruvates. Propargyl esters were found to be less efficient heterogeneous PHIP-SAH precursors than esters with C=C bond. As a result of these trends, the highest ^{1}H polarizations were detected for propyl acetate and ethyl acetate in CD_3OD (0.6 and 0.5%, respectively). Upon magnetic field cycling hyperpolarization was transferred to ^{13}C nuclei with the maximum attainable ^{13}C polarization levels of ~0.1% for the same two esters. In D_2O the maximum polarizations were 0.3% for ^{1}H and 0.07% for ^{13}C nuclei, respectively.

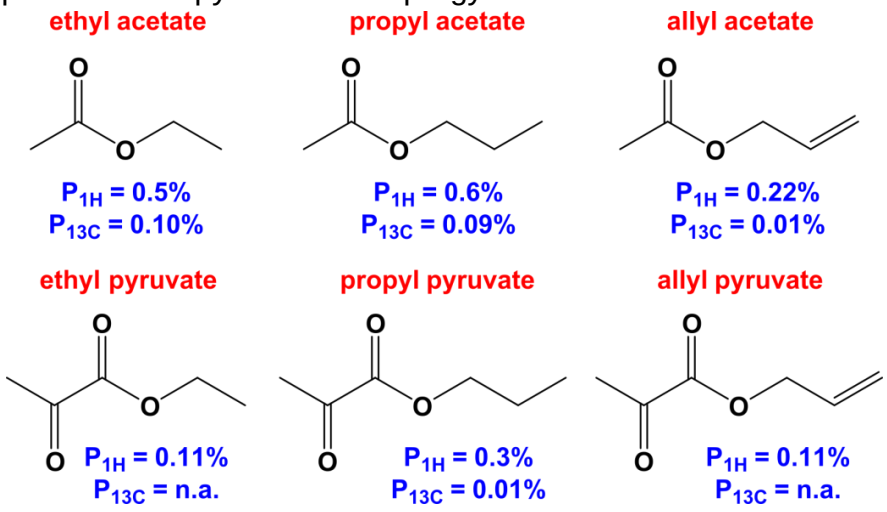

Figure 1. Hyperpolarized esters and their polarization levels in CD_3OD.

REFERENCES: (1) Reineri, F.; et al. *Nat. Commun.* **2015**, *6*, 5858. (2) Salnikov O. G.; et al. *ChemPhysChem* **2021**, DOI: 10.1002/cphc.202100156.

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Zero-field NMR Detection of Molecules Hyperpolarized by SABRE-relay

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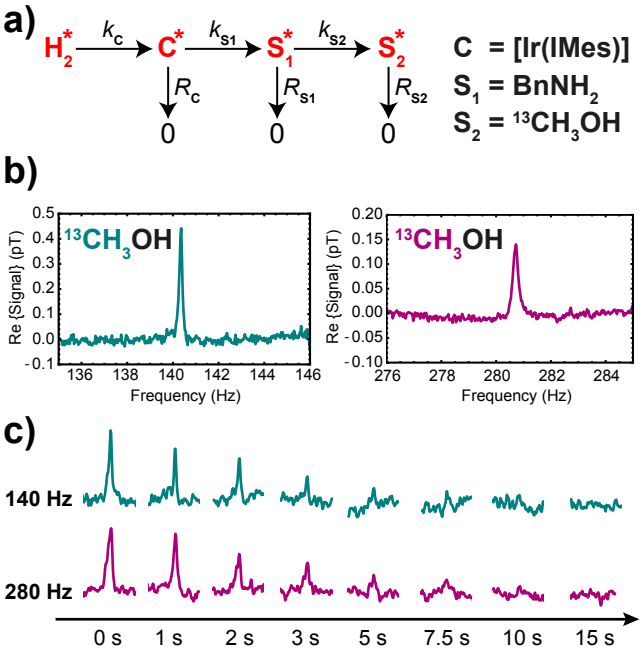

Figure 1. a) SABRE-relay process: nuclear spin-order (*) flow from hydrogen (H_2) to the SABRE complex (C), substrate-1 (S_1) and, eventually, substrate-2 (S_2). Specific examples of C, S_1, S_2 used in this work are shown on the right. b) Zero-field NMR *J*-spectrum of the SABRE-relay-hyperpolarized [^{13}C]-methanol. c) ZULF NMR signals of SABRE-relay-hyperpolarized [^{13}C]-methanol with a variable waiting time at zero field.

Zero- to ultralow-field (ZULF) nuclear magnetic resonance (NMR) is a variant of NMR in which measurements of nuclear spin signals are carried out at magnetic fields typically below ~100 nT [1]. Removing the requirement of having high fields while maintaining the ability to differentiate small molecules [2] makes ZULF NMR a promising detection modality with chemical specificity for applications outside of research laboratories [3]. However, since a magnetic field is the typical source of initial nuclear polarization, removing it necessitates other ways of polarizing spins. In this work we investigated combining the parahydrogen-based technique SABRE-relay [4] with ZULF NMR detection. In SABRE-relay, the initial non-equilibrium spin order of parahydrogen (Figure 1a) is transferred through a magnetization-transfer catalyst to the first substrate (in this work, benzylamine = $BnNH_2$) and, subsequently, through chemical exchange, to the second substrate (in this work, $^{13}CH_3OH$). We carried out ZULF NMR measurements of hyperpolarized [^{13}C]-methanol (Figure 1b) leveraging proton polarization obtained by parahydrogen bubbling at 6 mT in a sample with an optimized chemical composition. Spending a variable amount of time at zero field after manual sample transfer from 6 mT allowed recording time-traces of two zero-field peaks (at *J* and 2*J*) characteristic of A_3X chemical motif in [^{13}C]-methanol (Figure 1c).

While chemical exchange is a necessary requirement of the SABRE-relay process, it may detrimentally affect ZULF NMR spectra [5]. Nonetheless, we demonstrated feasibility of obtaining ZULF NMR spectrum of [^{13}C]-methanol by SABRE-relay via benzylamine. We anticipate a wide variety of chemical motifs to be polarizable using this approach. Opportunities for producing and storing non-equilibrium nuclear spin orders in $^{13}CH_3$ groups will also be discussed.

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Long-Lived ^{1}H Singlet Spin States Generated by Para-Hydrogen Induced Polarization in Symmetrical Molecules

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Parahydrogen induced singlet spin order provides an excellent tool for many NMR/MRI applications in natural sciences and medicine. The huge benefit of para-H_2 for NMR experiments is two-fold: it represents a pure spin state and can thus be very efficiently used in hyperpolarization experiments and its singlet spin order makes it long-lived because it is immune to most relaxation mechanisms of NMR. For symmetrical molecules, the hyperpolarized long-lived singlet spin state of para-H_2 can be preserved after the hydrogenation reaction at any magnetic field strength (see figure 1) [1]. The most promising example for this concept is hyperpolarized fumarate generated by PHIP [2] because it can be used for hyperpolarized metabolic imaging [3]. Fumarate has two magnetically equivalent proton sites, which form a very isolated spin system resulting in a long lifetime of the ^{1}H singlet state. To release the singlet spin order, the symmetry of the molecule and thereby also the magnetic equivalence of the strongly coupled spin pair need to be broken e.g. by enzymatic conversion to malate. In this contribution, the general concept of molecular ^{1}H singlet spin states originating from para-H_2 will be introduced. Thereafter, the generation of hyperpolarized fumarate from para-H_2 and the enzymatic readout of its singlet state will be shown and used in MRI experiments. This approach can allow hyperpolarization-enhanced ^{1}H MRI for metabolic imaging.

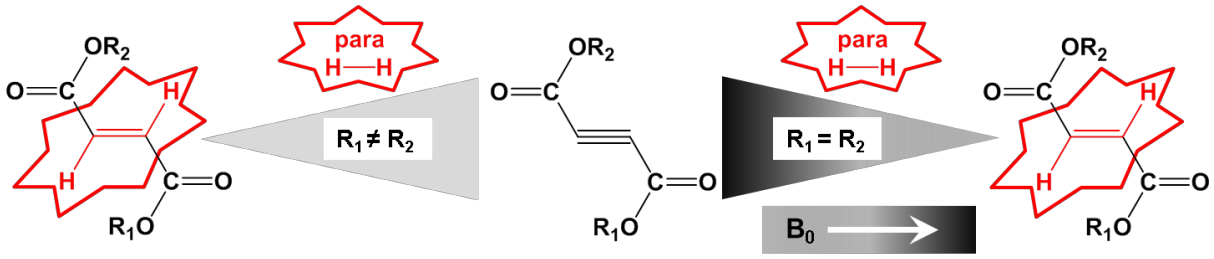

Fig. 1: *Sketch of PHIP reactions resulting in hyperpolarized singlet spin states in different molecules. Left hand side: p-H_2 is added to a nonsymmetrical molecule, where the protons remain only strongly coupled at low magnetic fields (B_0) resulting in singlet state preservation only at low magnetic fields. Right hand side: p-H_2 is introduced in a symmetrical molecule thus the protons are naturally strongly coupled leading to singlet state preservation at any magnetic field strength.*

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Long-term Generation of Longitudinal Spin Order Controlled by Ammonia Ligation Enables Rapid SABRE Hyperpolarized 2D NMR

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Symmetry breaking of parahydrogen using iridium catalysts converts singlet spin order into observable hyperpolarization. In low field conditions (< 20mT), coherent polarization transfer via SABRE occurs when the strong-coupling condition induce level-anti crossings (LAC's). At high magnetic field, when matching conditions are lifted and polarization transfer decreases in efficiency, singlet-triplet mixing governed by chemically inequivalent hydride positions can spontaneously generate non-thermal longitudinal two-spin order.[1]

Gaining accurate control over stereochemistry of SABRE Ir-catalysts is thus essential for generation of non-thermal longitudinal two-spin order. Here, iridium catalysts are designed to exhibit asymmetry in their hydrides, regulated by *in situ* generation of deuterated ammonia governed by ammonium buffers. The concentrations of ammonia (N) and pyridine (P) provide a handle to generate a variety of stereo-chemically asymmetric N-heterocyclic carbene iridium complexes, ligating either [3xP], [2xP;N], [P;2xN] or [3xN] in an octahedral SABRE type configuration.[2] [3]

The controlled ligation on iridium via deuterated ammonia provides a route to extend singlet-triplet mixing at high magnetic field and experimentally induce prolonged generation of non-equilibrium longitudinal two-spin order in order of minutes. This long-lasting magnetization can be exploited in hyperpolarized 2D-OPSY-COSY experiments providing direct structural information on the catalyst using a single contact with parahydrogen.[2]

Finally, controlling catalyst stereochemistry by introducing small and deuterated ligands, such as deuterated ammonia, simplifies the spin-system. This is shown to unify experimental and theoretically derived field-sweep experiments for a four-spin system.[2]

[1] Eshuis *et al. Angew. Chemie - Int. Ed.* **2015**, 54, 14527–14530.
[2] Vaneeckhaute *et al. ChemPhysChem* **2021**, doi: 10.1002/cphc.202100079
[3] Front Cover Special Collection of Parahydrogen Enhanced Spectroscopy. *ChemPhysChem* **2021**, doi: 10.1002/cphc.202100079

A study on the effect of the hydrogenation solvent in the application of the PHIP-SAH methodology to generate Hyperpolarized [1-^{13}C]pyruvate

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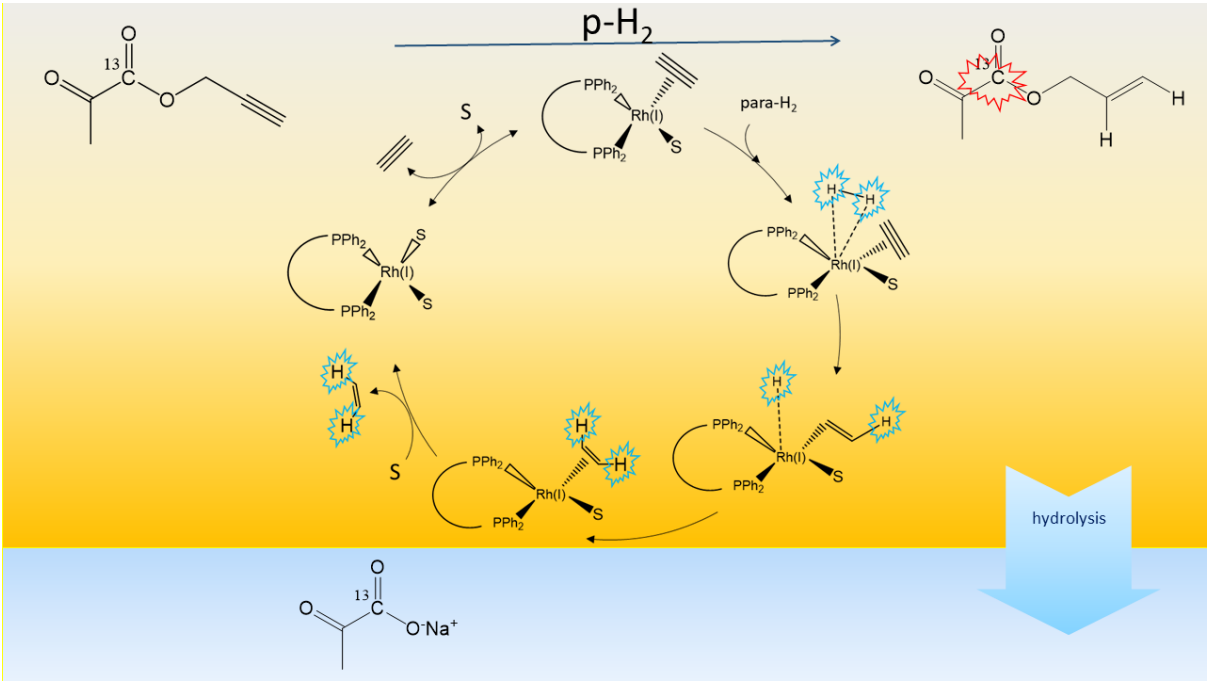
ParaHydrogen Induced Polarization-Side Arm Hydrogenation (PHIP-SAH) is an inexpensive tool to obtain hyperpolarized pyruvate (and other metabolites) that can be applied to in-vivo diagnostics, for the investigation of metabolic processes. This method is based on hydrogenation, using hydrogen enriched in the para-isomer, of an unsaturated ester derivative of pyruvate, catalyzed by a homogeneous rhodium(I) complex (Scheme 1). Then, the hyperpolarized ester is hydrolyzed using an aqueous base.



***Scheme 1**. PHIP-SAH procedure with hydrogenation pathway for the Rh(I) complex containing the chelating phosphine dppb (bis (diphenilphosphino)butane).*

The hyperpolarization level on the parahydrogenation products depends strongly on the solvent used. This is due not only to the solubility of hydrogen in a given solvent, but also to the binding interaction of the solvent molecules to the hydrogenation catalyst. In the catalytic cycle (scheme 1), the substrate coordinates to the metal center and displaces the solvent molecules. Coordinating solvents, as acetone, methanol, ethanol can lead to high efficiency, in terms of hydrogenation speed and polarization level. Unfortunately, these solvents are not compatible with the intended metabolic applications, because they are completely miscible with water. To obtained an aqueous solution of hyperpolarized pyruvate without the hydrogenation catalyst and solvents, hydrogenation has been carried out in hydrophobic solvents (chloroform and toluene) and 13C polarization on the ester was 5.2±0.6% in chloroform and 8.4±0.6% in the toluene/ethanol mixture.

After hydrolysis sodium [1-^{13}C]pyruvate is extracted in the aqueous phase which still contains traces of the organic solvents. Filtration of the aqueous solution of the hyperpolarized product through a lipophilic resin (Tenax TA) led to complete removal of the organic solvents, still maintaining a good hyperpolarization level sufficient for ^{13}C MRI studies.

Parahydrogen-polarized [1-^{13}C]fumarate – State-of-the-art and future directions

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Hyperpolarized fumarate is a promising biosensor for hyperpolarized carbon-13 magnetic resonance metabolic imaging. It has been shown to be a marker for imaging tumor response to therapy [1], as well as acute kidney injury [2] and myocardial infarction [3]. Dissolution dynamic nuclear polarization is the current state-of-the-art methodology for hyperpolarizing fumarate, but is expensive and some 30 minutes is required to produce each sample. Alternatively, fumarate can be hyperpolarized in a cheap and convenient manner using parahydrogen-induced polarization (PHIP). However, this process requires a chemical reaction, and the resulting solutions are contaminated with the catalyst, unreacted reagents, and reaction side-product molecules, and are hence unsuitable for use in vivo. We have recently shown that the hyperpolarized fumarate can be purified from these contaminants by acid precipitation as a pure solid, and later redissolved to a desired concentration in a clean aqueous solvent [4]. With this method we have shown it is possible to form hyperpolarized fumarate at ^{13}C polarization levels of 30-45%, and the turnover time between delivery of samples of a few millilitres is 1-2 minutes.

This technique should greatly accelerate preclinical studies into the uses of fumarate as a metabolic marker in vivo, and PHIP-polarized [1-^{13}C]fumarate has already been used in this context [5]. However, the precipitation of a hyperpolarized fluid as a pure solid carries further implications. Firstly the solid material can be stored at cryogenic temperatures to greatly extend the ^{13}C T_1 times, which opens the door to storage and transport. Secondly, the material can be redissolved at a chosen concentration. This means that for applications requiring high molar polarization (the product of concentration and polarization) this is an attractive option, especially given the short timescale on which the solutions can be produced.

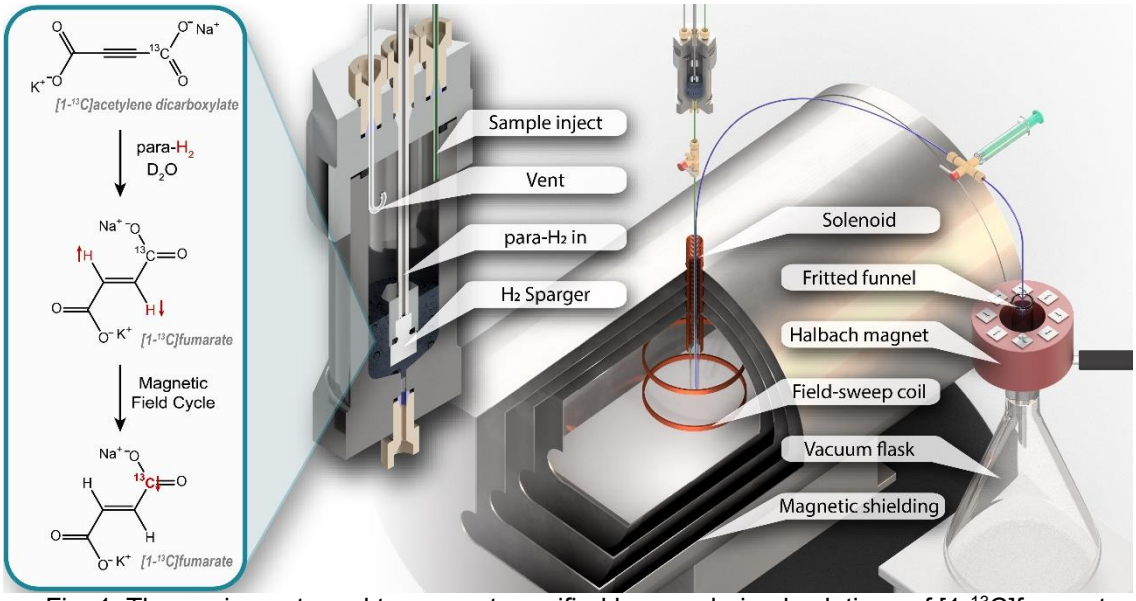

Fig. 1: The equipment used to generate purified hyperpolarized solutions of [1-^{13}C]fumarate.

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Large-scale synthesis of ^{15}N-labelled [$^{15}N_3$]metronidazole antibiotic

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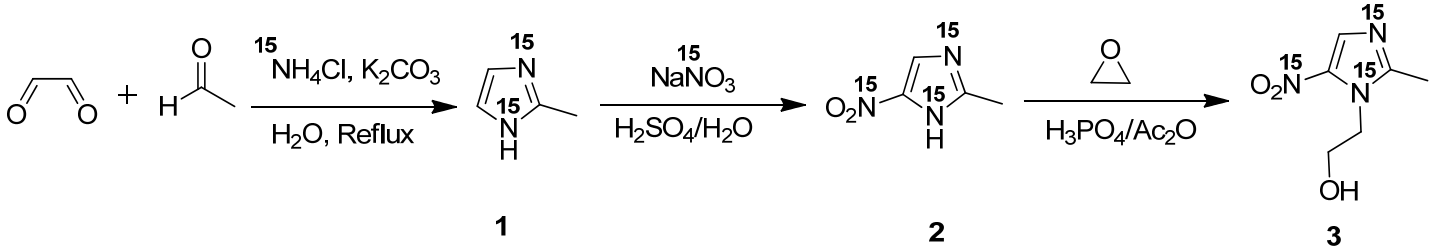
Introduction: [$^{15}N_3$]metronidazole has been shown to be readily amenable to SABRE hyperpolarization in microtesla magnetic fields with potential utility for a hypoxia sensor [1,2].
Objective: We aim to develop robust large-scale synthesis of ^{15}N-labelled [$^{15}N_3$]metronidazole for future *in vivo* studies including future clinical trials.
Methods: The synthesis of ^{15}N-labelled [$^{15}N_3$]metronidazole was approached through a 3-step process (Figure 1) based on previously published methods [1,2]. Briefly, first step was performed by reacting the mixtures of glyoxal solution and acetaldehyde with $^{15}NH_4Cl$ and K_2CO_3 to yield the heterocyclic product**1**, which was purified by sublimation. Nitration of **1** was carried out by using $Na^{15}NO_3$ and H_2SO_4 to form nitro intermediate**2**. Purification of **2** was conducted in recrystallization in HPLC-grade water. Finally, [$^{15}N_3$]metronidazole**3** was synthesized by reacting **2** very slowly with ethylene oxide under acidic conditions. Pure compound **3** was obtained by recrystallization in HPLC-grade water followed by recrystallization in methanol and ^{1}H, ^{13}C ,^{15}N NMR characterization.



1 **2** **3**

Figure 1. Scheme of three-step reaction used for the large-scale preparation of ^{15}N-labelled metronidazole.

Results: [$^{15}N_3$]metronidazole with 99% ^{15}N isotopic purity and ~99.7% chemical purity was obtained in quantity of ~50 g, which bodes well for clinical translation efforts of this potent hyperpolarized contrast agent for hypoxia sensing.
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