



HAL
open science

Hyperpolarized NMR metabolomics

Victor Ribay, Clément Praud, Marine P.M. Letertre, Jean-Nicolas Dumez,
Patrick Giraudeau

► **To cite this version:**

Victor Ribay, Clément Praud, Marine P.M. Letertre, Jean-Nicolas Dumez, Patrick Giraudeau. Hyperpolarized NMR metabolomics. *Current Opinion in Chemical Biology*, 2023, 74, pp.102307. 10.1016/j.cbpa.2023.102307 . hal-04086586

HAL Id: hal-04086586

<https://hal.science/hal-04086586>

Submitted on 2 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

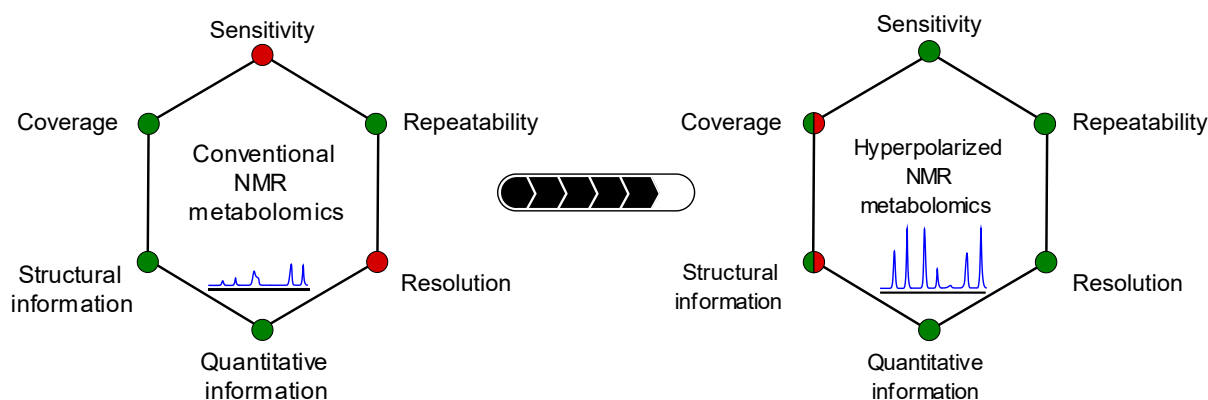
Hyperpolarized NMR metabolomics

Victor Ribay¹, Clément Praud¹, Marine Letertre¹, Jean-Nicolas Dumez¹, Patrick Giraudeau^{1*}

¹Nantes Université, CNRS, CEISAM UMR 6230, F-44000 Nantes, France.

*Email: patrick.giraudeau@univ-nantes.fr

Graphical abstract



Highlights

- Hyperpolarized techniques can break the sensitivity barrier of NMR metabolomics.
- Dissolution-DNP and parahydrogen-based methods are particularly promising.
- Recent applications include metabolic fingerprint and targeted quantification.

Abstract

Hyperpolarized NMR is a promising approach to address the sensitivity limits of conventional NMR metabolomics approaches, which currently fails to detect minute metabolite concentrations in biological samples. This review describes how tremendous signal enhancement offered by dissolution-dynamic nuclear polarization and parahydrogen-based techniques can be fully exploited for molecular omics sciences. Recent developments, including the combination of hyperpolarization techniques with fast multi-dimensional NMR implementation and quantitative workflows are described, and a comprehensive comparison of existing hyperpolarization techniques is proposed. High-throughput, sensitivity, resolution and other relevant challenges that should be tackled for a general application of hyperpolarized NMR in metabolomics are discussed.

Addresses

¹Nantes Université, CNRS, CEISAM UMR 6230, F-44000 Nantes, France.

Corresponding author: Giraudeau, Patrick
(patrick.giraudeau@univ-nantes.fr)

Keywords: NMR spectroscopy; Metabolomics; Hyperpolarization; Dissolution Dynamic Nuclear Polarization; Para-hydrogen

Introduction

Metabolomics belongs to the family of “omic sciences” (which address the global analysis of pools of biological molecules), and plays an important role for the global understanding of metabolism. It consists of the analysis of the small molecules present in biological samples in the most exhaustive manner. By analyzing those small molecules, the aim is to discriminate different classes of samples under different conditions, to discover new biomarkers [1] and ultimately to map metabolic pathways [2]. Metabolomics is applied in many different fields such as personalized medicine [3], plant sciences [4], and environment [5], among many others. Metabolomics studies can be implemented through untargeted or targeted approaches. An untargeted approach relies on the detection of the largest possible number of metabolites without any *a priori*, to eventually highlight biomarkers after statistical analysis [6]. A targeted approach consists in the accurate quantification of candidate biomarkers to confirm their significance, which explains that targeted analyses are often applied following untargeted ones [7]. Complementarily, stable isotope resolved metabolomics (SIRM) consists in detecting labeled metabolites after incorporation of a labeled precursor to obtain information on specific metabolic pathways [8].

Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) – often hyphenated with chromatography for the latter– are the two main analytical techniques in metabolomics [9,10]. MS benefits from an unmatched sensitivity, however, it is limited by its lower repeatability and the ambiguity of feature assignment, whereas NMR is highly reproducible and provides valuable structural information [11]. Consequently, MS and NMR are highly complementary and increasingly combined in multi-platform approaches [12–17]. However, this complementarity is limited, as is the general spread of NMR spectroscopy in the metabolomics field compared to MS techniques, by the low sensitivity of NMR and the ubiquitous peak overlaps that occur in ^1H NMR spectra of complex biological mixtures. 2D NMR can address overlap issues by spreading signals over a second frequency dimension and recently, many developments have been carried out to make 2D NMR more sensitive, faster and quantitative, in order to match metabolomics analysis requirements [18–20]. Nonetheless, all conventional NMR methods still suffer from an intrinsic low sensitivity due to the weak energy gap between spin states, leading to a poor nuclear polarization – *i.e.* $4 \cdot 10^{-4} \%$ for ^1H at 300 K at 9.4 T (400 MHz spectrometer). Hyperpolarized NMR makes it possible to increase the nuclear polarization by several orders of magnitude. Many solution-state hyperpolarization techniques exist, such as optical pumping of noble gases [21], optically polarized crystals [22], Overhauser dynamic nuclear polarization [23], dissolution Dynamic Nuclear Polarization (d-DNP) and para-hydrogen ($p\text{-H}_2$) hyperpolarization.

While all these methods can address the current sensitivity limitations of NMR, d-DNP and $p\text{-H}_2$ hold most promises for metabolomics and are the focus of this review. These two methods have been applied to a variety of fields such as magnetic resonance imaging or monitoring of (bio)chemical reactions [24]. However, the investigation of their potential for metabolomics is much more recent [25], and the last decade has witnessed many efforts to improve their suitability for the analysis of complex biological mixtures. This review presents the latest developments in hyperpolarized NMR methods and discusses the perspectives that could arise from their broad use in the field of metabolomics.

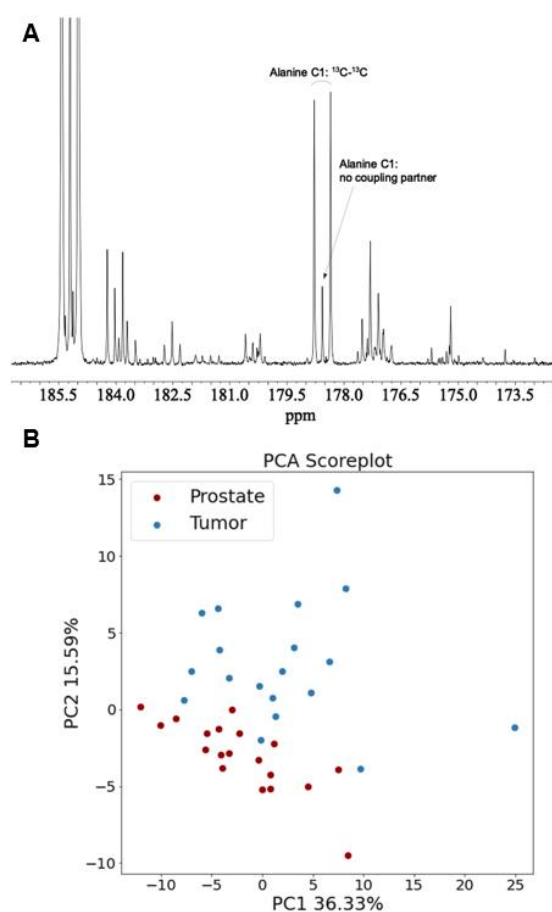
Making metabolomics more sensitive by d-DNP

DNP is a hyperpolarization method based on polarization transfer from unpaired electrons to nuclei, which provides a close-to-unity nuclear polarization in a non-selective way. Among the different experimental approaches to DNP, dissolution-DNP (d-DNP) is best suited to improve the sensitivity of liquid-state NMR. It consists in dissolving a hyperpolarized frozen sample with a hot solvent prior to a super-sensitive liquid-state NMR acquisition. The experiment benefits from enhanced nuclear polarization while retaining the resolution advantages of liquid-state NMR for complex mixture analysis such as those of interest in metabolomics [26].

The d-DNP workflow starts by mixing or dissolving the sample of interest in a water-glycerol matrix doped with stable organic radicals used as polarizing agents (PAs). This sample is cryogenically cooled inside a polarizer equipped with a cryostat, forming a glassy matrix. Hyperpolarization takes place inside the polarizer through microwave irradiation for a duration ranging from a few minutes to a few hours, allowing an efficient transfer of polarization from highly polarized electrons to nuclei. This is followed by a rapid dissolution of the sample in a hot solvent and its transfer to a nearby liquid-state spectrometer through a transfer line. After a short

stabilization delay, a super-sensitive liquid-state signal acquisition is performed at room temperature. However, the hyperpolarization is short-lived and decreases because of longitudinal relaxation with a time constant T_1 once the sample has been dissolved. Moreover, the irreversible character of the overall process allows recording only a single, or at most a few, scans. The quantitative nature of d-DNP is also a challenging question, since all signals with different T_1 have a different response factor, contrary to quantitative 1D NMR. As a consequence, an accurate knowledge of peak-specific response factor is required to recover quantitative information from d-DNP data [27]. Therefore, d-DNP applications mainly rely on ^{13}C detection since the ^{13}C hyperpolarization survives longer during the transfer between the two magnets due to their longer T_1 . Compared to a thermally polarized signal that would be obtained on the same sample by conventional ^{13}C NMR, the signal is typically enhanced by four orders of magnitude. In contrast, ^1H d-DNP enhancement is modest – one to two orders of magnitude – due to protons hyperpolarization fast decay [28]. However, recent developments, including faster transfer lines [29,30] and methods to extend the lifetime of hyperpolarization [31,32] could allow a more general use of ^1H hyperpolarization. Still, the significant sensitivity achieved by ^{13}C d-DNP NMR, associated with the wider spectral width of ^{13}C and its narrow signal lineshape has recently enabled several key applications of d-DNP in metabolomics.

Figure 1



(A) Carbonyl region of hyperpolarized 1D- ^{13}C NMR spectrum of prostate extract from mouse injected with $[\text{U}-^{13}\text{C}]$ glucose 30 min prior to tissue harvest. (B) Full Spectrum analysis of hyperpolarized 1D ^{13}C NMR spectra by principal component analysis (PCA) each point represents a single sample of which are determined by the collective contributions of all sample corresponding integrals. Data was mean centered and unit variance scaled before PCA was applied. Adapted from reference [35●●].

Determination of isotopic ratios can be easily carried out by integration of isotopic patterns of labeled samples in 1D ^{13}C NMR thanks to its resolution advantages (Fig. 1) [33]. Lerche's group reported a

method combining ^{13}C d-DNP with NMR-based SIRM using labelled $[\text{U-}^{13}\text{C}, \text{d}_7]\text{glucose}$ as a tracer and benefiting from the sensitivity boost offered by d-DNP [27]. With this method, the authors were able to distinguish different cancer cell types from ^{13}C -enriched tissues. Also, the authors proposed a method to provide quantitative information by d-DNP for identified labeled metabolites. To account for inhomogeneous signal decay during transfer, a signal to loss coefficient (SLC) was determined relative to an internal standard and compared to the ratio measured by thermal ^{13}C NMR [27]. In 2020, Frahm *et al.* applied a similar approach to the differentiation of aggressive and less-aggressive prostate cancer cell extracts, and managed to highlight biomarkers of this discrimination [34]. In 2021, mice tissues were analyzed for the first time with d-DNP, and the same group observed a tendency to separate healthy and cancer tissues by principal component analysis (Fig. 1B) and supervised analysis [35●●].

Natural abundance analysis by d-DNP is more challenging in terms of sensitivity due to the lower abundance of ^{13}C (1.1 %), but it still offers great perspectives for metabolomics. Indeed, ^{13}C NMR metabolomics – which is barely accessible without hyperpolarization for sensitivity reasons – would offer significant advantages compared to its ^1H NMR counterpart, such as reduced peak overlap that directly facilitates metabolite assignment and biomarker discovery.

Figure 2

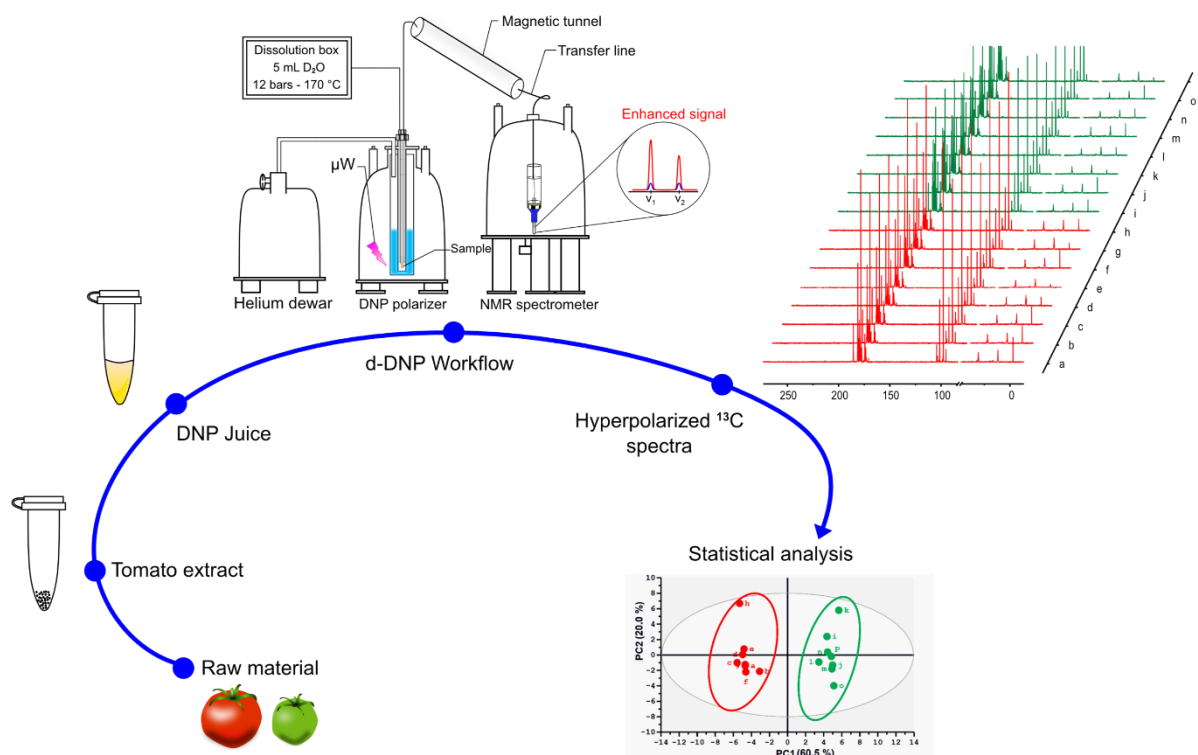


Illustration of untargeted metabolomics workflow on tomato extracts using ^{13}C d-DNP. Tomato extracts were prepared from a single pooled sample of tomato powder and mixed in a DNP juice ($\text{d}_8\text{-gly: D}_2\text{O: H}_2\text{O, 6:3:1, v/v}$). 200 μL of solution were polarized at 1.2 K and 7.5 T using cross-polarization. Sample were dissolved with 5 mL of hot D_2O , spectra were acquired after a total transfer time of 12.8 s. In spectra, letters a to h refer to red-ripe tomatoes, letters i to p refer to green tomatoes. Adapted with permission from reference [38●●].

A few years ago, a proof-of-concept work highlighted experimental conditions that make it possible to record hyperpolarized d-DNP ^{13}C NMR spectra of biological extracts at natural ^{13}C abundance, relying

on cross-polarization from ^1H to ^{13}C nuclei in the solid state. In 2015, Dumez et al. reported hyperpolarized ^{13}C NMR spectra acquired on plants and cancer cell extracts at natural abundance [36]. In 2016, Bornet et al. reported an average repeatability of 4% on normalized signal areas for such extracts, demonstrating the suitability of the d-DNP experimental setting for metabolomics [37].

In 2020, ^{13}C d-DNP was successfully incorporated into a full metabolomics workflow, allowing to separate red-ripe and mature-green tomatoes extracts (Fig. 2) [38●●]. Authors highlighted known biomarkers of two ripening stages of tomato, previously determined by ^1H NMR but so far inaccessible by ^{13}C NMR [38●●]. This proof-of-concept study represents an important step towards hyperpolarized ^{13}C NMR metabolomics. However, in spite of the high polarization reached in the solid-state – *c.a.* 60 % – part of the polarization was lost during the transfer time (12.8 s in this study), and the sample was diluted by a factor 25 during dissolution. This led to a limit of detection in the millimolar concentration range for ^{13}C d-DNP, roughly comparable to ^1H NMR. Nonetheless, the resolution advantages of ^{13}C detection increase the overall data quality and can facilitate the discovery of biomarkers. Moreover, the same authors recently reported that a fine optimization of the whole ^{13}C d-DNP workflow could further increase sensitivity and resolution, that could enlarge the application perspectives of d-DNP to metabolomics at natural ^{13}C abundance [39]. The ability of ^{13}C d-DNP to provide relevant information for both labelled and unlabeled samples is now proven, yet, there is plenty of room for improvement before its more general use in metabolomics. As discussed previously, d-DNP sensitivity is limited by the loss of hyperpolarization during the transfer between the two magnets – accelerated by paramagnetic phenomena induced by radicals – and by the non-negligible dilution factor. Several recent developments could help circumventing these limitations.

Sensitivity limits could be addressed by using fast-transfer lines, since the polarization decreases exponentially through longitudinal relaxation. Shortening the transfer time can improve the limits of detection for quaternary ^{13}C ($T_1 \approx 40$ s) but could also drastically facilitate the detection of aliphatic ^{13}C ($T_1 \approx 10$ s) and even ^1H ($T_1 \approx 2$ s). Different fast transfer lines have been described in the literature [29,40–42]. In particular, an automated liquid-driven system described by Ceillier *et al.* showed efficient transfer of hyperpolarized liquid within 2.1 seconds [3]. Nowadays, ^1H d-DNP is quite restricted to aromatic ^1H ($T_1 \approx 10$ s) [28,43●] but with fast-transfer lines and recent developments, general implementation of ^1H d-DNP could represent a major advance for hyperpolarized metabolomics as it would benefit from the higher intrinsic sensitivity of ^1H spectroscopy, albeit at the cost of stronger peak overlap. The loss of hyperpolarization between the two magnets is accelerated by the presence of paramagnetic radicals thus, limiting significantly the signal sensitivity. In order to prevent this phenomenon, chemical scavengers can be added to neutralize radicals after dissolution [44,45]. Also, porous material can be used to polarize metabolites efficiently while keeping away radicals from the sample [32].

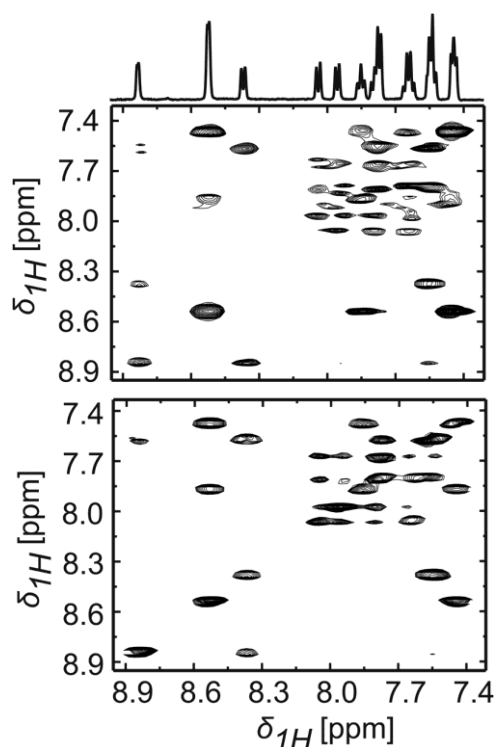
High throughput and reasonable experimental cost are also very important practical points for a general use of an analytical method in metabolomics. At the time of writing, there is a limited number of automated commercial instruments for d-DNP and research is limited by an important consumption of liquid He. However, cryogen-consumption free d-DNP prototypes were already reported in the literature, opening promising perspectives concerning throughput and experimental cost of d-DNP experiments [46–48].

The ability to provide valuable structural information is one of the most important features of NMR. 1D NMR experiments do provide structural information thanks to reliable chemical shifts, J-couplings values and peak integrals. However, they are not always sufficient and often limited by peak overlap.

Unambiguous structural information is generally obtained from 2D NMR experiments, either relying on homonuclear (COSY, TOCSY, ...) or heteronuclear couplings (HSQC, HMBC, ...), or on couplings through space (NOESY, ROESY) [49]. 2D NMR also offers an additional peak dispersion which is of great interest to overcome 1D signal overlap and facilitate metabolite identification in metabolomics [19,20]. Unfortunately, the irreversible character of d-DNP experiment is incompatible with conventional 2D NMR which requires multiple scans and long experiment times. Although small-angle strategies have been suggested in specific cases to record conventional 2D NMR spectra after hyperpolarization [50], a more general solution relies on Ultrafast 2D NMR (UF), which allows to record a full 2D spectra in a single-scan, replacing the time incrementation of conventional 2D NMR by spatial encoding using frequency-swept pulses and gradients [51,52]. The single-scan character of UF 2D NMR is compatible with the short-lived hyperpolarization of d-DNP [53] and proof-of-concept work on simple mixtures of natural products was demonstrated in 2009 [54] by analyzing natural abundance terpenes at 1 mM with entangled UF HMBC and UF HSQC, with a single d-DNP experiment. In 2015, Dumez et al. reported the first UF long-range heteronuclear correlation spectra of breast cancer cell extracts at natural abundance [36]. More recently, ^1H - ^1H d-DNP enhanced UF 2D spectra were reported by Singh et al. on a mixture of aromatic compounds, observing a signal enhancement higher than 150 (Fig. 3) [43•].

While not applied yet in a metabolomics context, these approaches could facilitate the identification of biomarkers in hyperpolarized metabolomics studies, or the determination of isotopic patterns in hyperpolarized SIRM. Challenges remain to be addressed, such as the impact of post-dissolution turbulences effects which can significantly affect the quality of spatial encoding in UF experiments [55].

Figure 3



2D ^1H - ^1H hyperpolarized UF TOCSY spectrum obtained in a single scan on model mixture of pyridine, quinoline and benzophenone at 85 mM before dissolution (top). Conventional 2D ^1H - ^1H TOCSY spectrum on the same sample after rethermalisation and shimming, in 4 hours and 25 minutes using 128 t_1 increments with 8 scans per increment (bottom). The data for obtaining the conventional spectra were processed with same acquisition (t_2) and evolution (t_1) times as used for its corresponding 2D hyperpolarized UF spectra. Shown on top of the 2D sets is the conventional 1D spectrum for this mixture. Adapted with permission from reference [43•].

Enhanced ^1H sensitivity with para-hydrogen

Hyperpolarization techniques based on $p\text{-H}_2$ can overcome the sensitivity barrier of conventional ^1H NMR using the large and long-lived spin order of dihydrogen gas in its para form, and metal-based catalysts that allow a hyperpolarized detection of specific analytes.

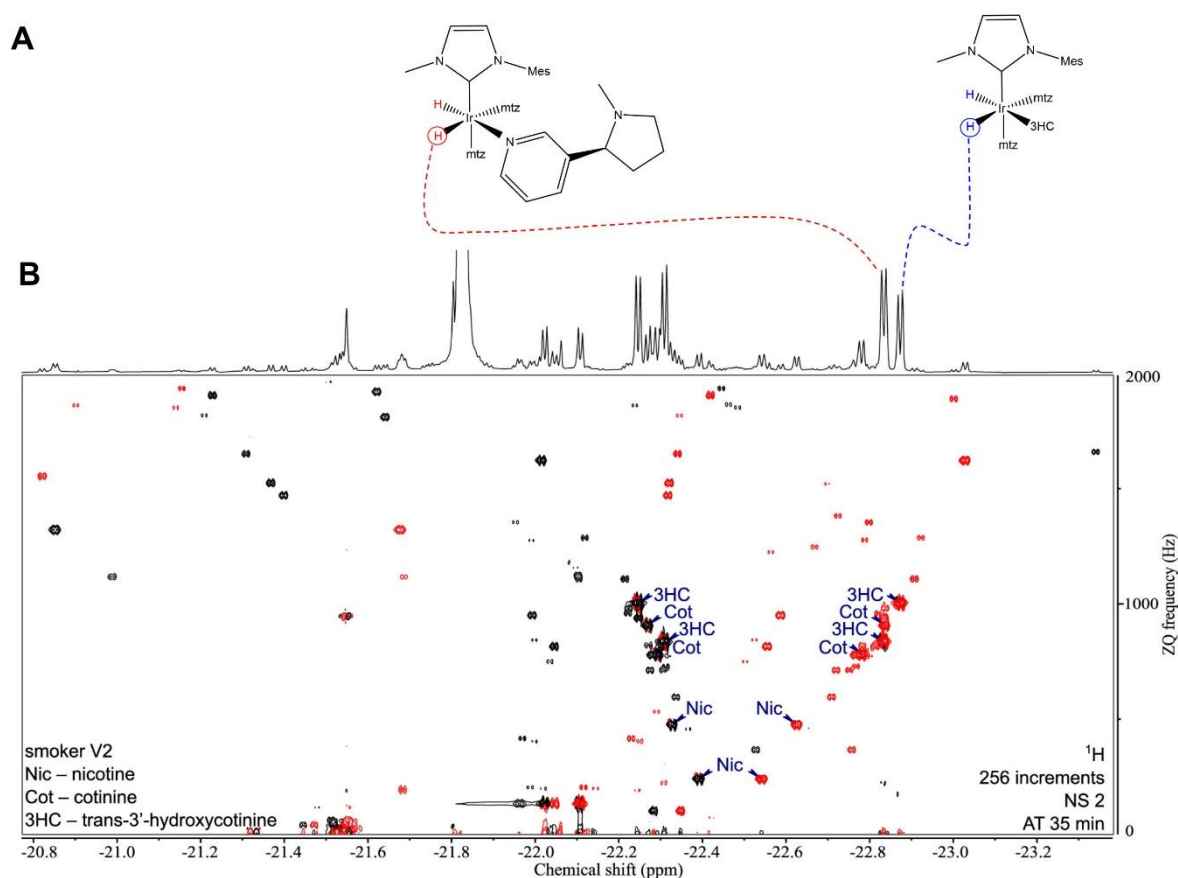
Among all the variants of $p\text{-H}_2$ techniques, non-hydrogenative Para-Hydrogen Induced Polarization (nh-PHIP) is particularly relevant and developed for mixture analysis. This is notably the case of Signal Amplification By Reversible Exchange (SABRE), which consists in using the reversible binding of $p\text{-H}_2$ and the substrates to a metal complex, to transfer spin order from $p\text{-H}_2$ to substrates through J-couplings, followed by the spontaneous conversion of spin order into observable magnetization, allowing the detection of substrates at low- μM detection in a single-scan [56,57]. SABRE is compatible with a wide set of conventional 2D NMR experiments (COSY, UF COSY and HMBC) [58]. However, SABRE techniques are quite restricted to organic solvents since water-soluble catalysts provide – at the time of writing – poorly efficient polarization transfer [59,60], limiting their application for biological sample analysis and metabolomics.

Several non-hydrogenative PHIP (nh-PHIP) based techniques rely on a direct observation of hyperpolarized hydrides (Fig. 4). Indirect detection of metabolites is possible since hydride chemical shifts are sensitive to bound metabolites. However, it makes signal assignment more challenging and – in the absence of rich libraries – internal standard additions are often mandatory for signal assignment [61●].

Fortunately, nh-PHIP based techniques are also compatible with 2D NMR experiments, especially 2D zero-quantum (ZQ) experiments. ZQ spectroscopy shows several advantages compared to more conventional 2D experiments, indeed ZQ coherences are not altered by unwanted inter-hydride J-couplings ($J_{\text{AX}} \approx 7.5$ Hz), moreover, they offer a linear pattern for similar metabolite structures that can aid for signal assignment (Fig. 4) [62].

Recently, Reimets et al. showed that 2D ZQ nh-PHIP based methods (Fig. 4) were suited to determine accurately the concentration of nicotine and its derivatives in smoker urine at sub- μM concentrations – using a standard additions workflow – as suitable for pharmacokinetics and targeted metabolomics [63●●]. However, both H_2 and parahydrogen catalysts are poorly soluble in aqueous solvents, limiting direct analysis of biofluids. Moreover, in the case of urine, the presence of urea and ammonium leads to intense unwanted signals and must be removed from the sample. As a consequence, a solid phase extraction (SPE) with an organic solvent (MeOH or CHCl_3) of urine sample is necessary prior to the analysis.

Figure 4



(A) Illustration of hyperpolarized hydrides showing different chemical shifts in function of bond metabolites, Cotinine (left, red) and 3HC (right, blue) (B) 2D ZQ p-H₂ spectrum of urine extract in CDCl₃. Each complex of an analyte gives rise to two opposite phase doublets at the same ZQ frequency. Chiral analytes (i.e., Nic, Cot, and 3HC) can combine in two ways due to stereogenic center on iridium in the complex, forming two diastereomers with different physical properties. Signals were assigned by internal standard addition during method development. Upper 1D trace recorded separately with the 1D Selective excitation of polarization using PASADENA (SEPP) sequence. Adapted from reference [63●●].

Hyperpolarization induced by p-H₂ provides an impressive ¹H enhancement – typically two orders of magnitude – and the recent developments focusing on biofluids make it a promising hyperpolarization technique for metabolomics thanks to its inexpensive and straightforward workflow despite the required extraction step. However, p-H₂ techniques are still limited by their selective character, indeed, only a few functional groups can efficiently bind to the organometallic complex and be subsequently detected, like amines, diazirines, amino-acids or nitrogen and sulfur heteroatomic compounds (Fig. 5). This limitation could be addressed by developing new chemosensors – including water-soluble ones – with different affinity to a wider range of chemicals. Predicting hydride chemical shifts values as a function of bound metabolites is another very challenging task, and only internal standard additions are currently used to assign nh-PHIP signals, however, chemical shifts are very trustful and libraries could be built to overcome this issue [61●].

Figure 5

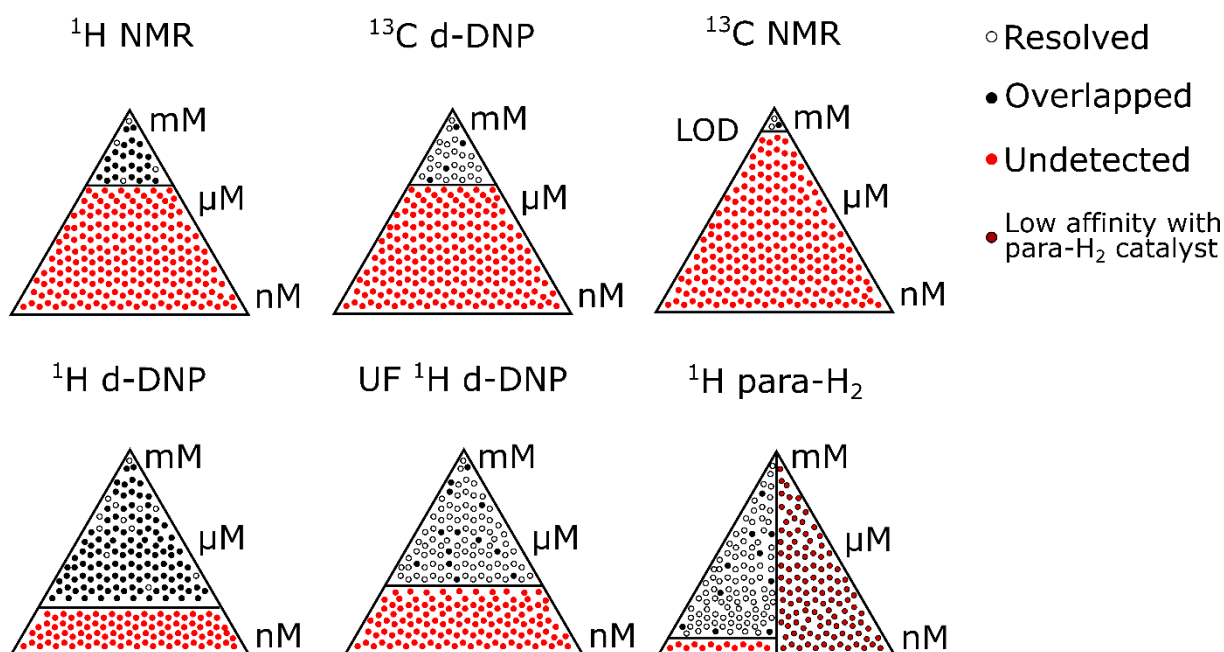


Illustration of the sensitivity, resolution and selectivity of main hyperpolarized techniques for metabolomics. White, black and red dots represent respectively resolved, overlapped and undetected metabolites signals. Dark red dots represent concentrated metabolites that are not detected by para-H₂ NMR because of its selectivity. For d-DNP schemes, estimated LOD are related to metabolite concentration before dilution at natural abundance. For conventional NMR schemes, estimated LOD are given for a 15 min acquisition time on a 700 MHz equipped with a cryoprobe in quantitative conditions. For p-H₂, the triangle is split in half to illustrate that compounds with a low affinity with the p-H₂ catalyst are not detected, regardless of their concentration.

Conclusion

Hyperpolarization NMR techniques offer great opportunities to break the sensitivity barrier of NMR. The last decades have witnessed many efforts to make hyperpolarized NMR suitable for metabolomics. In particular, dissolution-dynamic nuclear polarization and parahydrogen-based methods have been extensively explored.

These hyperpolarized techniques and their perspectives are compared in Fig. 5 to better understand their advantages and drawbacks in terms of sensitivity, resolution and selectivity. ¹³C d-DNP benefits from ¹³C resolution advantages while allowing to detect metabolites at sub-millimolar concentrations. In contrast to ¹³C, ¹H d-DNP will benefit from higher sensitivity of ¹H nuclei, improving theoretically the limit of detection by 3 orders of magnitude at the cost of stronger peak overlap. Moreover, homonuclear UF ¹H DNP (TOCSY, COSY) can reduce significantly strong peak overlap by spreading ¹H signal over a second dimension. p-H₂ also benefits from the natural sensitivity of ¹H nuclei and from 2D spectroscopy, an association that already demonstrated sub-μM limits of detection. However, p-H₂ methods are currently limited by their inherent selectivity, indeed, only selected functional groups of compounds can efficiently bind to organometallic complexes. Note that some degree of selectivity may actually be considered as a strength to observe accurately metabolites at very low concentration, especially if the selectivity is controlled.

At the time of writing, dissolution-dynamic nuclear polarization has shown its potential for both SIRM and metabolomics at natural abundance with super-sensitive ^{13}C detection. The implementation of ^1H d-DNP, multi-dimensional d-DNP and cryo-free systems are among most promising perspectives in the field as they can increase respectively sensitivity, resolution and through-put to another level. Parahydrogen-based methods are straightforward, cheap and naturally compatible with multi-scan approaches, allowing multi-dimensional NMR and an impressive sub- μM limit of detection. Signal assignment and intrinsic selectivity of parahydrogen-based methods are the most challenging limits to overcome and can be addressed respectively by parahydrogen chemical shifts libraries and a wider diversity of organometallic complexes. For both methods, we expect the coming years to be a source of both new developments and impacting applications for hyperpolarized NMR metabolomics. Along with the emergence of alternative hyperpolarization methods such as optical crystals, we expect hyperpolarized NMR, especially based on d-DNP and p- H_2 techniques, to be a source of both new developments and impacting applications for NMR metabolomics.

Acknowledgments

This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreements no 814747/SUMMIT and 801774/DINAMIX) and the Region Pays de la Loire (ConnectTalent/HPNMR). The authors also acknowledge the French National Infrastructure for Metabolomics and Fluxomics MetaboHUB-ANR-11-INBS-0010 (www.metabohub.fr) and the Corsaire metabolomics core facility (Biogenouest).

Competing interests statement

The authors have no competing interests to declare.

References and recommended reading

Papers of particular interest, published within the last two years, have been highlighted as:

● of special interest

●● of outstanding interest

1. Aderemi AV, Ayeleso AO, Oyedapo OO, Mukwevho E: **Metabolomics: A Scoping Review of Its Role as a Tool for Disease Biomarker Discovery in Selected Non-Communicable Diseases**. *Metabolites* 2021, **11**:418.
2. Mulvihill MM, Nomura DK: **Metabolomic strategies to map functions of metabolic pathways**. *Am J Physiol-Endocrinol Metab* 2014, **307**:E237–E244.
3. Puchades-Carrasco L, Pineda- Lucena A: **Metabolomics Applications in Precision Medicine: An Oncological Perspective**. *Curr Top Med Chem* 2017, **17**:2740–2751.
4. Allwood JW, De Vos RCH, Moing A, Deborde C, Erban A, Kopka J, Goodacre R, Hall RD: **Chapter sixteen - Plant Metabolomics and Its Potential for Systems Biology Research: Background Concepts, Technology, and Methodology**. In *Methods in Enzymology*. Edited by Jameson D, Verma M, Westerhoff HV. Academic Press; 2011:299–336.

5. Bundy JG, Davey MP, Viant MR: **Environmental metabolomics: a critical review and future perspectives.** *Metabolomics* 2009, **5**:3–21.
6. Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA: **Untargeted Metabolomics Strategies—Challenges and Emerging Directions.** *J Am Soc Mass Spectrom* 2016, **27**:1897–1905.
7. Griffiths WJ, Koal T, Wang Y, Kohl M, Enot DP, Deigner H-P: **Targeted Metabolomics for Biomarker Discovery.** *Angew Chem Int Ed* 2010, **49**:5426–5445.
8. Bruntz RC, Lane AN, Higashi RM, Fan TW-M: **Exploring cancer metabolism using stable isotope-resolved metabolomics (SIRM).** *J Biol Chem* 2017, **292**:11601–11609.
9. Gowda GAN, Raftery D (Eds): *NMR-Based Metabolomics: Methods and Protocols.* Springer New York; 2019.
10. Raja G, Jung Y, Jung SH, Kim T-J: **¹H-NMR-based metabolomics for cancer targeting and metabolic engineering –A review.** *Process Biochem* 2020, **99**:112–122.
11. Ren J-L, Zhang A-H, Kong L, Wang X-J: **Advances in mass spectrometry-based metabolomics for investigation of metabolites.** *RSC Adv* 2018, **8**:22335–22350.
12. Letertre MPM, Giraudeau P, de Tullio P: **Nuclear Magnetic Resonance Spectroscopy in Clinical Metabolomics and Personalized Medicine: Current Challenges and Perspectives.** *Front Mol Biosci* 2021, **8**:698337.
13. Fei Q, Wang D, Jasbi P, Zhang P, Nagana Gowda GA, Raftery D, Gu H: **Combining NMR and MS with Chemical Derivatization for Absolute Quantification with Reduced Matrix Effects.** *Anal Chem* 2019, **91**:4055–4062.
14. Bingol K, Bruschiweiler-Li L, Yu C, Somogyi A, Zhang F, Brüschiweiler R: **Metabolomics Beyond Spectroscopic Databases: A Combined MS/NMR Strategy for the Rapid Identification of New Metabolites in Complex Mixtures.** *Anal Chem* 2015, **87**:3864–3870.
15. Martias C, Baroukh N, Mavel S, Blasco H, Lefèvre A, Roch L, Montigny F, Gatien J, Schibler L, Dufour-Rainfray D, et al.: **Optimization of Sample Preparation for Metabolomics Exploration of Urine, Feces, Blood and Saliva in Humans Using Combined NMR and UHPLC-HRMS Platforms.** *Molecules* 2021, **26**:4111.
16. Marshall DD, Powers R: **Beyond the paradigm: Combining mass spectrometry and nuclear magnetic resonance for metabolomics.** *Prog Nucl Magn Reson Spectrosc* 2017, **100**:1–16.
17. Pan Z, Raftery D: **Comparing and combining NMR spectroscopy and mass spectrometry in metabolomics.** *Anal Bioanal Chem* 2007, **387**:525–527.
18. Hu K, Westler WM, Markley JL: **Simultaneous Quantification and Identification of Individual Chemicals in Metabolite Mixtures by Two-Dimensional Extrapolated Time-Zero ¹H–¹³C HSQC (HSQC0).** *J Am Chem Soc* 2011, **133**:1662–1665.
19. Marchand J, Martineau E, Guitton Y, Dervilly-Pinel G, Giraudeau P: **Multidimensional NMR approaches towards highly resolved, sensitive and high-throughput quantitative metabolomics.** *Curr Opin Biotechnol* 2017, **43**:49–55.

20. Martineau E, Dumez J-N, Giraudeau P: **Fast quantitative 2D NMR for metabolomics and lipidomics: A tutorial.** *Magn Reson Chem* 2020, **58**:390–403.
21. Walker TG, Happer W: **Spin-exchange optical pumping of noble-gas nuclei.** *Rev Mod Phys* 1997, **69**:629–642.
22. Eichhorn TR, Parker AJ, Josten F, Müller C, Scheuer J, Steiner JM, Gierse M, Handwerker J, Keim M, Lucas S, et al.: **Hyperpolarized Solution-State NMR Spectroscopy with Optically Polarized Crystals.** *J Am Chem Soc* 2022, **144**:2511–2519.
23. Ravera E, Luchinat C, Parigi G: **Basic facts and perspectives of Overhauser DNP NMR.** *J Magn Reson* 2016, **264**:78–87.
24. Kovtunov KV, Pokochueva EV, Salnikov OG, Cousin SF, Kurzbach D, Vuichoud B, Jannin S, Chekmenev EY, Goodson BM, Barskiy DA, et al.: **Hyperpolarized NMR Spectroscopy: d-DNP, PHIP, and SABRE Techniques.** *Chem – Asian J* 2018, **13**:1857–1871.
25. Ludwig C, Marin-Montesinos I, Saunders MG, Emwas A-H, Pikramenou Z, Hammond SP, Günther UL: **Application of ex situ dynamic nuclear polarization in studying small molecules.** *Phys Chem Chem Phys* 2010, **12**:5868–5871.
26. Ardenkjaer-Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K: **Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR.** *Proc Natl Acad Sci* 2003, **100**:10158–10163.
27. Lerche MH, Yigit D, Frahm AB, Ardenkjær-Larsen JH, Malinowski RM, Jensen PR: **Stable Isotope-Resolved Analysis with Quantitative Dissolution Dynamic Nuclear Polarization.** *Anal Chem* 2018, **90**:674–678.
28. Ragavan M, Chen H-Y, Sekar G, Hilty C: **Solution NMR of Polypeptides Hyperpolarized by Dynamic Nuclear Polarization.** *Anal Chem* 2011, **83**:6054–6059.
29. Ceillier M, Cala O, El Daraï T, Cousin SF, Stern Q, Guibert S, Elliott SJ, Bornet A, Vuichoud B, Milani J, et al.: **An automated system for fast transfer and injection of hyperpolarized solutions.** *J Magn Reson Open* 2021, **8–9**:100017.
30. Kouřil K, Kouřilová H, Bartram S, Levitt MH, Meier B: **Scalable dissolution-dynamic nuclear polarization with rapid transfer of a polarized solid.** *Nat Commun* 2019, **10**:1733.
31. Salvi N: **Chapter One - Applications of Hyperpolarisation and NMR Long-Lived States in Drug Screening.** In *Annual Reports on NMR Spectroscopy*. Edited by Webb GA. Academic Press; 2019:1–33.
32. Ji X, Bornet A, Vuichoud B, Milani J, Gajan D, Rossini AJ, Emsley L, Bodenhausen G, Jannin S: **Transportable hyperpolarized metabolites.** *Nat Commun* 2017, **8**:1–7.
33. Heux S, Bergès C, Millard P, Portais J-C, Létisse F: **Recent advances in high-throughput ¹³C-fluxomics.** *Curr Opin Biotechnol* 2017, **43**:104–109.
34. Frahm AB, Jensen PR, Ardenkjær-Larsen JH, Yigit D, Lerche MH: **Stable isotope resolved metabolomics classification of prostate cancer cells using hyperpolarized NMR data.** *J Magn Reson* 2020, **316**:106750.

35. Frahm AB, Hill D, Katsikis S, Andreassen T, Ardenkjær-Larsen JH, Bathen TF, Moestue SA, Jensen PR, Lerche MH: **Classification and biomarker identification of prostate tissue from TRAMP mice with hyperpolarized ^{13}C -SIRA.** *Talanta* 2021, **235**:122812.

[35●●] In this article, the authors present a SIRM workflow using d-DNP experiments. Enriched mice tissues were analyzed by ^{13}C d-DNP and both unsupervised and supervised analysis helped to highlight candidate biomarkers of cancer.

36. Dumez J-N, Milani J, Vuichoud B, Bornet A, Lalande-Martin J, Tea I, Yon M, Maucourt M, Deborde C, Moing A, et al.: **Hyperpolarized NMR of plant and cancer cell extracts at natural abundance.** *The Analyst* 2015, **140**:5860–5863.

37. Bornet A, Maucourt M, Deborde C, Jacob D, Milani J, Vuichoud B, Ji X, Dumez J-N, Moing A, Bodenhausen G, et al.: **Highly Repeatable Dissolution Dynamic Nuclear Polarization for Heteronuclear NMR Metabolomics.** *Anal Chem* 2016, **88**:6179–6183.

38. Dey A, Charrier B, Martineau E, Deborde C, Gandriau E, Moing A, Jacob D, Eshchenko D, Schnell M, Melzi R, et al.: **Hyperpolarized NMR Metabolomics at Natural ^{13}C Abundance.** *Anal Chem* 2020, **92**:14867–14871.

[38●●] This publication shows the suitability of ^{13}C d-DNP for untargeted metabolomics at natural abundance. The authors were able to highlight biomarkers of two ripening stages of tomato, previously determined by ^1H NMR, but so far inaccessible by conventional ^{13}C NMR.

39. Dey A, Charrier B, Lemaître K, Ribay V, Eshchenko D, Schnell M, Melzi R, Stern Q, Cousin SF, Kempf JG, et al.: **Fine optimization of a dissolution-DNP experimental setting for ^{13}C NMR of metabolic samples.** *Magn Reson Discuss* 2022, doi:10.5194/mr-2022-14.

40. Granwehr J, Panek R, Leggett J, Köckenberger W: **Quantifying the transfer and settling in NMR experiments with sample shuttling.** *J Chem Phys* 2010, **132**:244507.

41. Chen H-Y, Hilty C: **Implementation and Characterization of Flow Injection in Dissolution Dynamic Nuclear Polarization NMR Spectroscopy.** *ChemPhysChem* 2015, **16**:2646–2652.

42. Katsikis S, Marin-Montesinos I, Pons M, Ludwig C, Günther UL: **Improved Stability and Spectral Quality in Ex Situ Dissolution DNP Using an Improved Transfer Device.** *Appl Magn Reson* 2015, **46**:723–729.

43. Singh K, Jacquemmoz C, Giraudeau P, Frydman L, Dumez J-N: **Ultrafast 2D ^1H - ^1H NMR spectroscopy of DNP-hyperpolarised substrates for the analysis of mixtures.** *Chem Commun* 2021, **57**:8035–8038.

[43●] This article demonstrates the promising compatibility between UF-NMR-based techniques and ^1H d-DNP for complex mixture analysis.

44. Miéville P, Ahuja P, Sarkar R, Jannin S, Vasos PR, Gerber-Lemaire S, Mishkovsky M, Comment A, Gruetter R, Ouari O, et al.: **Scavenging Free Radicals To Preserve Enhancement and Extend**

- Relaxation Times in NMR using Dynamic Nuclear Polarization.** *Angew Chem Int Ed* 2010, **49**:6182–6185.
45. Negroni M, Turhan E, Kress T, Ceillier M, Jannin S, Kurzbach D: **Frémy's Salt as a Low-Persistence Hyperpolarization Agent: Efficient Dynamic Nuclear Polarization Plus Rapid Radical Scavenging.** *J Am Chem Soc* 2022, doi:10.1021/jacs.2c07960.
 46. Baudin M, Vuichoud B, Bornet A, Bodenhausen G, Jannin S: **A cryogen-consumption-free system for dynamic nuclear polarization at 9.4 T.** *J Magn Reson* 2018, **294**:115–121.
 47. Ardenkjær-Larsen JH, Bowen S, Petersen JR, Rybalko O, Vinding MS, Ullisch M, Nielsen NChr: **Cryogen-free dissolution dynamic nuclear polarization polarizer operating at 3.35 T, 6.70 T, and 10.1 T.** *Magn Reson Med* 2019, **81**:2184–2194.
 48. Ferrari A, Peters J, Anikeeva M, Pravdivtsev A, Ellermann F, Them K, Will O, Peschke E, Yoshihara H, Jansen O, et al.: **Performance and reproducibility of ¹³C and ¹⁵N hyperpolarization using a cryogen-free DNP polarizer.** *Sci Rep* 2022, **12**:11694.
 49. Elyashberg M: **Identification and structure elucidation by NMR spectroscopy.** *TrAC Trends Anal Chem* 2015, **69**:88.
 50. Zeng H, Bowen S, Hilty C: **Sequentially acquired two-dimensional NMR spectra from hyperpolarized sample.** *J Magn Reson* 2009, **199**:159–165.
 51. Giraudeau P, Frydman L: **Single-scan 2D NMR: An Emerging Tool in Analytical Spectroscopy.** *Annu Rev Anal Chem Palo Alto Calif* 2014, **7**:129–161.
 52. Frydman L, Scherf T, Lupulescu A: **The acquisition of multidimensional NMR spectra within a single scan.** *Proc Natl Acad Sci* 2002, **99**:15858–15862.
 53. Frydman L, Blazina D: **Ultrafast two-dimensional nuclear magnetic resonance spectroscopy of hyperpolarized solutions.** *Nat Phys* 2007, **3**:415–419.
 54. Giraudeau P, Shrot Y, Frydman L: **Multiple Ultrafast, Broadband 2D NMR Spectra of Hyperpolarized Natural Products.** *J Am Chem Soc* 2009, **131**:13902–13903.
 55. Jacquemmoz C, Giraud F, Dumez J-N: **Online reaction monitoring by single-scan 2D NMR under flow conditions.** *Analyst* 2020, **145**:478–485.
 56. Eshuis N, Hermkens N, van Weerdenburg BJA, Feiters MC, Rutjes FPJT, Wijmenga SS, Tessari M: **Toward Nanomolar Detection by NMR Through SABRE Hyperpolarization.** *J Am Chem Soc* 2014, **136**:2695–2698.
 57. Reile I, Aspers RLEG, Tyburn J-M, Kempf JG, Feiters MC, Rutjes FPJT, Tessari M: **DOSY Analysis of Micromolar Analytes: Resolving Dilute Mixtures by SABRE Hyperpolarization.** *Angew Chem* 2017, **129**:9302–9305.
 58. Lloyd LS, Adams RW, Bernstein M, Coombes S, Duckett SB, Green GGR, Lewis RichardJ, Mewis RE, Sleight CJ: **Utilization of SABRE-Derived Hyperpolarization To Detect Low-Concentration Analytes via 1D and 2D NMR Methods.** *J Am Chem Soc* 2012, **134**:12904–12907.

59. Fekete M, Gibard C, Dear GJ, Green GGR, Hooper AJJ, Roberts AD, Cisnetti F, Duckett SB: **Utilisation of water soluble iridium catalysts for signal amplification by reversible exchange.** *Dalton Trans* 2015, **44**:7870–7880.
60. Hövener J-B, Bär S, Leupold J, Jenne K, Leibfritz D, Hennig J, Duckett SB, von Elverfeldt D: **A continuous-flow, high-throughput, high-pressure parahydrogen converter for hyperpolarization in a clinical setting.** *NMR Biomed* 2013, **26**:124–131.
61. Ausmees K, Reimets N, Reile I: **Understanding Parahydrogen Hyperpolarized Urine Spectra: The Case of Adenosine Derivatives.** *Molecules* 2022, **27**:802.

[61●] In this communication, the authors present an early example of identification by parahydrogen NMR for metabolomics purpose. Adenosine and its derivatives were successfully assigned in a urine matrix.

62. Sellies L, Reile I, Aspers RLEG, Feiters MC, Rutjes FPJT, Tessari M: **Parahydrogen induced hyperpolarization provides a tool for NMR metabolomics at nanomolar concentrations.** *Chem Commun* 2019, **55**:7235–7238.
63. Reimets N, Ausmees K, Vija S, Reile I: **Developing Analytical Applications for Parahydrogen Hyperpolarization: Urinary Elimination Pharmacokinetics of Nicotine.** *Anal Chem* 2021, **93**:9480–9485.

[63●●] This recent article shows the ability of parahydrogen-based methods to break NMR sensitivity limits in biological sample. The authors illustrate its capabilities by following sub- μM nicotine and its derivatives in urine in a pharmacokinetics workflow.