Introduction to PHIP (& NVision)

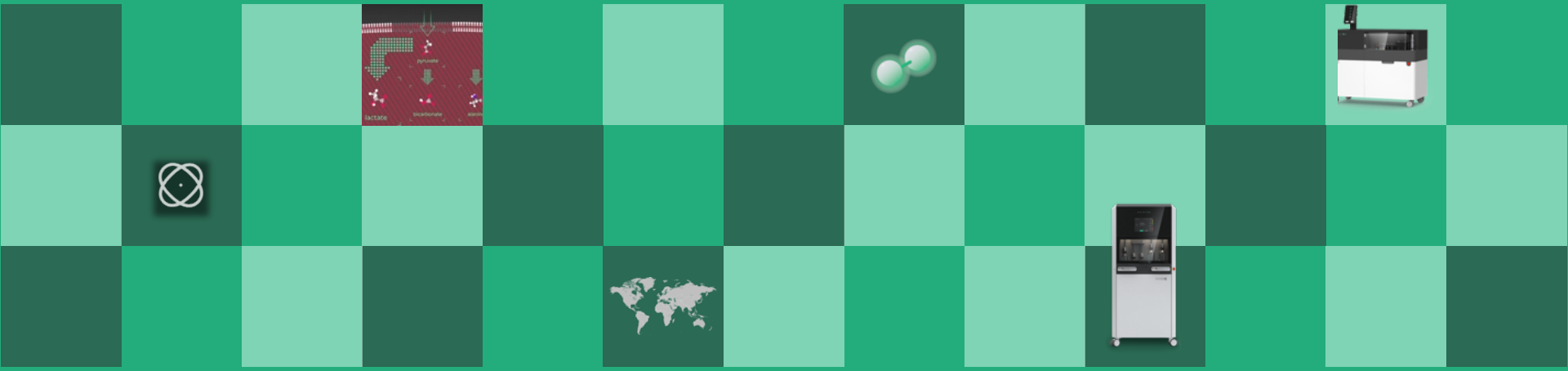
International Training Course 2024

MR Research Centre, Aarhus University

7th October 2024

NVISION

Hyperpolarized MRI made simple



NVISION

NVision is a multinational, interdisciplinary team of physicists, chemists, engineers and life scientists, collocated in Ulm, Germany

82 team members

23 nationalities

40% PhD level

NVISION

Headquartered in Ulm

NVISION

Introducing
POLARIS

Unlocking the
full potential of MRI

robust | effective | fast | easy to use | cost efficient

NVISION

- ✓ ROBUST
- ✓ EFFECTIVE
- ✓ FAST
- ✓ EASY TO USE
- ✓ COST EFFICIENT

NVISION

Our product is based on Parahydrogen Induced Polarization (PHIP)

PHIP was first introduced in the eighties, and further developed in the last decade with the Side Arm Hydrogenation strategy (PHIP-SAH), pioneered by Reineri et al

1986

J|A|C|S

Parahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment

C. Russell Bowers and D. P. Weitekamp*

2001

Magnetic Resonance in Medicine

Parahydrogen-Induced Polarization in Imaging: Subsecond ^{13}C Angiography

K. Golman,* O. Axelsson, H. Jóhannesson, S. Månsson, C. Olofsson, and J.S. Petersson

2015

nature communications

ParaHydrogen Induced Polarization of ^{13}C carboxylate resonance in acetate and pyruvate

Francesca Reineri[1], Tommaso Boi[2] & Silvio Aime[1]

2018

nature SCIENTIFIC REPORTS

The ^{13}C hyperpolarized pyruvate generated by ParaHydrogen detects the response of the heart to altered metabolism in real time

Eleonora Cavallari[1], Carla Carrera[1], Matteo Sorge[1], Gisèle Bonne[2], Antoine Muchir[2], Silvio Aime[1] & Francesca Reineri[1]

2021

PNAS

Rapid hyperpolarization and purification of the metabolite fumarate in aqueous solution

Stephan Knecht[a,1], John W. Blanchard[b,1], Danila Barskiy[c], Eleonora Cavallari[d], Laurynas Dagys[e], Erik Van Dyke[c], Maksim Tsukanov[c], Bea Bliemel[c], Kerstin Münnemann[f], Silvio Aime[d], Francesca Reineri[d], Malcolm H. Levitt[e], Gerd Buntkowsky[a], Alexander Pines[c,2], Peter Blümler[g], Dmitry Budker[b,g], and James Eills[b,g,2]

2023

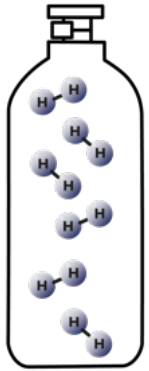
J|A|C|S

Parahydrogen-Polarized Fumarate for Preclinical *in Vivo* Metabolic Magnetic Resonance Imaging

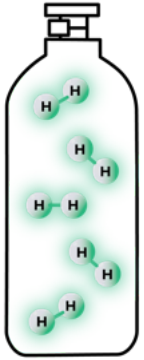
Martin Gierse, Luca Nagel, Michael Keim, Sebastian Lucas, Tobias Speidel, Tobias Lobmeyer, Gordon Winter, Felix Josten, Senay Karaali, Maximilian Fellermann, Jochen Scheuer, Christoph Müller, Frits van Heijster, Jason Skinner, Jessica Löffler, Anna Parker, Jonas Handwerker, Alastair Marshall, Alon Salhov, Bilal El-Kassem, Christophoros Vassiliou, John W. Blanchard, Román Picazo-Frutos, James Eills, Holger Barth, Fedor Jelezko, Volker Rasche, Franz Schilling,* Ilai Schwartz,* and Stephan Knecht*

NVision's scalable process uses transportable polarized hydrogen to enable room-temperature hyperpolarization in 2-3 minute

The source of polarization is parahydrogen, produced centrally

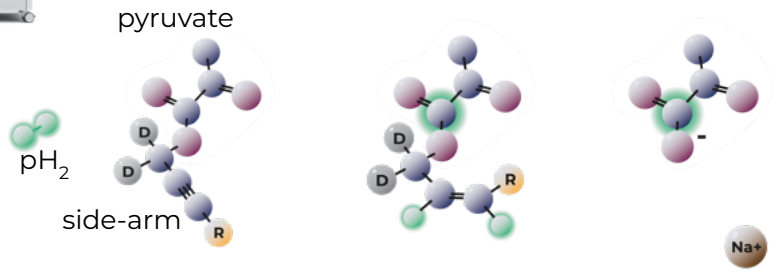


Hydrogen



Parahydrogen (pH_2)

On site, polarization is transferred from parahydrogen to the natural metabolite



pH_2 + pyruvate precursor

Hyperpolarized precursor

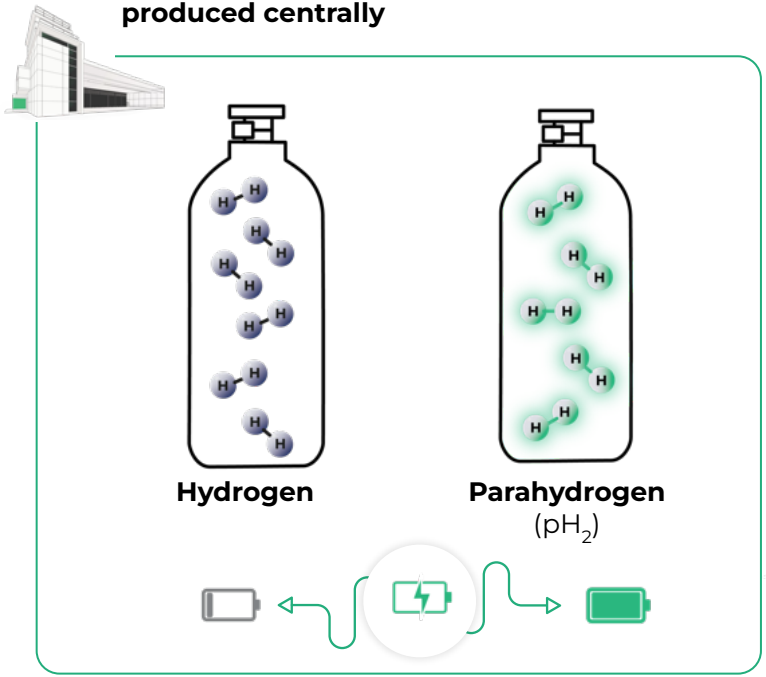
Hyperpolarized pyruvate

NVISION

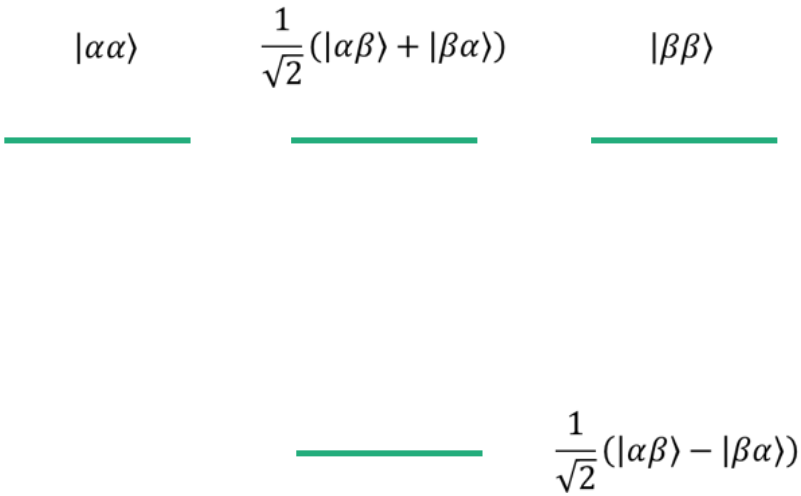
PHIP-SAH = ParaHydrogen Induced Polarization-Side Arm Hydrogenation.

We utilize parahydrogen - a remarkable quantum state of hydrogen

The source of polarization is parahydrogen, produced centrally



Four nuclear spin states in each H2 molecule - huge energy difference due to molecule symmetry and Pauli's exclusion principle*



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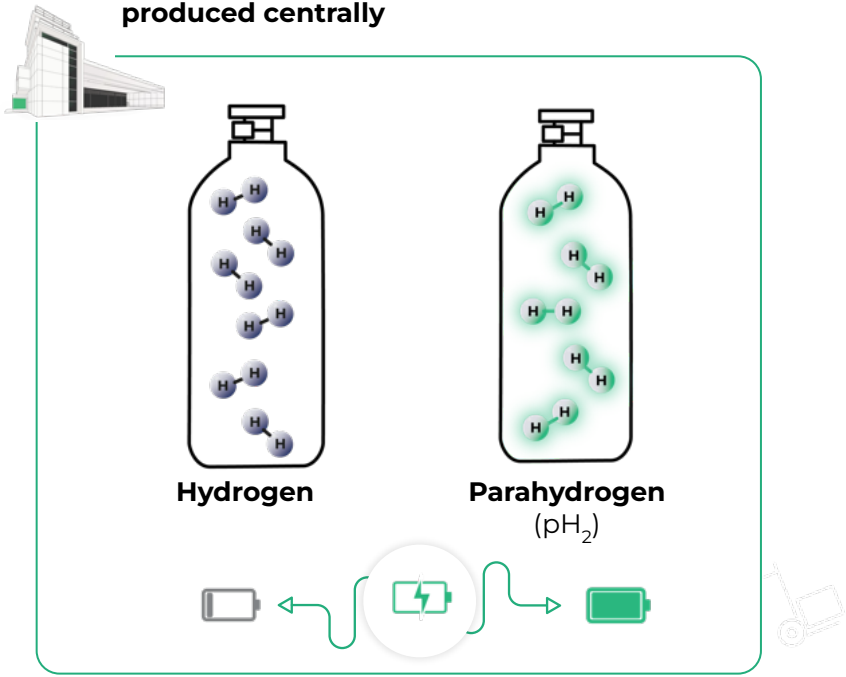
* see e.g. nuclear statistics in Atkins, Peter William, Julio De Paula, and James Keeler. *Atkins' physical chemistry*. Oxford university press, 2023.

By flowing the hydrogen to cold temperature, and then **back to room temperature**, we obtain 99% population in the nuclear spin singlet state

The source of polarization is parahydrogen, produced centrally



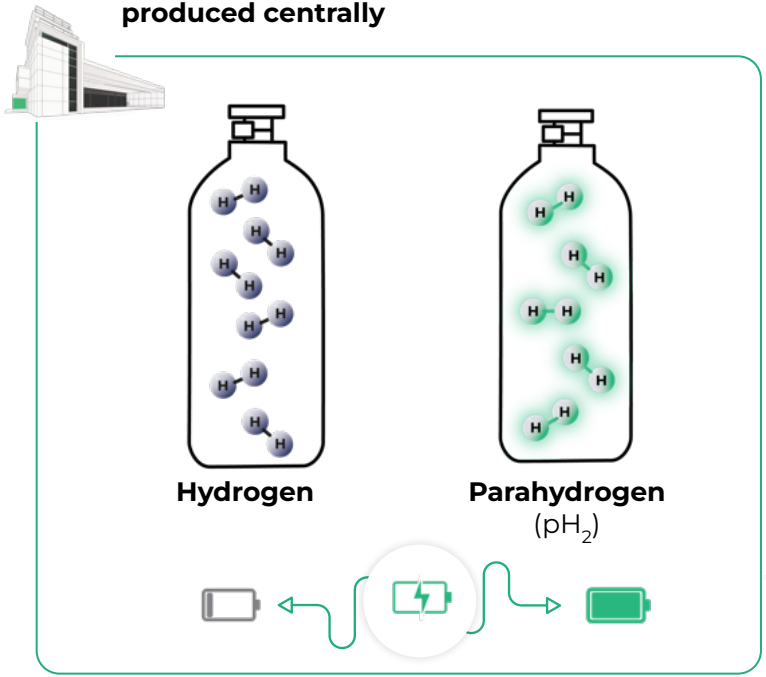
$|\alpha\alpha\rangle$ $\frac{1}{\sqrt{2}}(|\alpha\beta\rangle + |\beta\alpha\rangle)$ $|\beta\beta\rangle$

>99% population

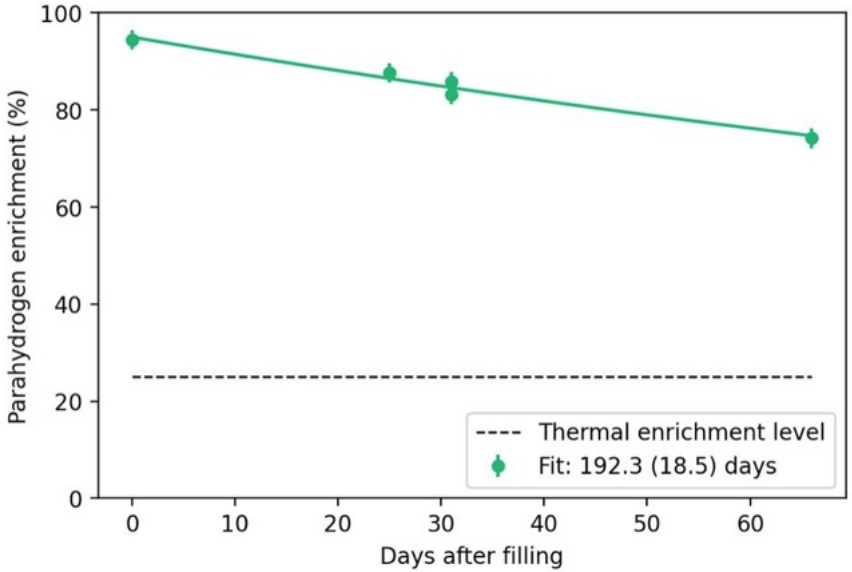
$\frac{1}{\sqrt{2}}(|\alpha\beta\rangle - |\beta\alpha\rangle)$

Remarkably, para-H2 is the only known material maintaining a coherent entangled quantum state at room temperature for over 6 months!

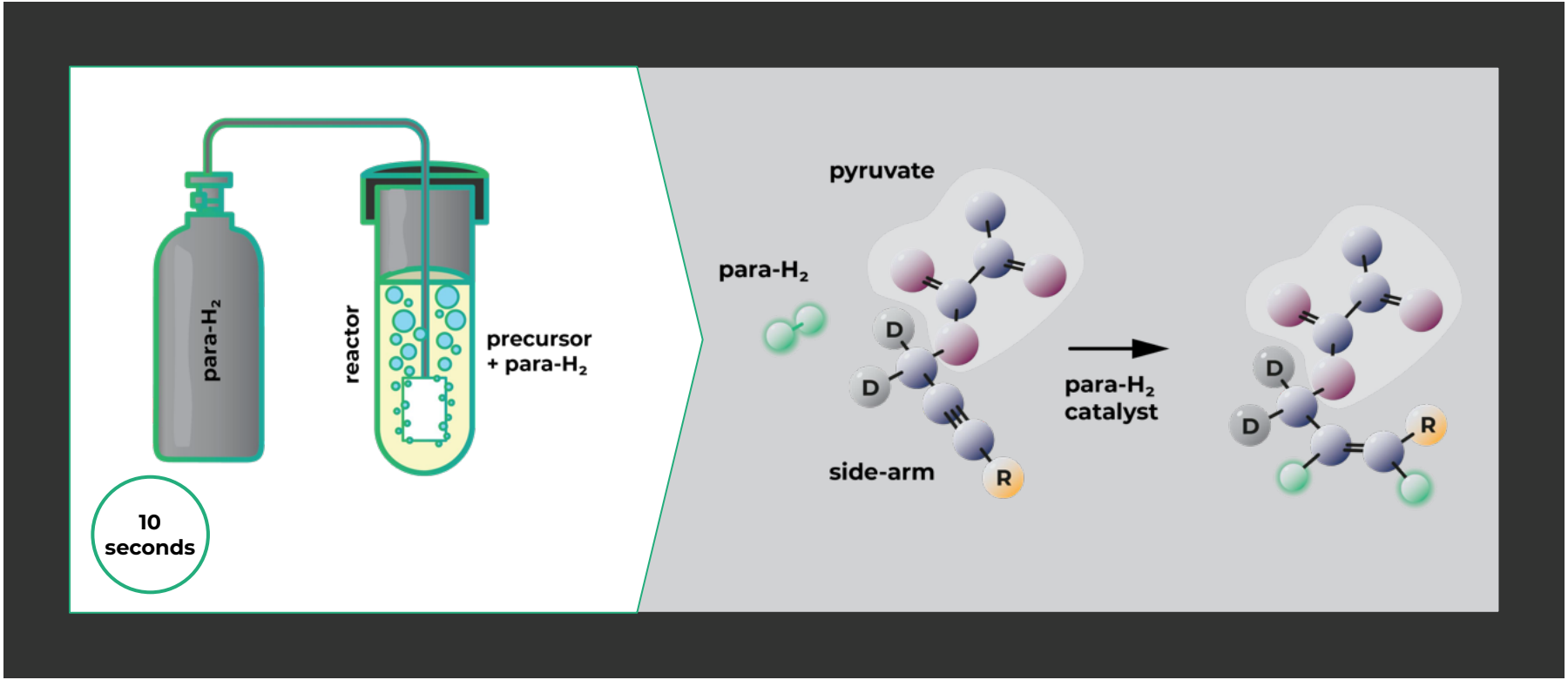
The source of polarization is parahydrogen, produced centrally



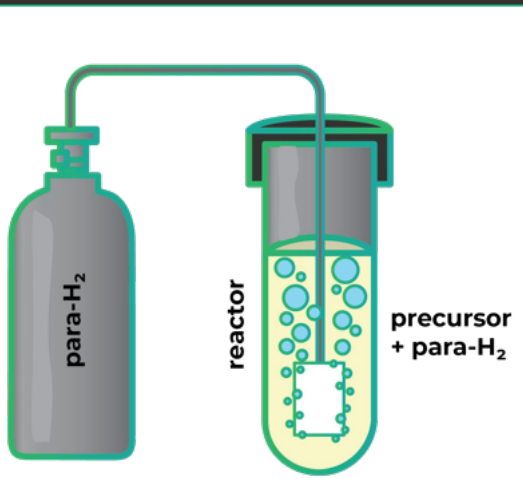
At room temperature in standard cylinders, relaxation time >6 months

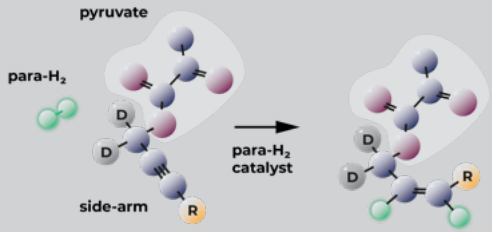


Step 1: Hydrogen is bubbled through a solution containing the precursor and the catalyst, initiating the hydrogenation reaction



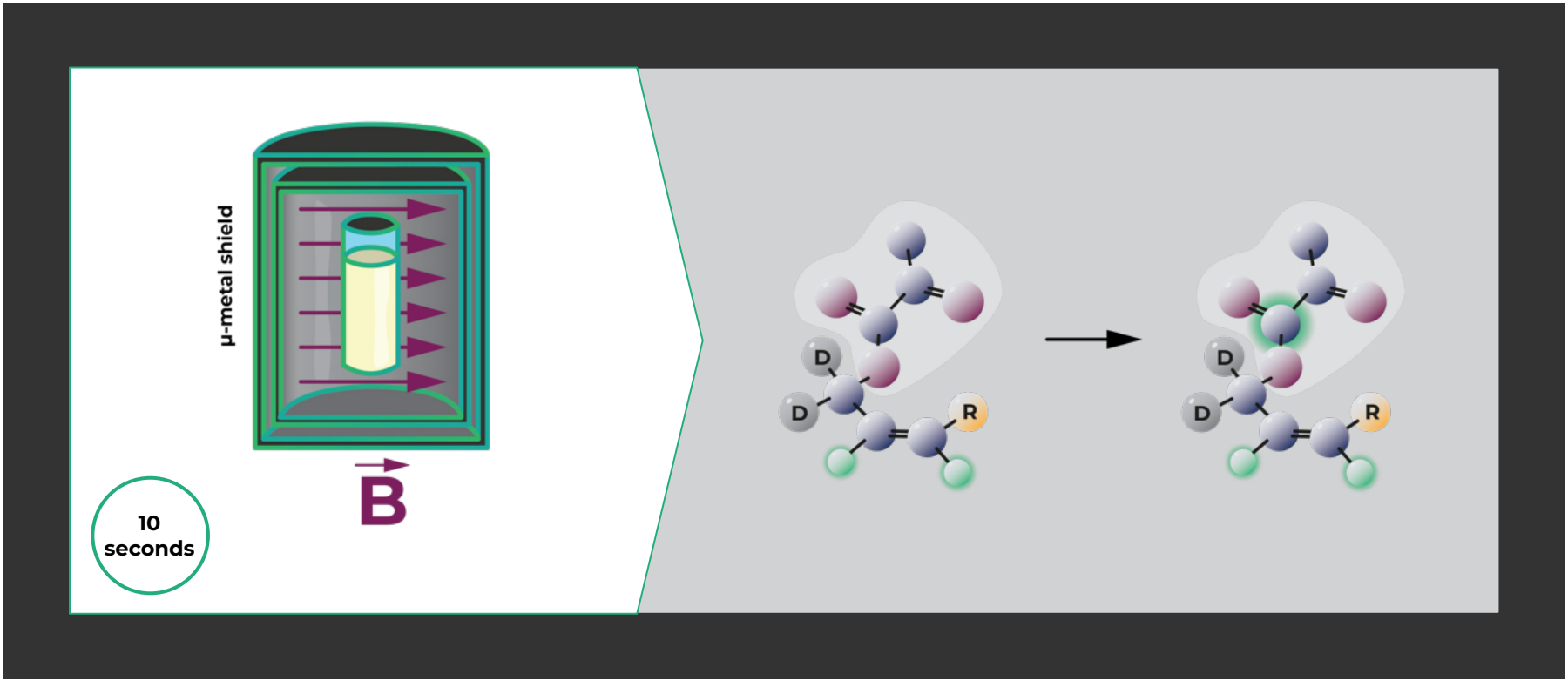
Hydrogenation is performed at high pressure



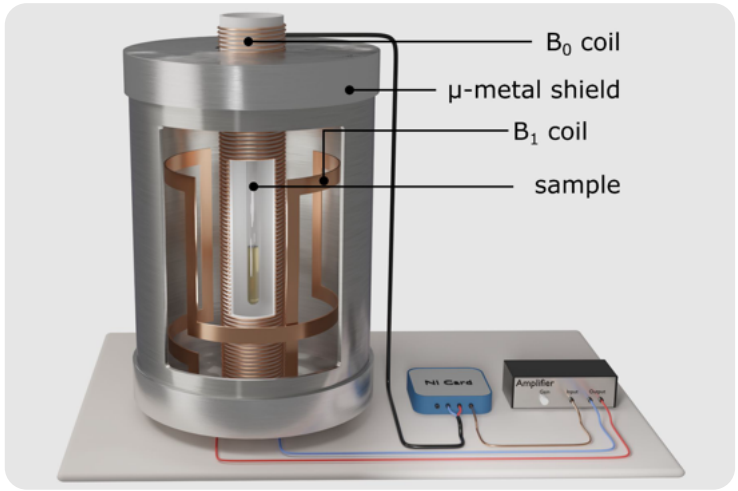


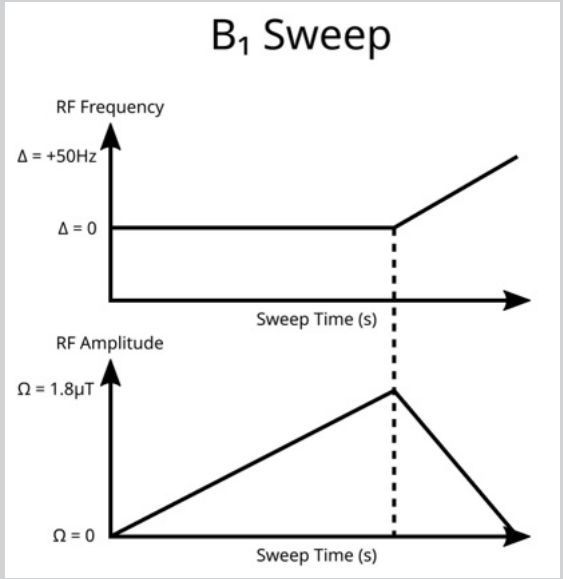
- Hydrogenation of up to 250 mM precursor in 5-10s
- Hydrogen pressure of 10 bar
- Reaction temperature 55 degree Celsius
- Acetone-d6 is used as a solvent

Step 2: polarization is transferred to the carbon nuclear spin by using RF sequences



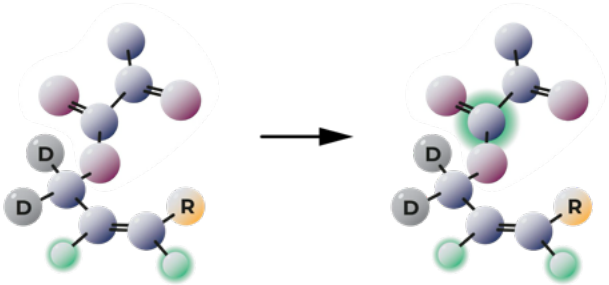
We use Low-field NMR as an efficient and scalable polarization transfer method



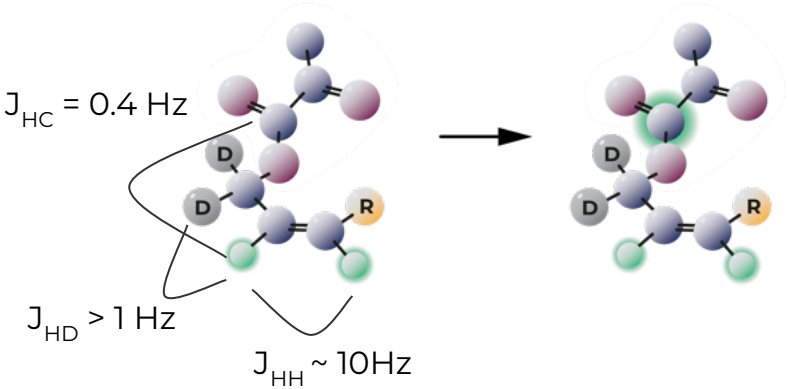


Static field is 50 μT. Oscillating field < 2 μT

For optimal polarization, we need to efficiently transfer the spin order from the hydrogenated protons (in the singlet state) to polarization on the highlighted carbon



However, the couplings between the 5-spin network (2 hydrogens, 2 deuterium, 1 carbon) involved in the polarization transfer are challenging



Challenges in polarization transfer

- J-coupling between hydrogen and 13C very weak (J_{HC} = 0.4 Hz), much weaker than the coupling between the hydrogenated protons spins J_{HH} ~ 10Hz
- Relatively strong J-coupling to fast relaxing deuterium (J_{HD} > 1 Hz)
- Clinical scale magnetic (B_0) and RF (B_1) inhomogeneities are substantial, significantly reducing transfer efficiency with current quantum control sequences

We recently realized a full Hamiltonian equivalence between pulsed-DNP (used for NV centers) and chemically-equivalent PHIP, enabling access to new powerful quantum control sequences

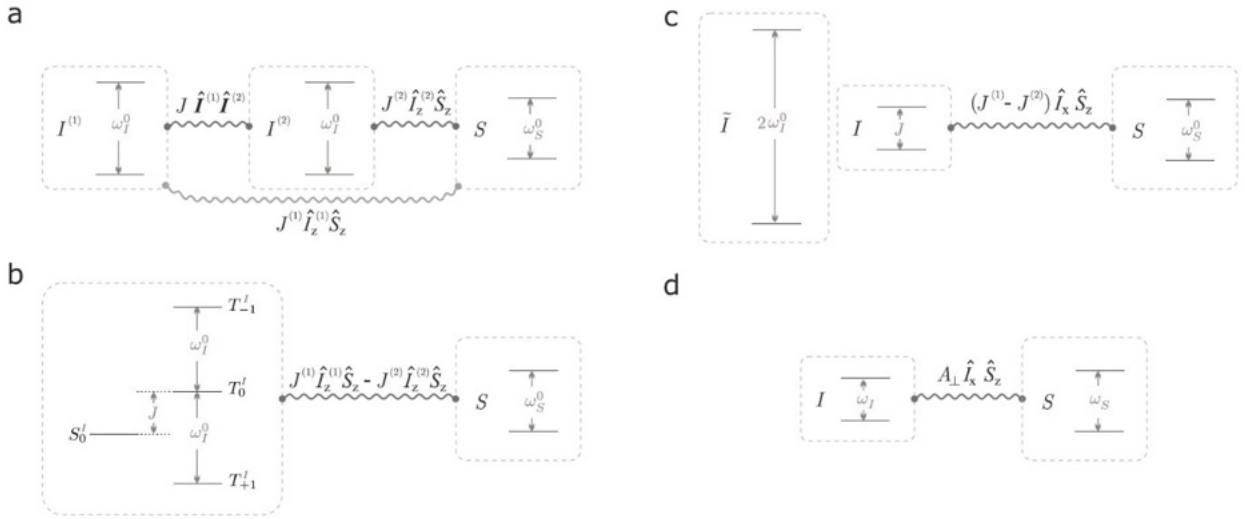
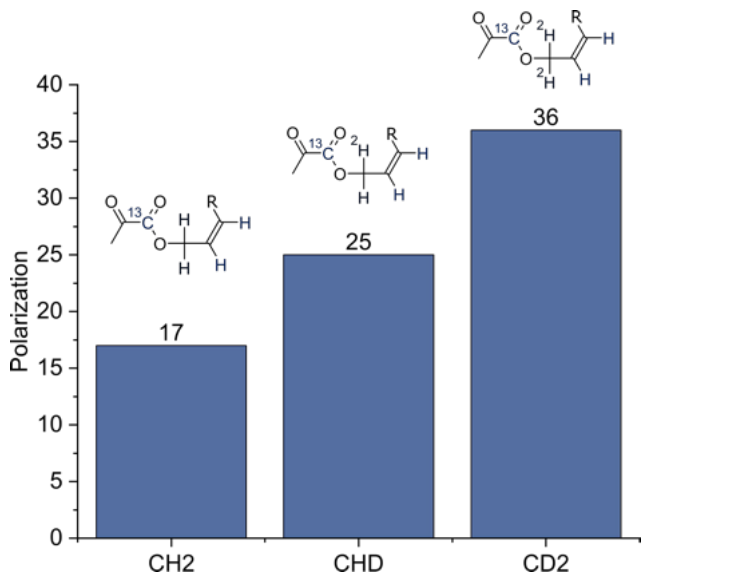


Fig. 1. Depiction of the spin system, energy levels and relevant Hamiltonian terms, demonstrating the mapping between DNP and PHIP. (a) the system of two hydrogen spins $I^{(1)}$, $I^{(2)}$ and one heteronuclear spin S. (b) The same system with the hydrogen spins $I^{(1)}$, $I^{(2)}$ in the singlet–triplet basis notation, as typically done in PHIP. (c) decomposing the hydrogen energy states into the interacting (I) and non-interacting ($\tilde{I}$) pseudospins. (d) Hamiltonian of nuclear spin and electron spin in typical DNP systems. From (c), (d) it becomes evident that the pseudospin formalism for PHIP has significant similarities to a DNP system.

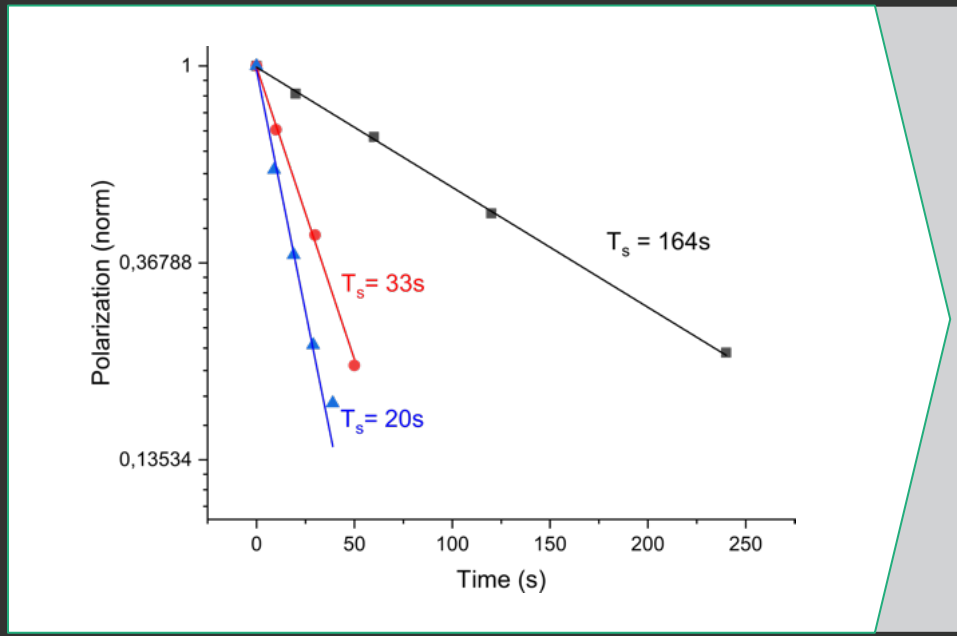
Korzeczek, Martin C., et al. *Journal of Magnetic Resonance* 362 (2024): 107671.

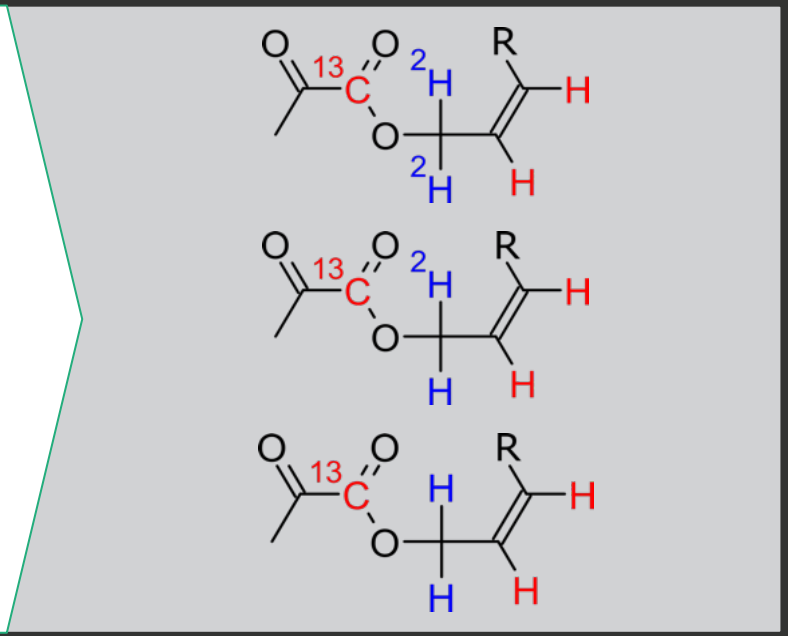
Pyruvate precursor design improves transfer efficiency



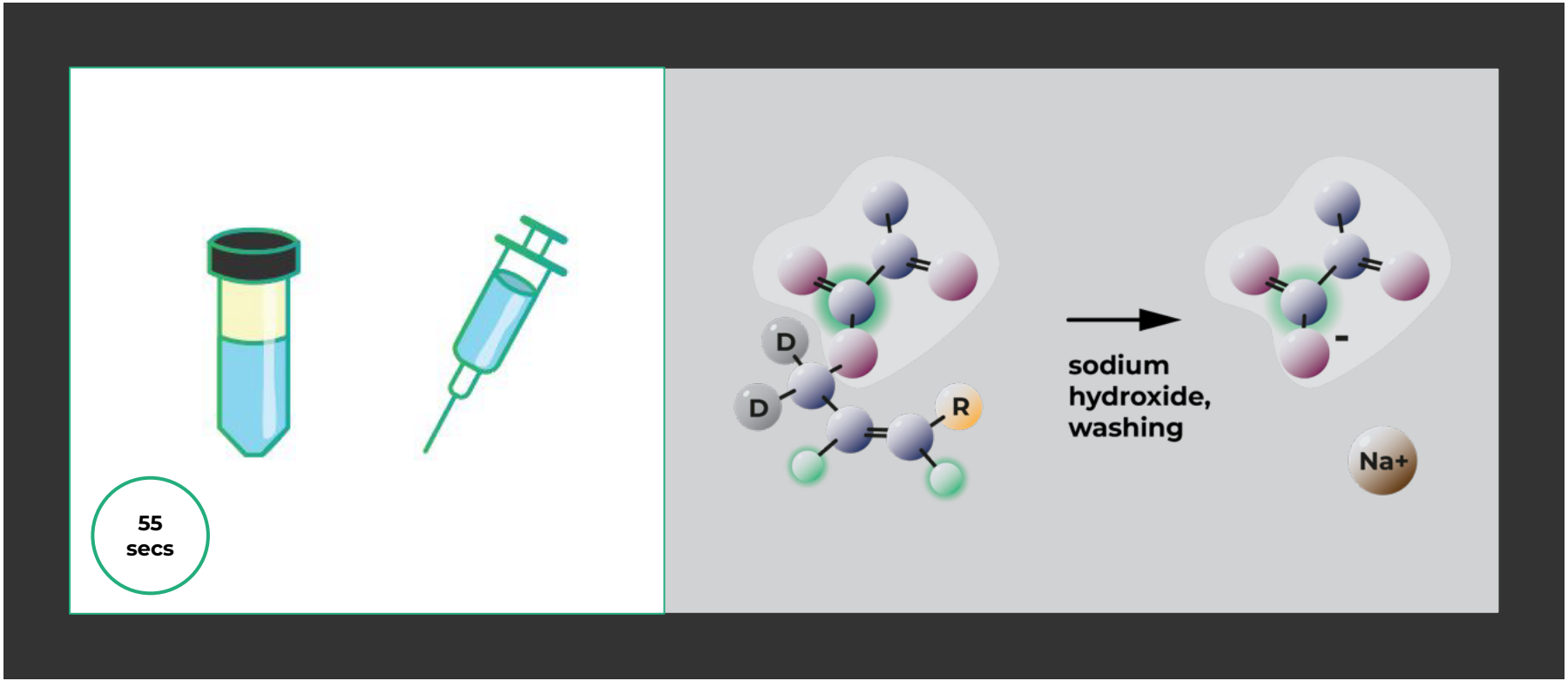
Design of partially deuterated molecules to increase polarization transfer efficiency

Deuteration improves relaxation times





Step 3: The polarized metabolite is separated from the contaminants and the sidearm in multistep process, resulting in a pure drug product



NVision's safety strategy includes regulated thresholds, optimized processes and toxicological studies - supported by certified players in the field

Safe process and thresholds in place

PERMITTED DAILY EXPOSURE

ICH based toxicological permitted daily exposure thresholds for organic, elemental and residual solvents impurities

ICH harmonisation

+

Toxicological studies by certified CRO

Bacterial reverse mutation (Ames) test and *in vitro* micronucleus test

Local lymph node assay in mice

High dosing study in rats, including safety margin of impurities, injected daily over 14 days

labcorp charles river

▶ **NVision provides these results in a CMC / non-clinical data package ready to be included in your IND submission**

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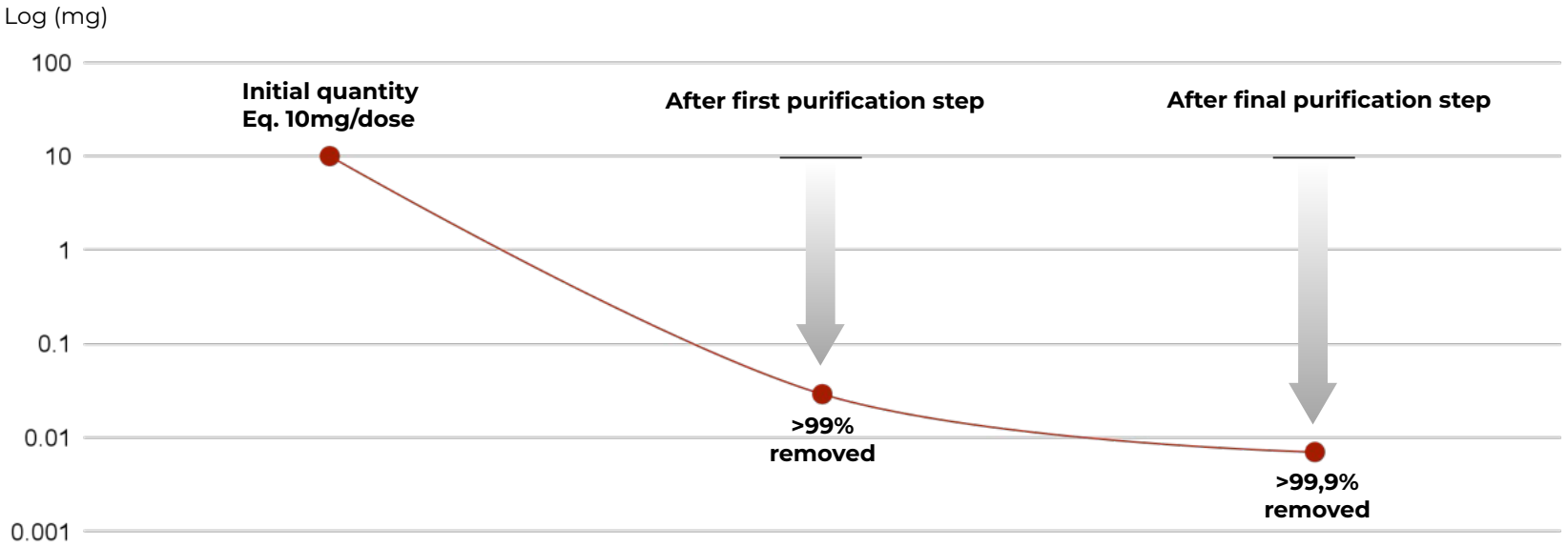
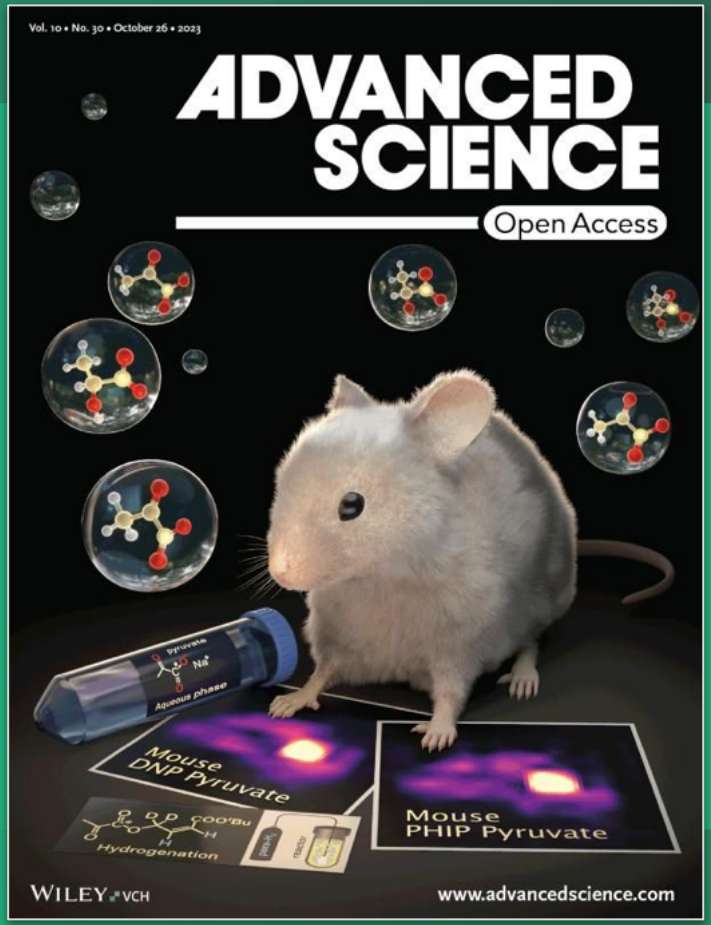
Residual Rhodium is below safe toxicological thresholds



(ICP-OES measurements)	Batch 1	Batch 2	Batch 3	Batch 4
Rhodium concentration [µg/dose]	8.82	5.38	10.11	5.16



RESEARCH ARTICLE

ADVANCED SCIENCE
www.advancedscience.com

Parahydrogen-Polarized [1-^{13}C]Pyruvate for Reliable and Fast Preclinical Metabolic Magnetic Resonance Imaging

Luca Nagel, Martin Gierse, Wolfgang Gottwald, Zumrud Ahmadova, Martin Grashei, Pascal Wolff, Felix Josten, Senay Karaali, Christoph A. Müller, Sebastian Lucas, Jochen Scheuer, Christoph Müller, John Blanchard, Geoffrey J. Topping, Andre Wendlinger, Nadine Setzer, Sandra Sühnel, Jonas Handwerker, Christophoros Vassiliou, Frits H.A. van Heijster, Stephan Knecht, Michael Keim,* Franz Schilling,* and Ilai Schwartz**

Hyperpolarization techniques increase nuclear spin polarization by more than four orders of magnitude, enabling metabolic MRI. Even though hyperpolarization has shown clear value in clinical studies, the complexity, cost and slowness of current equipment limits its widespread use. Here, a polarization procedure of [1-^{13}C]pyruvate based on parahydrogen-induced polarization by side-arm hydrogenation (PHIP-SAH) in an automated polarizer is demonstrated. It is benchmarked in a study with 48 animals against a commercial dissolution dynamic nuclear polarization (d-DNP) device. Purified, concentrated (≈70–160 mM) and highly hyperpolarized (≈18%) solutions of pyruvate are obtained at physiological pH for volumes up to 2 mL within 85 s in an automated process. The safety profile, image quality, as well as the quantitative perfusion and lactate-to-pyruvate ratios, are equivalent for PHIP and d-DNP, rendering PHIP a viable alternative to established hyperpolarization techniques.

1. Introduction

Since its introduction in 1973, magnetic resonance imaging (MRI) has provided non-invasive insights into living organisms with high soft-tissue contrast by means of low-energy radiofrequency fields.[1] Imaging physiological functions and microstructure continues to be a major motivation for innovation in the field and has resulted in a plethora of technologies and techniques that have reached clinical applications, including functional MRI (fMRI),[2] diffusion-weighted imaging (DWI),[3] and dynamic contrast-enhanced (DCE)-MRI.[4] A unique capability of magnetic resonance is the ability to assess molecular composition of tissue, using differences in the local magnetic fields experienced by nuclear spins, generating a difference in resonance frequency, also known as chemical shift. This enables liquid-state NMR spectroscopy techniques that are routinely used in various fields of chemistry, but have not yet been exploited for routine diagnostics in the clinic. This is partly due to the low sensitivity of NMR, resulting from the intrinsic small nuclear spin polarization at thermal equilibrium at clinically achievable field strengths, which prohibits molecular imaging at sufficient resolution.[5] ^{1}H-MR spectroscopy suffers from long acquisition times and crowded spectra due to the relatively small spectral range, an abundance of different ^{1}H-nuclei and strong *J*-coupling. Conversely, positron emission tomography (PET) offers very high sensitivity, but involves patient exposure to potentially harmful ionizing radiation and cannot directly distinguish different molecules and

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/advs.202303441

DOI: 10.1002/advs.202303441

Recent results published in Advanced Science

NVISION

We’re reaching 20% 13C-pyruvate polarization at injection

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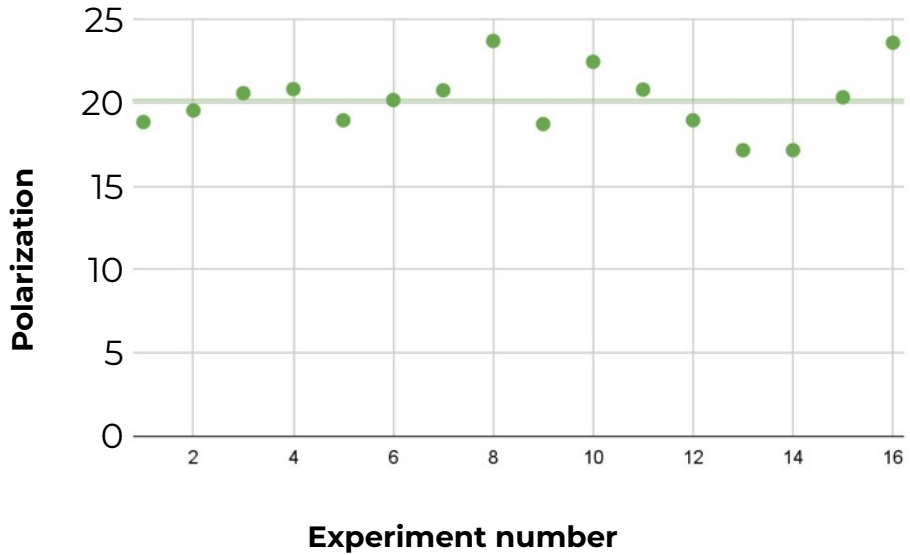

Concentration: 75 +- 8 mM
Stable physiological pH values for all samples

Reproducible 20% polarization levels of purified pyruvate

Effectiveness of HP-pyruvate produced by PHIP

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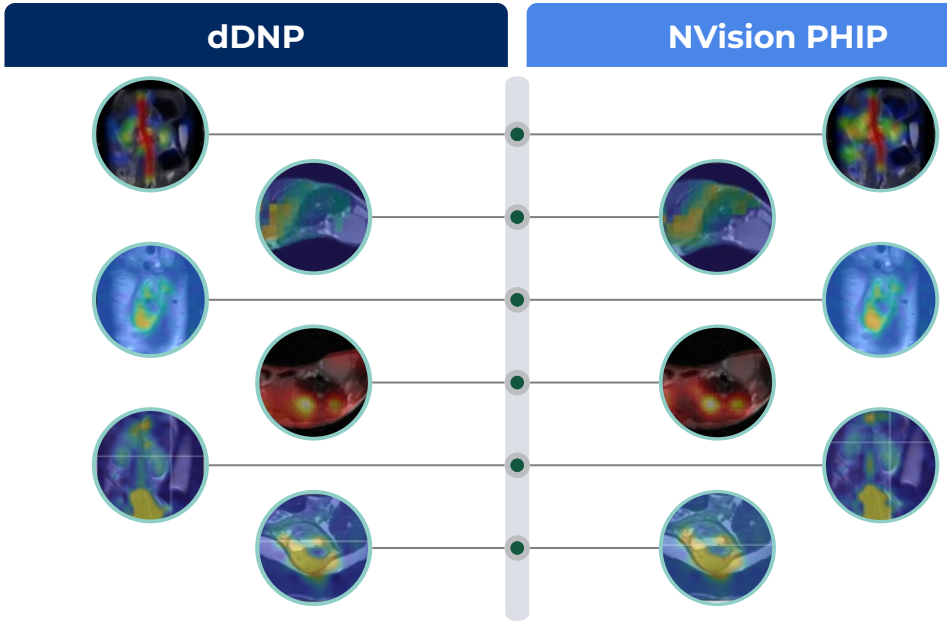

Average polarization (%)

dDNP	NVision PHIP
25.9 +- 2.7	20.2 +- 1.9

Comparable % polarization between dDNP and PHIP

Yielding the desired metabolic data and images

Comparison of PHIP / d-DNP imaging in animal tumor model

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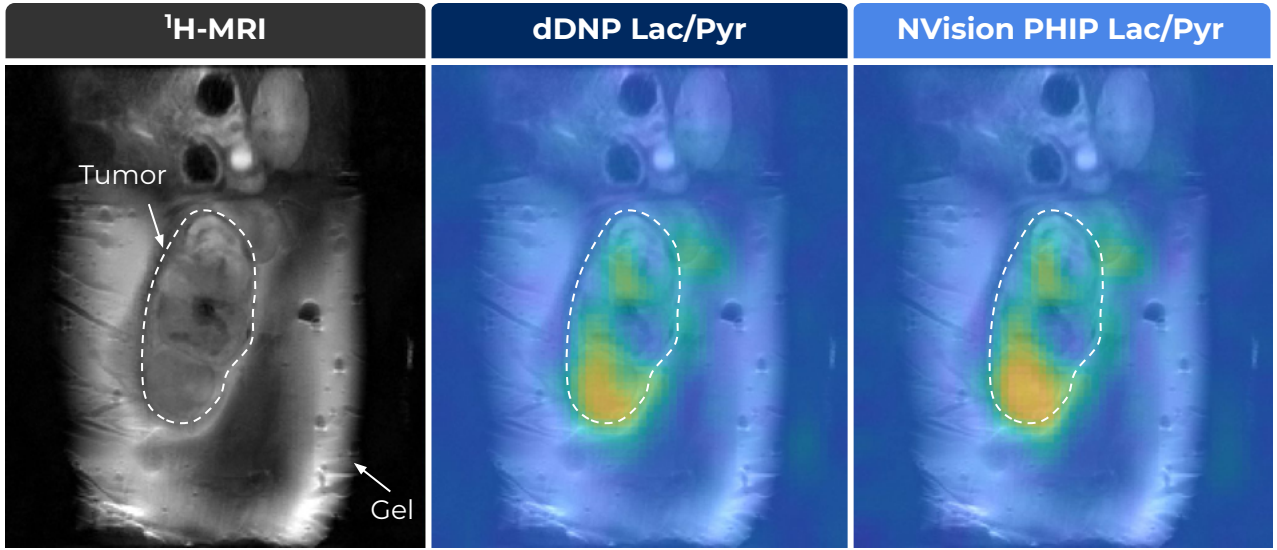

Left	Coronal slice of a subcutaneous MATBIII breast cancer model (rat) surrounded by gel for B_0 homogeneity.
Center	Lactate/pyruvate ratio measured from 3D dynamic bSSFP after injection of d-DNP pyruvate.
Right	Lactate/pyruvate ratio after injection of PHIP pyruvate (same location, same sequence).

Comparable imaging of metabolism in tumor model

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Luca Nagel, Department of Nuclear Medicine, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany

Dynamic metabolic information and metabolic imaging in a animal tumor model

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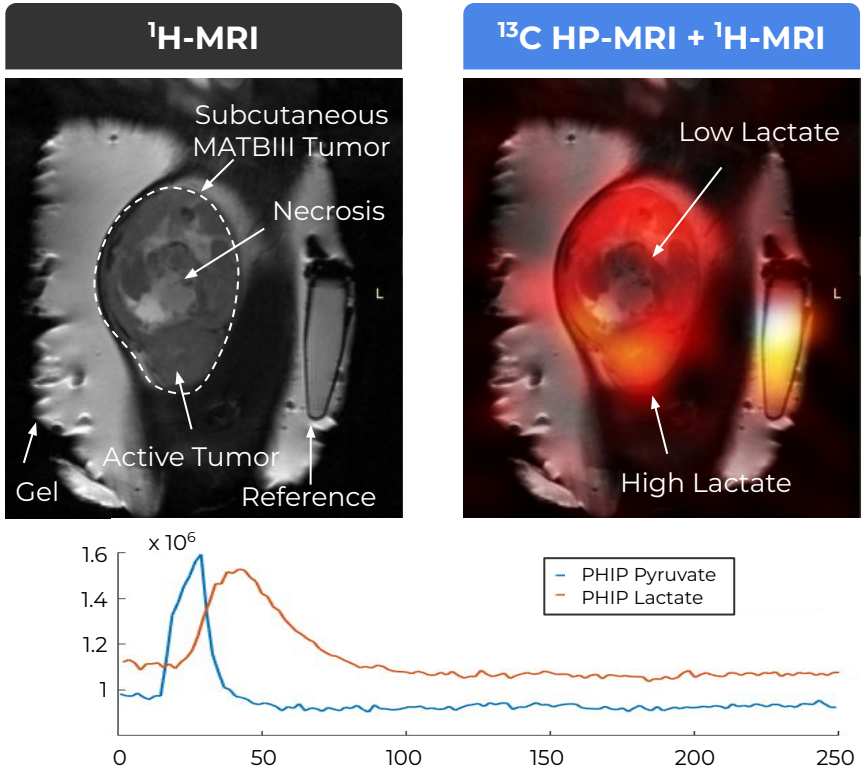
13C-HP MR Imaging (lactate)

Dynamic metabolic data



Results of tumor metabolism assessment as expected

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Top Left: A coronal slice of a subcutaneos MATBIII breast cancer model (rat) surrounded by gel.
Top right: Local distribution of lactate signals at the same slice (measured via 3D dynamic bSSFP).
Bottom: Extracted total pyruvate and lactate signal intensities as function of time after injection.

PHIP process speed enabled 4 injections in 1 hour in the same rat, revealing consistent metabolic behaviour over 1 hour

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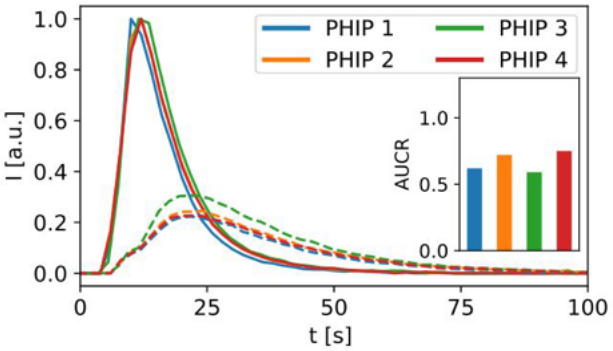
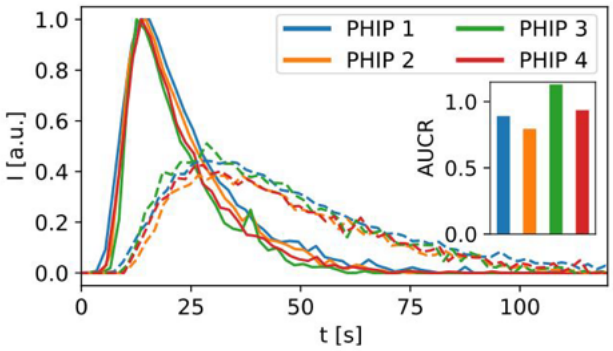
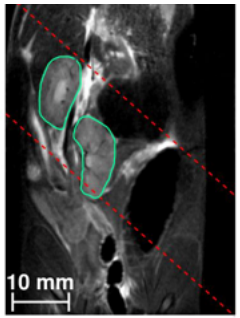
Pyruvate and lactate dynamics

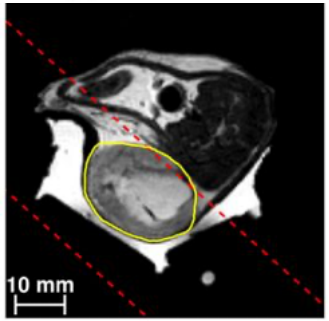




Healthy rat



Subcutaneous MATBIII breast cancer tumor rat model



New applications enabled by fast dose production

NVISION

Pyruvate peaks normalized to 1; **Wolfgang Gottwald**, Department of Nuclear Medicine, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany

Safety of HP-pyruvate produced by PHIP

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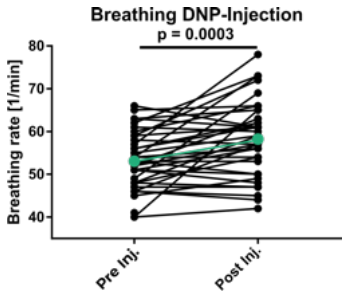
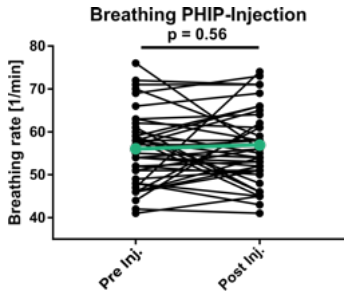
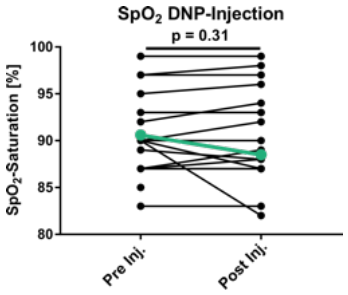
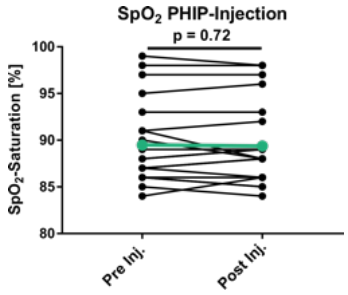
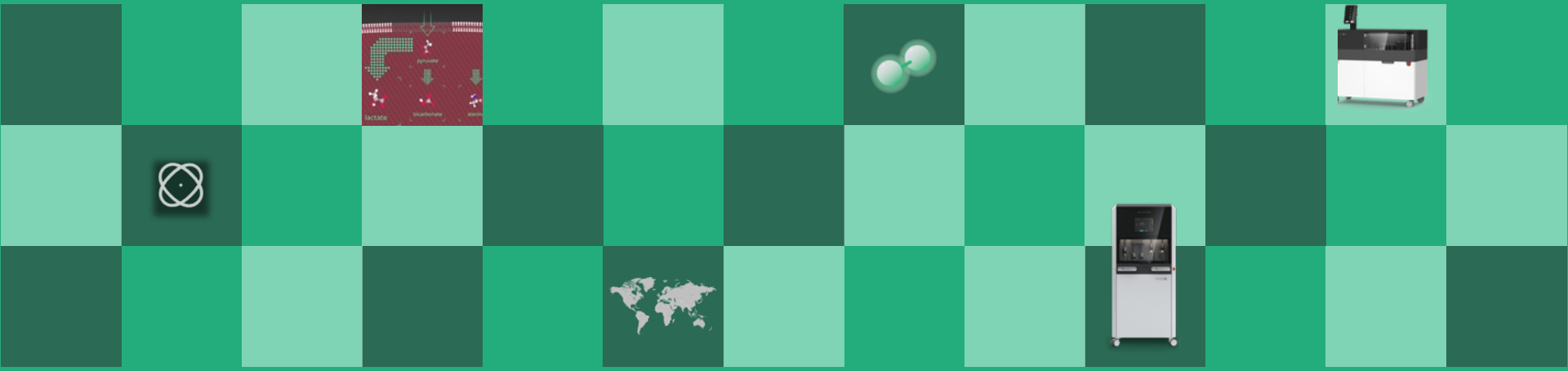
Technische Universität München

	dDNP	NVision PHIP
Breathing rate		
SpO_2 saturation		

Animals stable post injection with stable breathing rate and SpO_2 levels

Our RoadMap



NVISION

NVision product roadmap

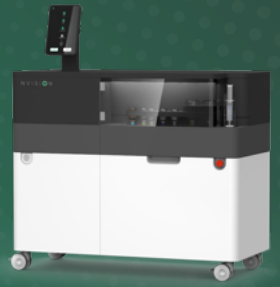
Research system
- Fully automated
- Integrated purification

2023

First customer delivery in 2024

POLARIS PRECLINICAL
- Preclinical volumes
- Research grade

2024



POLARIS CLINICAL
- Clinical volumes & purity
- cGMP grade

2025

NVISION

A first glimpse ...

NVISION

NVision provides an end-to-end product

POLARIZER

Active ingredients[1]
Ready to use ^{13}C-pyruvate precursor and catalyst

Para-hydrogen cylinders[1]
Compliant to medical gases requirements

Off the shelf reagents[2]
MTBE, Acetone-D6, D20, NaOH and phosphate buffer

CONSUMABLES

Service

Maintenance

Training

Consulting
(regulatory, quality, scientific)

Research collaborations

SUPPORT

NVISION

1 Supplied by NVision
2 NVision provides full “shopping list” and can support in the purchasing process

Preclinical kits

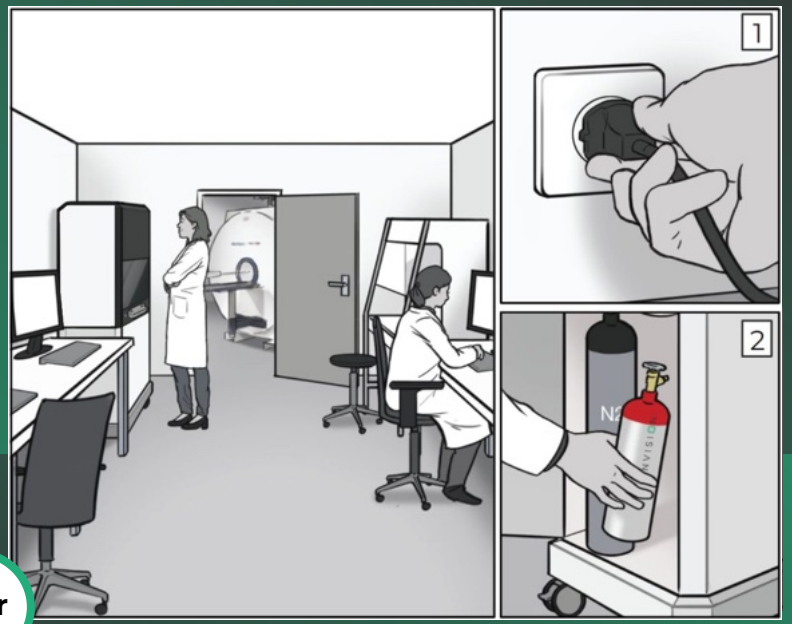
Kit box including 20 doses (3 vials each)

Cylinders of paraH2

Installation: The NVision polarizer is small and mobile, installation can be done in less than one day

Wheeling the polarizer to the lab

Setup in 2 simple steps



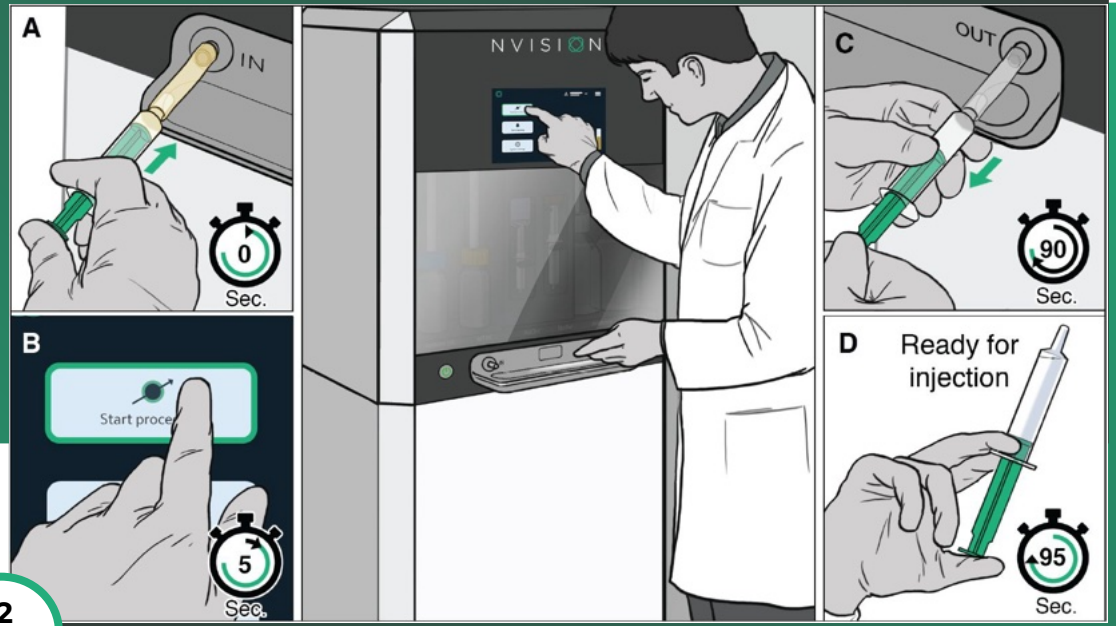
< 1 hr

Production of a dose: Easy and requires minimal preparation

Pre-mixing precursor, catalyst and acetone-d6 in advance

< 1 min

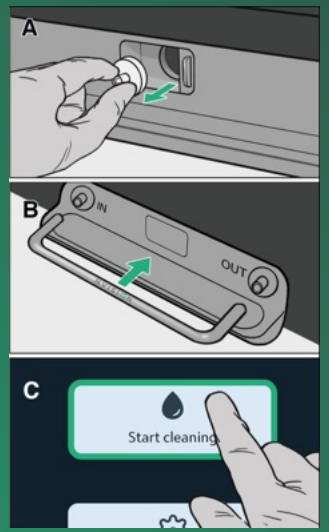
Dose production in 3 simple short steps



< 2 min

Cleaning between doses: Semi-automated and takes < 10 minutes

Cleaning after a dose, minimal manual manipulation required

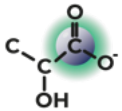
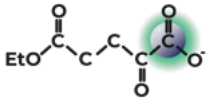
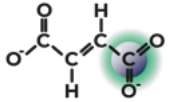


Replacing filter

< 10 min

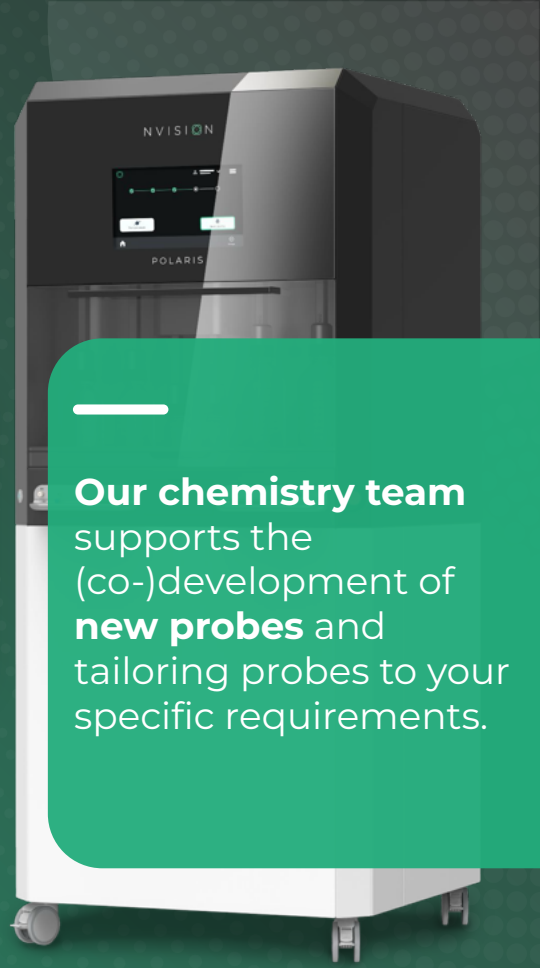
Ready for next dose...

POLARIS provides strong pipeline of probes beyond pyruvate - and NVision is continuing to expand its pipeline according to customer needs

Pyruvate	Lactate	α-Ketoglutarate	Fumarate
			

Proof-of-concept successfully demonstrated:

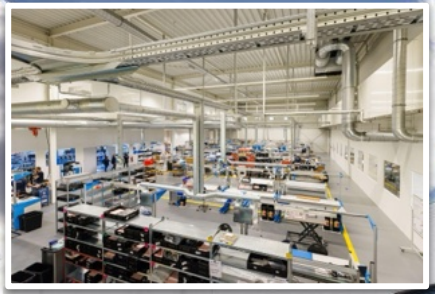
- Z-OMPD
- Glutamine
- Acetate
- Alanine
- [2-13C] Pyruvate
- Glutamate
- Acetoacetic acid



Our chemistry team supports the (co-)development of **new probes** and tailoring probes to your specific requirements.

NVISION

Productization and manufacturing of the polarizers by our partner DEMCON – specialist for medical and lab equipment



GMP / ISO manufacturing

ISO9001:2015 – quality management

ISO13485:2016 – medical devices – quality mgmt

ISO45001:2018 – occupational health & safety mgmt

1993 established

1000 employees

25 Demcon companies

A first glimpse ...

NVISION

NVision product roadmap

Research system
- Fully automated
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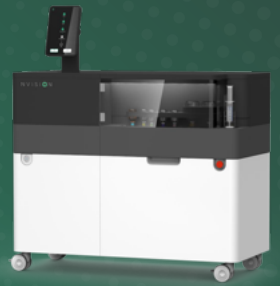
2023

POLARIS PRECLINICAL
- Preclinical volumes
- Research grade

2024



POLARIS CLINICAL
- Clinical volumes & purity
- cGMP grade

2025

NVISION

NVision's clinical polarizer POLARIS Clinical offers a simple workflow with ready-to-use kits and no need for a clean room

Ready to use kits

Kit features:

- Ready-to-use, single-use
- No manual compounding
- No clean room needed

QUALITY CONTROL (QC)

QC features:

- Semi-automated and fast
- Integrated automated filter integrity test

Efficient and safe clinical use with high throughput, reliability and ease of use

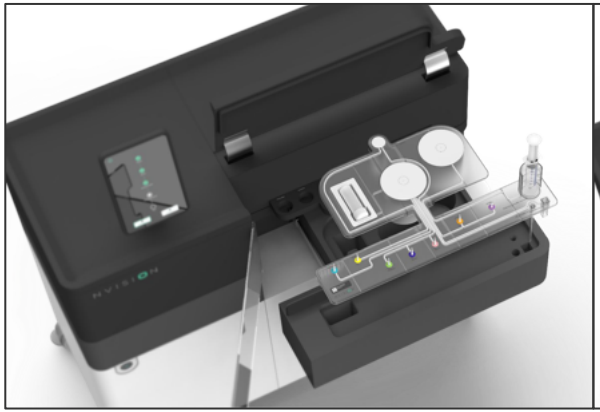
NVISION

Production of a dose: Easy and requires minimal preparation

Step 1: Load sterile cassette

Open drawer

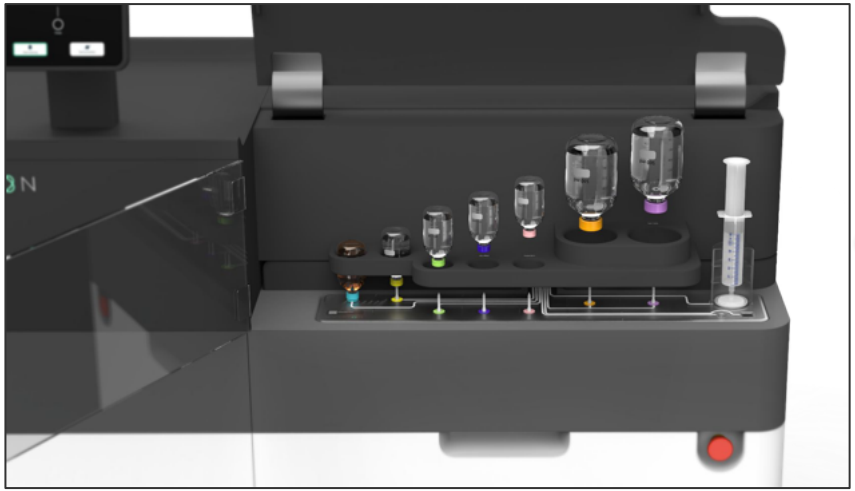
Insert cassette



Production of a dose: Easy and requires minimal preparation

Step 2: Load reagents

Close the drawer and spike reagents on cassette



Production of a dose: Easy and requires minimal preparation

Step 3: Start the automated process

Start the polarization process by the push of a button ...

After 2 minutes extract the polarized dose...

Pivotal industrial and research collaborations

Collaboration agreement with Siemens Healthineers

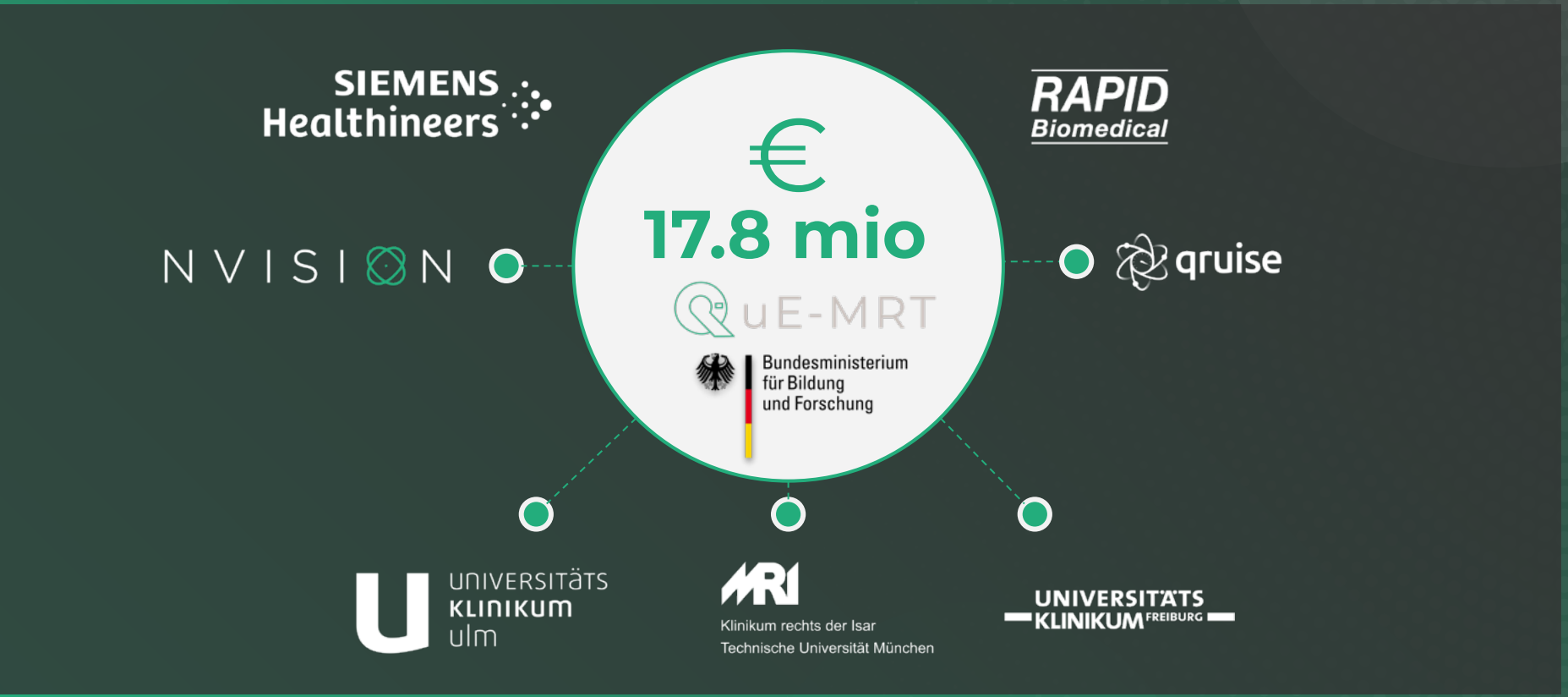
NVISION

SIEMENS Healthineers

Collaboration to bring NVision's polarizers to Siemens centers, initiated in 2022

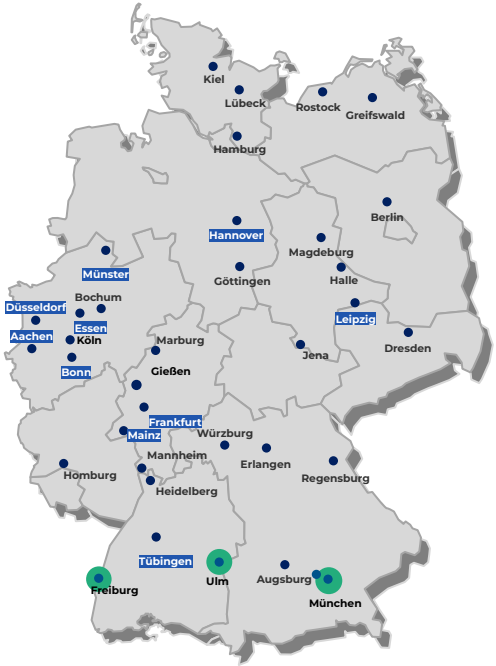
Pivotal industrial and research collaborations

In Germany, BMBF-funded “QuE-MRT” project running full steam with key partners



https://www.quantentechnologien.de/forschung/foerderung/leuchtturmprojekte-der-quantenbasierten-messtechnik/que-mrt.html

Upcoming clinical use in Germany



DFG Deutsche Forschungsgemeinschaft

Funding for new generation of polarizers made available

>12 sites applied for a polarizer

JÜLICH Forschungszentrum
Universitätsklinikum Leipzig
UNIVERSITÄTSmedizin.
Universitätsmedizin Essen
UKM
MHH Medizinische Hochschule Hannover
UNIVERSITÄTS KLINIKUM ulm
GOETHE UNIVERSITÄT FRANKFURT AM MAIN
UNIVERSITÄTS KLINIKUM
MRI Klinikum rechts der Isar Technische Universität München
Universitätsklinikum Tübingen
universitäts klinikum bonn

THANK YOU

THANK YOU

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NVISION