Biochemical phosphates observed using hyperpolarized $^{31}$P in physiological aqueous solutions

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The dissolution-dynamic nuclear polarization technology had previously enabled nuclear magnetic resonance detection of various nuclei in a hyperpolarized state. Here, we show the hyperpolarization of $^{31}$P nuclei in important biological phosphates (inorganic phosphate and phosphocreatine) in aqueous solutions. The hyperpolarized inorganic phosphate showed an enhancement factor $>11,000$ (at 5.8 T, 9.3% polarization) in D$_2$O ($T_1$ 29.4 s). Deuteration and the solution composition and pH all affected the lifetime of the hyperpolarized state. This capability opens up avenues for real-time monitoring of phosphate metabolism, distribution, and pH sensing in the live body without ionizing radiation. Immediate changes in the microenvironment pH have been detected here in a cell-free system via the chemical shift of hyperpolarized inorganic phosphate. Because the $^{31}$P nucleus is 100% naturally abundant, future studies on hyperpolarized phosphates will not require expensive isotope labeling as is usually required for hyperpolarization of other substrates.
Recent advances in the dissolution-dynamic nuclear polarization (d-DNP) and para-hydrogen-induced polarization (PHIP)-based hyperpolarization techniques have revolutionized the field of liquid-state nuclear magnetic resonance (NMR) spectroscopy. By providing signal enhancements of up to four orders of magnitude, such methods enable the detection of small amounts of materials as well as detections made on very short time scales. The d-DNP technology, pioneered by Ardenkjaer-Larsen et al., has been predominantly focused on metabolic work using hyperpolarized $^{13}$C sites. A few studies looked at metabolites labeled with $^{15}$N. In the field of contrast material, hyperpolarized yttrium showed beneficial relaxation properties and water protons were also hyperpolarized in solution.

PHIP is a method of creating highly polarized nuclear spins in solution utilizing the spin order of para-hydrogen. The polarization transfer occurs following a hydrogenation reaction of an unsaturated molecular precursor with the para-hydrogen molecule. A variant of the PHIP approach uses a reversible interaction with the para-hydrogen molecule and is termed signal amplification by reversible exchange (SABRE). Both techniques offer orders of magnitude increase in the NMR signal for multinuclei. To date, we have seen a couple of reports of hyperpolarized $^{31}$P in solution—done by the SABRE method. However, the $^{31}$P hyperpolarization achieved in these studies was of molecules that are not water soluble and do not present any biomedical relevance.

Phosphate-containing compounds are of key importance in biology and medicine and the 100% natural abundance of their NMR-active $^{31}$P nucleus make them affordable, attractive targets. Here, we show that observation of hyperpolarized $^{31}$P in important phosphate-containing molecules is indeed possible with the d-DNP methodology under physiologically relevant conditions. We present a potential application of this capability for fast pH sensing. We note that there are numerous important phosphate-containing metabolites in biology and medicine. Further research into their metabolism and bodily distribution is likely to benefit from the approach described herein.

**Results**

Inorganic phosphate (Pi) is observed in a hyperpolarized state in aqueous media. To explore the hyperpolarized state of Pi in solution, the following formulation was prepared: 120.1 µmol KH$_2$PO$_4$, 15.1 µmol OXXO63 radical, 5.4 mmol glycerol, and 33.1 mmol H$_2$O. A vitriolate buffer was placed in a sample cup and polarized for 1.5–3 h with a microwave frequency of 94.097 GHz, a power of 100 mW, and a temperature of 1.46 K at a 3.35 T magnetic field using a d-DNP setup. Then the formulation was quickly dissolved in 4 ml of aqueous media at a temperature of 170 °C and a pressure of 10 bar. The dissolved hyperpolarized solution was directly injected to a 10-mm NMR tube in a 5.8 T NMR spectrometer and $^{31}$P spectra were continuously recorded. The hyperpolarized signals were recorded starting about 16 s after the start of the dissolution process. When dissolved in D$_2$O, the Pi signal was clearly visible for about 50 s across 50 scans, each acquired with a 10° nutation angle. The $T_1$ of this hyperpolarized site was about 15 s (Fig. 1).

**Fig. 1** Summary of $T_1$ relaxation time constants for hyperpolarized phosphates in aqueous media. The average value of $n$ measurements is given and the error bars represent the standard deviation. The hyperpolarized decay curves were recorded at room temperature. The concentration of the hyperpolarized phosphate, the pH, and the number of measurements for each condition was as follows: KH$_2$PO$_4$ in D$_2$O: 0.94 ± 0.14 mM, pH 5.1, $n = 2$; KH$_2$PO$_4$ in saline and citrate-Tris buffer: 0.43 ± 0.07 mM, pH 4.5, $n = 2$; includes two experiments with N$_2$ bubbling; $K_H$PO$_4$ in D$_2$O: 1.80 ± 0.90 mM, pH 8.1, $n = 5$; $K_H$PO$_4$ in pure H$_2$O: 2.95 ± 0.03 mM (n = 3), 34.93 mM (n = 1); $K_H$PO$_4$ in physiological saline: 2.78 ± 0.00 mM, pH 7.4, $n = 2$; $PCr$ in D$_2$O with phosphate buffer: 120 ± 0.17 mM, pH 7.4, $n = 2$. Pi inorganic phosphate, $PCr$ phosphocreatine.
with water instead of D$_2$O. The dissolution medium consisted of 90% physiological saline and 10% D$_2$O with 100 mg/l EDTA, which were combined in the NMR tube with 5 ml of citrate-Tris buffer (altogether 300 mOsm and a pH of 4.5). Both the dissolution solvent and the citrate-Tris buffer were bubbled with N$_2$ for 1 h prior to use. The hyperpolarized Pi signal was again clearly visible but the T$_1$ appeared to be shortened to about 6 s (Fig. 1). This result could be expected as T$_1$ shortening by protonated solvents and by the addition of osmotic pressure to the solution was previously seen for other hyperpolarized compounds$^{23, 24}$. To check for possible effects of oxygen depletion, the experiment was repeated twice more in fresh solutions without N$_2$ bubbling. The resulting T$_1$ of the hyperpolarized Pi was similar at 6 s. Therefore, the results with and without N$_2$ bubbling were combined and are presented in Fig. 1.

A basic environment prolonged the T$_1$ of hyperpolarized Pi. We further explored the effect of pH of the solid-state formulation (and the resulting hyperpolarized media) on the production and maintenance of the hyperpolarized state. To this end we used K$_2$HPO$_4$ to prepare the formulation for polarization instead of KH$_2$PO$_4$, keeping all other conditions the same. When dissolved in D$_2$O, this formulation resulted in a pH of 8.1. This change led to an improvement in the ability to observe the hyperpolarized state of Pi, as the T$_1$ in solution increased to about 29 s (Fig. 1). Figure 2 demonstrates a typical experiment done at these conditions. The hyperpolarized signal of Pi in D$_2$O is clearly visible for more than 2 min across more than 120 acquisitions, each acquired with a 10° nutation angle. The thermal equilibrium spectrum of this sample is shown in Fig. 2b. The enhancement factor for the hyperpolarized Pi signal at these conditions was calculated to be 11,364 ± 438 (at 5.8 T, n = 2, 1.5 h of polarization), equivalent to 9.3 ± 0.3% polarization. In addition, we observed two other hyperpolarized signals downfield from Pi, likely corresponding to contaminations of the K$_2$HPO$_4$ batch.

The effect of water protons on the hyperpolarized Pi decay. To explore the effects of solvent (water) protonation in the basic condition, the experiment was repeated using pure water for dissolution. The T$_1$ of the hyperpolarized Pi decreased twofold to about 15 s (Fig. 1). This effect can be explained by inter-molecular dipolar interaction between the water protons and the hyperpolarized $^{31}$P nucleus. The effect of dissolution osmolarity was also tested (using physiological saline as the dissolution medium, ca. 300 mOsm), and the T$_1$ was found to be shortened slightly to about 13 s (Fig. 1). Despite this T$_1$ shortening effect of using water instead of D$_2$O and using iso-osmotic solutions, the T$_1$ of the basic formulation was still comparable to the T$_1$ of the acidic formulation in D$_2$O. We conclude that the basic formulation is preferential for preparing Pi solutions for hyperpolarization.

Media components affect the Pi T$_1$ relaxation rate too. Thus far we investigated the T$_1$ of Pi under varied solution conditions in a hyperpolarized state. These conditions included protonation of the solvent, mixture pH, and osmotic pressure. However, the T$_1$ of Pi could be sensitive to other medium components as well. To explore this aspect further we performed a preliminary investigation comparing two different types of media (water and citrate-Tris media). This investigation was carried out at thermal equilibrium and is summarized in Supplementary Note 3 and Supplementary Figs. 3 and 4. Briefly, we have found a surprising T$_1$ prolongation of the citrate-Tris media on the T$_1$ of Pi at the acidic to neutral pH values (1 ≤ pH and lower than 7). The T$_1$ values determined at thermal equilibrium (in both types of media) are in agreement with the T$_1$ values determined in the hyperpolarized state (which are summarized in Fig. 1).

Hyperpolarized Pi reports on instantaneous pH changes. The chemical shift of Pi is known to be sensitive to pH at the most physiologically relevant pH ranges$^{25}$ (Supplementary Note 2 and Supplementary Fig. 1). To illuminate an aspect of biomedical significance, we decided to demonstrate that hyperpolarized Pi can report on instantaneous pH changes. To this end we carried out a couple of experiments in which the hyperpolarized Pi signal underwent through varying and controlled pH regimes within
one hyperpolarization decay time and was recorded continuously. The first experiment is described below and the second is shown in Supplementary Note 4 and Supplementary Fig. 5. In the experiment depicted in Fig. 3, a basic formulation of Pi was hyperpolarized as described in the “Methods”. The hyperpolarized sample was dissolved in 4 ml of medical grade saline solution with 10% D_2O and immediately transferred from the d-DNP device to the NMR spectrometer. The pH of this solution composition was determined independently prior to the hyperpolarized experiment to be 8.1. About 9 s after the appearance of the hyperpolarized signal, the pH in the sample tube was quickly adjusted by means of a quick injection of 0.5 ml of citrate-Tris buffer of pH 4.5. In response, the continuously recorded 31P spectra show two spectra with broad lines attributed to air bubbles introduced during mixing and then the following spectra show the hyperpolarized 31P signal at a new chemical shift representing the new pH of 4.8, determined in the same sample immediately after the end of the hyperpolarized decay. This result demonstrates the potential for instantaneous pH sensing of aqueous solutions using hyperpolarized Pi. Since Pi is the ultimate pH sensor for biology, this result brings hope that hyperpolarized Pi can be used for clinical imaging of pH.

### 31P Hyperpolarization of Phosphocreatine (PCr) is Feasible.

PCr is a phosphate-containing molecule of key biochemical importance. Its main biochemical activity relates to the creatine kinase (CK) reaction, which serves to regenerate ATP in cells that rapidly consume ATP such as brain and muscle cells. To test the ability to observe the PCr phosphate in a hyperpolarized state, the following formulation was prepared: 25.4 µmol PCr, 1.8 µmol OXO63, 0.9 mmol glycerol, 3.6 mmol D_2O. About 40–80 mg of this formulation were polarized in the same conditions described above. Indeed, we were able to detect the hyperpolarized state of the 31P nucleus in the PCr molecule in aqueous media as well. Figure 4 demonstrates the hyperpolarized signal of PCr, visible for about 40 s, when dissolved in D_2O containing 50 mM phosphate buffer and 2 mg of the CK protein (not active at the time of measurement), at a final pH of 7.4. The T_1 of this hyperpolarized site was about 11 s (Fig. 1). The CK protein was added to the NMR tube to test the ability to observe not only the PCr phosphate in a hyperpolarized state but also in the presence of the enzyme that may bind it and therefore may affect the decay of the hyperpolarized state.

### Discussion

Magnetic resonance (MR) imaging provides the most exquisite anatomical soft tissue details and therefore contrast in medical imaging that relies on MR—and can be fused to conventional magnetic resonance imaging (MRI) images—is preferential. Hyperpolarized compounds have the potential to serve as molecular imaging probes on clinical imaging and to provide unique contrast mechanisms that will report on tissue functionality without ionizing radiation. Here, we capitalized on the d-DNP technique to create an unexplored type of molecular imaging probes for MR, i.e., hyperpolarized phosphates. We demonstrated the ability to hyperpolarize the 31P nucleus in Pi and PCr, which warrants research into many more endogenous phosphates.

We note that a key factor in the success of hyperpolarization of phosphates for use in solutions is a relatively long T_1 in solution. This requirement applies to any spin hyperpolarized MR molecular imaging probe. Indeed, Pi and PCr have a relatively long T_1 in aqueous solutions at 5.8 T compared to other phosphates; although in the d-DNP field these are considered among the shortest T_1 that have been amenable for investigation. A detailed investigation of 31P T_1 in aqueous media is provided in Supplementary Notes 1–3, Supplementary Table 1, and Supplementary Figs. 2–4. Therefore, as a first step in the development of further phosphate compounds for hyperpolarization it is important to characterize their T_1 time constants.

Another key factor for the utilization of hyperpolarized phosphates is the actual percent polarization achievable in...
solution. This polarization level is intimately dependent on the solution state $T_1$ of the particular nucleus in the specific molecule, solvent, mixture, temperature, pressure, and magnetic field throughout the path of the hyperpolarized media to the spectrometer and within it. For d-DNP studies, the polarization level in solution is primarily dependent on the polarization level achieved in the solid state, which is dependent on the formulation, microwave irradiation frequency, temperature, magnetic field, and the duration of polarization. Nevertheless, the following is a brief review of previously reported polarization levels in solution. We have demonstrated here $^{31}$P polarization levels of 9.3%. This polarization level is within the same order of magnitude of $^{13}$C polarization achieved on d-DNP studies (e.g., 5.5%26, 4.2%25, and 24%27) and is higher than the $^{31}$P polarization level achieved using para-hydrogen-based experiments (2.3%20, 0.3%21). Other examples of polarization levels previously documented in solutions are 1.3–9.1% for $^{13}$C24, and 0.00929 to 2% for $^1$H.

To demonstrate one potential biochemical application of hyperpolarized Pi, we carried out experiments in which the hyperpolarized Pi reported on the pH of the microenvironment in a rapid pH change process, with a temporal resolution of 1 s. pH sensing in intact tissues in living subjects is a long sought target with immediate diagnostic and therapeutic implications in various diseases and conditions.25 Pi has long been recognized as a promising probe for imaging tissue pH18, 25, as (1) its chemical shift is sensitive to changes of pH in the range of 6.4–7.6, which is the relevant physiological range (Supplementary Note 2 and Supplementary Fig. 1), (2) it is a non-toxic endogenous substrate present in high concentrations physiologically (0.3–1.1 mM in blood30), (3) it requires no expensive isotopic labeling as $^{31}$P is 100% naturally abundant, and (4) its gyromagnetic ratio is high ($\gamma_{31P}/\gamma_{1H} = 0.40$). However, the clinical relevance of Pi for pH sensing has been limited by two factors. First, the signal-to-noise ratio (SNR) of in vivo $^{31}$P spectra is low due to the relatively long $T_1$ relaxation time of Pi combined with the inherently low SNR ratio of thermal-equilibrium $^{31}$P MR, dictating acquisition times that are too long to be clinically useful. Second, previous studies have shown that the endogenous Pi signal appearing on in vivo $^{31}$P spectra predominantly originates from the extracellular space29, which is not a sensitive indicator of disease, unlike extracellular pH that has been shown to vary in a large number of important diseases and to be a marker of tissue changes in response to ischemia31, malignancy32, and metastasis33. The approach described herein surmounts these two obstacles: (1) the d-DNP method provides a very high SNR and thus allows detection of lower quantities (which can be translated to higher spatial and temporal resolution on imaging); (2) unlike endogenous Pi, the hyperpolarized Pi developed here could serve as a potential reporter of extracellular pH, as, depending on transport kinetics, it will likely be primarily located in the extracellular space during the short $T_1$ limited window of observation.

The following rough calculation is provided to give an estimate for the potential utility of Pi in vivo, considering the signal enhancement demonstrated herein. The concentration of Pi in the hyperpolarized media measured here was ca. 1 mM. We shall consider the case in which such a concentration is reasonably achieved in the tissue. The detected volume in our probe was about 1.3 ml. Let us consider a potential spatial resolution of 5 x 5 mm$^2$ and a slices thickness of 5 mm. This results in voxels with a volume of 125 mm$^3$. Thus, in the volume detected in the current experiment (1.3 ml), there are about 10 such potential voxels. All of the hyperpolarized studies presented here show an SNR of more than 100 (per 1 mM), even after ca. 15–20 s that would be required for circulation through the vasculature. Therefore, it appears that an in vivo observation is warranted with a potential SNR higher than 10 (per 1 mM per 5 x 5 x 5 mm$^3$ voxels). Many more factors should be taken into account: one factor that will increase the SNR on imaging is the fact that one actually excites each voxel many times, depending on the acquisition sequence, thereby increasing the SNR of each voxel correspondingly. Factors that may reduce the potential signal are (1) a reduced sensitivity of the in vivo radio frequency (RF) coils in a pre-clinical MRI compared to the high resolution NMR spectrometer and 10-mm probe used here, and (2) possible $T_1$ shortening by blood and tissues. The sensitivity of RF coils can be optimized and should be checked back-to-back with the relevant coils. The main shortening effect in blood and tissues is likely due to oxygen. In our hands, oxygen did not dramatically shorten the $T_1$ of hyperpolarized Pi (Supplementary Note 5). In short, in vivo experimentation is warranted keeping in mind that each part of the detection system (sequences and hardware) should be optimized for the detection of hyperpolarized $^{31}$P nuclei.

We note that NMR chemical shifts are relative properties and therefore a chemical shift standard would be required for hyperpolarized Pi to actually serve as such a reporter on tissue pH. However, the SNR of the hyperpolarized Pi is much larger than the thermal equilibrium SNR meaning that other endogenous phosphates, which could potentially serve as references for chemical shift, are unlikely to be observed simultaneously with the hyperpolarized Pi. One potential solution to this problem would be to generate a map of relative differences of all of the observed chemical shifts. Another potential solution would be to combine two agents with different chemical shifts and different chemical shift pH sensitivities, one could accurately determine the pH of the microenvironment.

We note that using the d-DNP technique1, it was previously shown that detection of hyperpolarized bicarbonate and carbon dioxide provides a measure of tissue pH12, 33. However, the particular tissue compartment inspected in this way is inherently dependent on the hyperpolarized substrate administered to the subject (pyruvic acid32 or bicarbonate12), and the kinetics of membrane transport and metabolism in the specific tissue inspected31.

Here, we propose that hyperpolarized Pi could serve as a robust pH sensor for tissue pH. The data suggest that hyperpolarized Pi could be imaged with high SNR on a time scale of seconds (calculation above). In addition, co-polarization and dissolution of Pi with PCr can provide an accurate internal standard for chemical shift.

The solid-state polarization, and therefore the SNR of hyperpolarized phosphates in solution, could likely be improved by further formulation trials. However, we note that in the current state of the art of d-DNP instruments, it is not possible to monitor the solid-state build-up of $^{31}$P polarization. This makes formulation development for $^{31}$P DNP cumbersome and costly as the polarization build-up can only be monitored after dissolution of the hyperpolarized sample. Further studies are required to robustly characterize the solid-state build-up time constants of Pi and PCr and the factors that may affect them. Such studies are currently underway in our laboratory.

In summary, we have observed here a DNP-driven hyperpolarized state of $^{31}$P in aqueous solutions. Using this technology, this state was observed in two biochemically important molecules, Pi and PCr. Factors such as solvent de-protonation, a basic pH, and media composition increased the
T_1 of Pi and therefore also increased the time duration at which this signal could be observed. While the comparably short T_1 remains a challenge, this work warrants further research in other important phosphate-containing biological molecules that could potentially shed light on fundamental biochemical and physiological processes.

**Methods**

**Chemicals.** K_2HPO_4, KH_2PO_4, and PCr (as diosodium salt) were purchased from Sigma-Aldrich, Rehovot, Israel. The OXO63 radical (GE Healthcare, UK) was obtained from Oxford Instruments Molecular Biotools (Oxford, UK). The citrate-Tris medium used here contained 100 mM citrate, 10 mM KCl, and 95 mM TRIS, all purchased from Sigma-Aldrich.

**DNP spin polarization and dissolution.** Spin polarization and fast dissolution were carried out in a d-DNP set-up (HyperSense, Oxford Instruments Molecular Biotools, Oxford, UK). A microwave frequency of 94.097 GHz was chosen based on the work of Reynolds et al.\(^4\). In this report, a solid-state polarization of 10\(^{10}\) was achieved when the microwave irradiation frequency was set at 0.017 GHz below the microwave frequency required to achieve solid-state polarization of the 31\(^\text{P}\) nucleus of pyridic acid. In our spin polarizer, the latter frequency corresponds to 94.114 GHz.

The dissolution process was performed as previously described\(^1\) and detailed in the results section. Briefly, 40–120 mg of the 31\(^\text{P}\) containing formulation which were placed in a the polarization sample cup were quickly dissolved in 4 ml of superheated aqueous media (170 °C and 10 bar). The dissolved hyperpolarized solution was directly injected to a screw cup 10-mm NMR tube in a 5.8 T NMR superheated aqueous media (170 °C and 10 bar). The dissolved hyperpolarized NMR of C-13-enriched Y-89-DOTA complex: experimental and theoretical considerations. Appl. Magn. Reson. 43, 69–79 (2012).

Harris, T., Giradeau, P. & Frydman, L. Kinetics from indirectly detected hyperpolarized NMR spectroscopy by using spatially selective coherence transfer. J. Magn. Chem. 127, 621–624 (2007).

Lumata, L. et al. DNP by thermal mixing under optimized conditions yields >60,000-fold enhancement of Y-89 NMR signal. J. Am. Chem. Soc. 133, 8673–8680 (2011).

Lumata, L., Merritt, M., Malloy, C., Sherry, A. D. & Kovacs, Z. Fast dissolution dynamic nuclear polarization NMR of C-13-enriched Y-89-DOTA complex: experimental and theoretical considerations. Appl. Magn. Reson. 43, 69–79 (2012).

Harris, T., Szekely, O. & Frydman, L. On the potential of hyperpolarized water in biomolecular NMR studies. J. Phys. Chem. B 118, 3281–3290 (2014).

Lipso, K. W., Bowen, S., Rybakko, O. & Ardenkjaer-Larsen, J. H. Large dose hyperpolarized water with dissolution-DNP at high magnetic field. J. Magn. Reson. 274, 65–72 (2016).

Chappuis, Q. et al. Hyperpolarized water to study protein-ligand interactions. J. Phys. Chem. Lett. 6, 1674–1678 (2015).

McCardy, E. R., Armstrong, B. D., Lingwood, M. D. & Han, S. Hyperpolarized water as an authentic magnetic resonance imaging contrast agent. Proc. Natl. Acad. Sci. USA 104, 1754–1759 (2007).

Lumata, L., Merritt, M. E., Hashami, Z., Ratnakar, S. J. & Kovacs, Z. Production and NMR characterization of hyperpolarized 10\(^{10}\)Ag complexes. Angew. Chem. Int. Ed. 51, 525–527 (2012).

van Heeswijk, R. B. et al. Hyperpolarized lithium-6 as a sensor of nanomolar contrast agents. Magn. Reson. Med. 61, 1489–1493 (2009).

Balzan, R. et al. Hyperpolarized Li-6 as a probe for hemoglobin oxygenation level. Contrast Media Mol. Imaging 11, 41–46 (2016).

Atkins, T. M. et al. Synthesis of long T-1 silicon nanoparticles for hyperpolarized Si-29 magnetic resonance imaging. ACS Nano 7, 1609–1617 (2013).

Lee, Y., Zeng, H. F., Ruedisser, S., Gossel, A. D. & Hilny, C. Nuclear magnetic resonance of hyperpolarized fluorene for characterization of protein-ligand interactions. J. Am. Chem. Soc. 134, 17448–17451 (2012).

Reynolds, S. & Patel, H. Monitoring the solid-state polarization of C-13, N-15, H-2, Si-29 and P-31. Appl. Magn. Reson. 34, 495–508 (2008).

Andrew, E. R. & Gaspar, R. Mechanisms of 31P relaxation in phosphorus metabolites. Magn. Reson. Mater. Phys. 2, 421–423 (1994).

Gamilie, A. et al. An apparatus for production of isotopically and spin enriched hydrogen for induced polarization studies. Appl. Magn. Reson. 39 329–345 (2010).

Adams, R. W. et al. Reversible interactions with para-hydrogen enhance NMR sensitivity by polarization transfer. Science 322, 1708–1711 (2009).

Burns, M. J. et al. Improving the hyperpolarization of (31)P nuclei by synthetic design. J. Phys. Chem. B 119, 5020–5027 (2015).

Zhivonitko, V. V., Skovpin, I. V. & Koptyug, I. V. Strong P-31 nuclear spin hyperpolarization produced via reversible chemical interaction with para-hydrogen. Chem. Commun. 51, 2508–2509 (2015).

Karlsson, M., Jensen, P. R., Duus, J. O., Meier, S. & Lerche, M. H. Development of dissolution DNP-MSR substrates for metabolic research. Appl. Magn. Reson. 43, 223–236 (2012).

Allouche-Aron, H. et al. In vivo magnetic resonance imaging of glucose—initial experience. Contrast Media Mol. Imaging 8, 72–82 (2013).

Lingwood, M. D. et al. Hyperpolarized water as an MR imaging contrast agent: feasibility of in vivo imaging in a rat model. Radiology 265, 418–425 (2012).

Robitaille, P. M. L., Robitaille, P. A., Brown, G. G. & Brown, G. G. An analysis of the pH-dependent chemical-shift behavior of phosphorus-containing metabolites. J. Magn. Reson. 92, 73–84 (1991).

Kumagai, K. et al. Synthesis and hyperpolarized N-15 NMR studies of N-15-choline-d13. Tetrahedron 69, 3806–3900 (2013).

Allouche-Aron, H. et al. A hyperpolarized choline molecular probe for monitoring acetylcysteine synthesis. Contrast Media Mol. Imaging 6, 139–147 (2011).

Gillies, R. J., Raghunand, N., Garcia-Martin, M. L. & Gatenby, R. A. pH imaging. IEEE Eng. Med. Biol. Mag. 23, 57–64 (2004).

Hashmi, A. I., Zhang, X. M., Woytowick, J. W., Martinez, G. V. & Gilles, R. J. Imaging pH and metastasis. NMR Biomed. 24, 582–591 (2011).

Human Metabolome Data Base, http://www.hmdb.ca/metabolites/HMDB02142#concentrations and http://www.hmdb.ca/metabolites/HMDB01429#concentrations (Accessed July 2017).

Schroeder, M. A. et al. Measuring intracellular pH in the heart using hyperpolarized carbon dioxide and bicarbonate: a C-13 and P-31 magnetic resonance spectroscopy study. Cardiovasc. Res. 86, 82–91 (2010).

Day, S. E. et al. Detecting tumor response to treatment using hyperpolarized C-13 magnetic resonance imaging and spectroscopy. Nat. Med. 13, 1382–1387 (2007).

Gallagher, F. A., Kettunen, M. I. & Brindle, K. M. Imaging pH with hyperpolarized C-13. NMR Biomed. 24, 1006–1015 (2011).

Received: 7 February 2017 Accepted: 24 June 2017 Published online: 24 August 2017

**References**

1. Ardenkjaer-Larsen, J. H. et al. Increase in signal-to-noise ratio of > 10,000 times in liquid-state NMR. Proc. Natl. Acad. Sci. USA 100, 10158–10163 (2003).

2. Wilson, D. M. et al. Multi-compound polarization by DNP allows simultaneous assessment of multiple enzymatic activities in vivo. J. Magn. Reson. 205, 141–147 (2010).

3. Gabelli, C. et al. Therapeutic target metabolism observed using hyperpolarized N-15 choline. J. Am. Chem. Soc. 130, 4598 (2008).
34. Daly, P. F., Lyon, R. C., Straka, E. J. & Cohen, J. S. P-31-NMR spectroscopy of human cancer-cells proliferating in a basement-membrane gel. FASEB J. 2, 2596–2604 (1988).

35. Massiot, D. et al. Modelling one- and two-dimensional solid-state NMR spectra. Magn. Reson. Chem. 40, 70–76 (2002).

Acknowledgements
This study was supported by the European Research Council (ERC award number 338040 to R.K.B.).

Author contributions
A.G., A.N.-S., and R.K.-B. conceived ideas and designed the studies. R.K.-B., A.G., and A.N.-S. performed the experiments. A.N.-S., G.S., and T.H. performed the analyses. R.K.-B. directly supervised the study. J.S. and J.M.G. supported and supervised this study. All authors approved the final version of the manuscript.

Additional information
Supplementary Information accompanies this paper at doi:10.1038/s41467-017-00364-3.

Competing interests: The authors declare no competing financial interests.

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