Development of Hyperpolarized Metabolic Contrast Agents Using PASADENA

Eduard Y. Chekmenev,1,2 Pratip Bhattacharya,2 Brian D. Ross2
1. California Institute of Technology, Pasadena, CA 91125 USA
2. Huntington Medical Research Institutes, Pasadena, CA 91105 USA

Nuclear magnetic resonance (NMR) is a powerful method not only for high-resolution structure elucidation methods on atomic and molecular scales, but also for in vivo magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) in biomedicine. Although NMR has a number of benefits for in vivo applications being nonradioactive and nontoxic compared to other imaging modalities, such as positron emission tomography (PET) and computed tomography (CT), the main weakness of NMR method is its relatively low sensitivity compared to the above methods. Low sensitivity is largely determined by poor alignments of the nuclear spins, which contribute to the formation of the NMR signal in a static magnetic field $B_0$. For example, only 10 out of one million proton spins are aligned with respect to the applied magnetic field $B_0 = 3$ tesla (T) at room temperature (Figure 1).

Nuclear spin alignment is even worse for other biologically relevant nuclei with low gyromagnetic ratio, $\gamma$, such as $^{13}$C and $^{15}$N by four and ten times, respectively, compared to protons. In addition to worse nuclear alignment, NMR detection of low-$\gamma$ nuclei is further exacerbated by lower receptivity, as overall sensitivity usually scales with $\gamma$. Thus, $^{13}$C and $^{15}$N are rarely used for direct detection in clinical practice and biomedical in vivo research in general. However, low-$\gamma$ also yields a much longer spin-lattice relaxation time $T_1$ for $^{13}$C and $^{15}$N since the dominating mechanism of dipole-dipole relaxation results in $T_1$ being inversely proportional to $\gamma$. As a result, $T_1$ of $^{13}$C and especially $^{15}$N of some molecular sites can reach several minutes in vivo.

While conventional in vivo NMR, such long $T_1$ is prohibitively inconvenient for signal recording and signal averaging, it offers unique advantages and opportunities for NMR methods enhanced by hyperpolarization. The goal of hyperpolarization techniques is to increase the nuclear spin alignment from several parts per million (ppm) to the order of unity (Figure 1). Both dynamic nuclear polarization (DNP) and parahydrogen and synthesis allow dramatically enhanced nuclear alignment (PASADENA) have recently been demonstrated to reach spin polarization of order unity on $^{13}$C and $^{15}$N sites. This corresponds to a signal enhancement by a factor of ~100,000 on currently utilized MRI scanners. This dramatic signal enhancement brings the sensitivity of $^{13}$C and $^{15}$N to the realm of research and potentially clinical in vivo tools.

While DNP inherently requires expensive cryogenic equipment, a homogeneous high-field magnet and sample polarization for a few tens of minutes, PASADENA offers an economical advantage of low magnetic field of several milliteslas (mT) and ultrafast sample polarization in just seconds. However, the main shortcoming of PASADENA implementation in practice is the requirement for unsaturated alkene or alkene bonds of the molecular precursor that leads to hyperpolarized $^{13}$C or $^{15}$N molecules after molecular hydrogenation. This application note provides the reader with essential steps for preparation of novel hyperpolarized $^{13}$C molecules using PASADENA.

Parahydrogen is routinely produced by a passage of hydrogen (25% para and 75% ortho at room temperature) gas through a catalyst chilled to cryogenic temperatures of 5-20K under 20-40 atm, allowing fast conversion of ortho-para mixture of hydrogen gas to parahydrogen. Produced in this fashion, parahydrogen gas is collected and stored in an aluminum cylinder under 20-30 atm pressure for convenient storage, transportation and production of $^{13}$C hyperpolarized compounds. The parahydrogen quality is characterized by $^1H$ spectroscopy. Since parahydrogen is NMR silent, proton spectroscopy measures the concentration of orthohydrogen, which is then used to calculate the percentage of parahydrogen in the mixture. An example of such measurement is shown in Figure 2.
The spin order transfer sequence requires ~0.35 second, which takes 1-3 seconds for >99% conversion of the molecular precursor under conditions of pHs near the pKa value(s). For example, no signal could be further extended by deuteration of the aqueous medium used during molecular addition of parahydrogen and during the delivery of the hyperpolarized contrast agent in vivo. For example, $^{13}$C, T$_1$ can be extended from 27 seconds (in H$_2$O) to 56 seconds (in D$_2$O, >99%) in succinic acid at 4.7T.

Intermediate chemical exchange, observed as line-broadening (Figure 6), can potentially preclude accurate extraction of the J-couplings at pHs near the pKa value(s). Moreover, it can also make the spin-order transfer extremely inefficient for the target chemical compound at such pH conditions. For example, no signal enhancement for $^{13}$C was observed at pHn7 using a 1.8 mT magnetic field in the PASADENA polarizer. Therefore, transfer of spin order should be carried out at pH $<<$ pKa, where the J-couplings necessary for PASADENA are best resolved.

The entire procedure should be automated for routine application of the hyperpolarized metabolic contrast agents to allow reproducibility and quality assurance of PASADENA-based hyperpolarization for in vivo work.
A number of hyperpolarized molecules have already shown early evidence of utility in biomedical research. The best known metabolic agent, hyperpolarized $^{1,13}$C-pyruvic acid by DNP, measures the lactate dehydrogenase-catalyzed flux of $^{13}$C label between the carboxyl groups of pyruvate and lactate in the tumor using $^{13}$C magnetic resonance spectroscopy and spectroscopic imaging.\(^{16}\)

Nontoxic hyperpolarized $^{1,13}$C-succinate-$D_2$, is the most developed metabolic agent with routinely obtained polarization (P>18%) by PASADENA.\(^{13}\) It can potentially assess the in vivo activity of succinate dehydrogenase (SDH), the enzyme that was recently tagged as an oncogene due to its crucial role in cell energetics (Figure 7).\(^{19, 20}\) These mutations in SDH result in the increase of mitochondrial succinate pool that eventually leaks to cytosol, where it inhibits prolyl hydroxylase (PH) (Figure 7). The reduced level of prolyl hydroxylation stabilizes otherwise constantly depleting hypoxia-inducible factor (HIF)$_a$, causing pseudo-hypoxia and hypoxia-inducible factor induction allowing tumor proliferation by means of anaerobic glycolysis. As a result, the metabolic profile is different from non-mutated cells, and it could be potentially measured using hyperpolarized metabolic contrast agents such as succinate. Moreover, it is possible to test the potency of these $^{13}$C hyperpolarized agents by conventional $^{13}$C NMR spectroscopy in cellular models of cancer.

Our preliminary results shown in Figure 8 obtained in cell cultures demonstrate different metabolic profiles when $^{13}$C label of $1,4,13$C-succinate enters the TCA cycle in normal and cancer cells.\(^{21}\) The normal product of the TCA cycle bicarbonate is detected in both pancreatic cancer cells and non-cancer fibroblast. In addition, pancreatic cancer cell line spectrum exhibits enrichment of glutamate and citrate. Based on the direct link between TCA cycle dysfunction due to impaired SDH transmembrane assembly (Figure 7), it will therefore be possible to provide metabolic markers for dysfunctional SDH complex, thereby providing an opportunity to detect genetic mutations in action. Specifically, it is anticipated that ultra-fast localized and non-localized spectroscopy utilizing hyperpolarized $^{13}$C-succinate will demonstrate the appearance of early key products of the TCA cycle, as well other mitochondrial and cytosolic pathways utilizing and TCA cycle intermediates. When translated to a clinical environment, these new metabolic markers could additionally allow detection of spatial distribution of such genetic mutations by means of ultra-fast chemical shift imaging (CSI), providing an opportunity to image active genetic mutations in humans. Besides an obvious utility to diagnose deficient SDH complex in vivo, such diagnostic methods could additionally guide surgery, radiation and especially gene therapy, as well as monitor the response to treatment, because multiple examinations would not result in additional exposure to radiation (in contrast to PET), toxic agents and would require only several minutes of patient and radiologist time.

Figure 4. Molecular hydrogenation scheme using Rh-based catalyst.

Figure 5. The scheme of the spin-order transfer from singlet states of parahydrogen spins to $^{13}$C. RF pulses\(^{9,15}\) are applied to transfer the spin order inside an 1.8 mT electromagnet following by ejection of hyperpolarized $1,13$C-succinic acid-$D_2$. The entire process is automated.

Figure 6. (Left) three spin system and three J-couplings necessary for spin order transfer sequence;\(^{9,15}\) (right) proton coupled $^{13}$C multiplets (experimental in black and simulated fits in red) of C$_1$ carbon collected in samples of natural-abundance succinic acid under various pH conditions at 14T.
References


