Efficient Spin-Relayed Heteronuclear Long-Range Signal Amplification by Reversible Exchange

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NMR hyperpolarization techniques transiently increase nuclear spin polarization (*P*) by several orders of magnitude. This significant *P* increase enables metabolic magnetic resonance spectroscopy (MRS) and MRS imaging (MRSI) after a bolus injection of a hyperpolarized (HP) compound to detect abnormal metabolism in cancer and other diseases. The ^{13}C isotope has been widely employed in a number of biomolecules, most notably in 1-^{13}C-pyruvate. However, this approach has a number of shortcomings. First, the lifetime of HP states (governed by the spin-lattice constant of exponential decay) is relatively short, with T_1 on the order of 1 min. As a result, only a limited number of metabolic pathways have been amenable to MRSI, because sufficient levels of nuclear spin polarization must persist until detection. Here, we employed the Signal Amplification by Reversible Exchange in SHield Enables Alignment Transfer to Heteronuclei (SABRE-SHEATH) hyperpolarization technique (1,4), which relies on performing simultaneous chemical exchange of parahydrogen (pH_2) and to-be-hyperpolarized biomolecules (without altering their structure) within the ligand sphere of a catalyst within micro-Tesla (μT) magnetic fields (Figure 1). SABRE-SHEATH has been shown to produce ^{15}N HP states with up to P≥30% in under one minute although these high polarization levels have been previously obtained at relatively low concentration of ^{15}N spins (7,8). Unlike other hyperpolarization techniques, SABRE-SHEATH requires relatively simple and inexpensive hardware (1,4). Although to date ^{15}N hyperpolarization of several biomolecules has been shown via SABRE-SHEATH, which indeed opens up a range of potential contrast agents, nearly all previous reports have been focused on hyperpolarization of ^{15}N sites that directly interact with the Ir catalytic center of the Ir-IMes polarization transfer catalyst, i.e. the catalyst typically used in most SABRE experiments. The direct polarization transfer from pH_2-derived hydrides to these ^{15}N sites is efficient (i.e. fast and yielding high P_{15N}), because the two-bond ^1H-^{15}N spin-spin couplings are relatively strong (e.g. 10-20 Hz) and sufficiently different from each other as to render the exchangeable catalyst binding sites magnetically inequivalent from one another (1). Although direct SABRE-SHEATH of remote spin-½ sites over ≥3 chemical bonds is inefficient in general (8), a network of *J*-coupled spin-½ nuclei can transmit polarization at least several chemical bonds away from pH_2-derived hydrides (2,3,5,6). Here, we show that uniformly ^{15}N-labeled metronidazole-$^{15}N_3$ (MNZ-$^{15}N_3$) can be efficiently hyperpolarized via the SABRE-SHEATH approach with P_{15N} >16% for each of the three ^{15}N sites using 87% pH_2, i.e. pH_2-derived hyperpolarization can be efficiently transmitted over six chemical bonds via a network of two-bond spin-spin couplings (Figure 1). In less than a minute of parahydrogen bubbling at ~0.4 μT, a high level of nuclear spin polarization P_{15N} of ~16% is achieved on all three ^{15}N sites of metronidazole-$^{15}N_3$ at up to ~41 mM concentration. At 1.4 T, the HP state of the $^{15}NO_2$ group persists for tens of minutes (T_1~10 min). Metronidazole is an FDA-approved antibiotic, and it can be safely administered orally and intravenously in large (multi-gram) doses. We envision a potential use of this contrast agent for hypoxia sensing (the focus of our future studies) in a manner similar to that of ^{18}F-fluoromisonidazole (FMISO) and other nitro-group containing radiotracers via Positron Emission Tomography (PET). The results presented here (P_{15N} ~ 16% at ≥98% ^{15}N enrichment, T_1 ~ 10 min, fast polarization, and straightforward isotopic enrichment) bode well for such envisioned cellular and *in vivo* experiments.

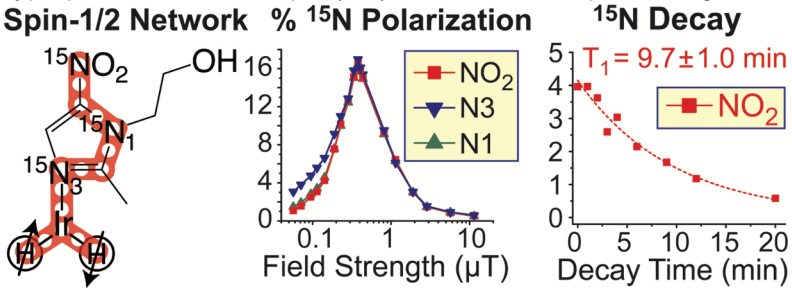

Figure 1. (**left**) the path (in red color) of chemical bonds over which the network of *I*=1/2 spin relays is established in metronidazole-$^{15}N_3$ (MNZ) undergoing SABRE hyperpolarization; (**right**) the decay curve of the HP $^{15}NO_2$ group in MNZ antibiotic.

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