Quantifying the Effects of Quadrupolar Sink via 15N Relaxation Dynamics in Metronidazoles Hyperpolarized via SABRE-SHEATH

<table>
<thead>
<tr>
<th>Journal:</th>
<th>ChemComm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>CC-COM-06-2020-003994.R1</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Communication</td>
</tr>
</tbody>
</table>
Quantifying the Effects of Quadrupolar Sink via $^{15}$N Relaxation Dynamics in Metronidazoles Hyperpolarized via SABRE-SHEATH

Jonathan R. Birchall,$^{a}$ Mohammad S. H. Kabir,$^{a}$ Oleg G. Salnikov,$^{b,c,d}$ Nikita V. Chukanov,$^{b,c}$ Alexandra Svyatova,$^{b,c}$ Kirill V. Kevtunov,$^{b,c}$ Igor V. Koptyug,$^{b,c}$ Juri G. Gelovani,$^{a,e}$ Boyd M. Goodson,$^{f}$ Wellington Pham,$^{g}$ and Eduard Y. Chekmenev$^{b,h}$

$^{15}$N spin-lattice relaxation dynamics in metronidazole-$^{15}$N$_2$ and metronidazole-$^{15}$N$_2$ isotopologues are studied for rational design of $^{15}$N-enriched biomolecules for Signal Amplification by Reversible Exchange in microtesla fields. $^{15}$N relaxation dynamics mapping reveals the deleterious effects of interactions with polarization transfer catalyst and quadrupolar $^{15}$N nucleus within the spin-relayed $^{14}$N-$^{15}$N network.

The nuclear spin polarization $P$ at thermal equilibrium is governed by a Boltzmann distribution of nuclear spins among Zeeman energy levels. $P$ increases linearly with magnetic field strength. For a conventional high-field NMR spectrometer (e.g., 9.4 T) or clinical Magnetic Resonance Imaging (MRI) scanner (e.g., 3 T) at room temperature, $P$ is typically on the order of $10^{-5}$ to $10^{-3}$, resulting in a relatively low sensitivity of NMR-based applications. For NMR applications where this sensitivity is too low to be useful, various hyperpolarization strategies may be employed to increase $P$ by as much as 4-5 orders of magnitude,$^{1,2}$ with corresponding signal gains.

One such technique is Signal Amplification by Reversible Exchange (SABRE), pioneered by Duckett et al. in 2009,$^{3}$ which utilizes simultaneous reversible chemical exchange of parahydrogen (p-H$_2$) and to-be-hyperpolarized substrate molecules at a metal center. In SABRE, the transfer of nuclear spin polarization from parahydrogen-derived hydrides to a spin-polarizable substrate occurs spontaneously via the network of spin-spin couplings established in a transient polarization transfer catalyst (PTC) complex (Figure 1a).$^{3,5}$ While several approaches have been developed for polarization transfer in SABRE,$^{5,11}$ a variant of SABRE, SABRE-SHEATH (SABRE in SHield Enables Alignment Transfer to Heteronuclei),$^{12,13}$ facilitates the generation of highly polarized spin states of heteronuclei$^{14-17}$ including nitrogen-15 with high polarization ($P_{15N} > 30\%$)$^{18}$ persisting for tens of minutes.$^{19}$ Nitrogen is found in a wide range of biomolecules including nucleic acids, amino acids, proteins, and drugs. Since SABRE-SHEATH is performed at very low magnetic fields (< 1 µT) and near room temperature, the production of such HP $^{15}$N spin-labeled biomolecules is comparatively simple, fast and inexpensive.$^{10}$ Nitroimidazoles can be readily reduced in anaerobic environments. This property has been widely employed in a number of antibiotic drugs,$^{20}$ Positron Emission Tomography (PET) tracers for hypoxia sensing,$^{21}$ and also in a number of emerging cancer therapeutics: e.g., evofosfamide (a.k.a. TH-302)$^{22}$ and the radiosensitizer nimorazole.$^{23}$

Metronidazole is an FDA-approved antibiotic,$^{24}$ belonging to the nitroimidazole class of compounds. We envision that $^{15}$N-hyperpolarized metronidazole can be potentially employed for hypoxia sensing in a manner similar to that of nitroimidazole-based Positron Emission Tomography (PET) tracers. One such tracer, $^{18}$F-fluoromisonidazole (FMISO),$^{21}$ undergoes reduction in hypoxic environment (including most notably hypoxic tumors) and the metabolic products of this reduction process become trapped in hypoxic cells, providing contrast in FMISO PET images.$^{25}$ The enormous potential for using HP MRI to sense metabolic transformations in vivo has been well demonstrated$^{10,26}$; correspondingly, HP MRI of metronidazole may obviate the limitations of FMISO PET imaging, including the use of ionizing radiation, the requirement for long clearance time from surrounding tissues, and the inability to spectrally...
distinguish parent compounds from downstream products. We have demonstrated efficient SABRE-SHEATH hyperpolarization of the $^{15}\text{N}_3$ site in natural abundance metronidazole with $\%P_{\text{SN}}$ exceeding 30%.\textsuperscript{18} This nitrogen site directly interacts with the PTC, and therefore gains its polarization directly from p-H$_2$-derived hydrides. Subsequently, commercially available metronidazole-$^{15}\text{N}_2$-$^{13}\text{C}$ (Sigma-Aldrich, \#32744) was employed for SABRE-SHEATH, but unfortunately yielded significantly lower $\%P_{\text{SN}}$ (roughly by an order of magnitude), although all $^{15}\text{N}$- and $^{13}\text{C}$-labeled sites have been successfully hyperpolarized.\textsuperscript{27, 28} Most recently we have synthesized metronidazole-$^{15}\text{N}_3$ and demonstrated $^{15}\text{N}\rightarrow^{15}\text{N}_3$ spin-relayed SABRE-SHEATH hyperpolarization via two-bond $^{15}\text{N}$-$^{15}\text{N}$ spin-spins couplings, Figure 1d. This metronidazole-$^{15}\text{N}_3$ isotopologue exhibited a remarkable $\%P_{\text{SN}}$ of $\sim$16% on all three $^{15}\text{N}$ sites including $^{15}\text{NO}_2$, which has a polarization relaxation decay constant $T_1$ approaching 10 minutes. However, in order to facilitate more effective production of HP biomolecules for bioimaging applications, it is clearly necessary to gain improved understanding of the underlying spin-relaxation phenomena to inform the rational design of these promising imaging agents. Here, we report a quantitative study of spin relaxation dynamics of metronidazole-$^{15}\text{N}_2$ and metronidazole-$^{13}\text{N}_3$ isotopologues, Figure 1c and Figure 1d respectively, using previously described experimental setup (Figure S2).\textsuperscript{27, 29} Catalyst activation was performed for approximately 2 h for each sample studied to ensure reproducibility, see Figure S1 in the Supporting Information (SI). Other experimental parameters (temperature, p-H$_2$ pressure, flow rate and in-shield magnetic field) were optimized for each isotopologue, Figure S1. Using an overpressure of 94 psig, p-H$_2$ was bubbled through a solution of MNZ-$^{15}\text{N}$-isotopologue substrate and Ir-IMes catalyst in 0.6 mL methanol-d$_4$ for one minute to facilitate polarization transfer in microtesla fields. Following polarization build-up, the sample solution was rapidly transferred (2-4 s to minimize the relaxation losses) to a 1.4 T NMR Pro (Nanalysis, Canada) benchtop NMR spectrometer for $^{15}\text{N}$ NMR detection (Figure 1h). Each relaxation/build-up curve was obtained by varying the time duration that the sample spent in a given magnetic field. The key results related to $^{15}\text{N} T_1$ relaxation at the optimal magnetic field during the SABRE-SHEATH polarization transfer process (ca. 0.4 $\mu$T, Figure S1) for metronidazole-$^{15}\text{N}_3$ and metronidazole-$^{13}\text{N}_3$ are shown in Figures 2e and 2f, respectively. As expected for microtesla magnetic fields, all $^{15}\text{N}$ sites within a given molecule share approximately the same relaxation rate (e.g., 13.8-15.4 s for MNZ-$^{15}\text{N}_3$, Figure 2e). However, the $^{15}\text{NO}_2$ group replacement by $^{14}\text{NO}_2$ leads to dramatic, 3-fold shortening of the $^{15}\text{N} T_1$ trend (4.3-4.8 s corresponding $T_1$ values for MNZ-$^{15}\text{N}_3$, Figure 2f). This striking effect can be explained by the enhanced scalar relaxation of the second kind\textsuperscript{30, 31} induced by the quadrupolar $^{14}\text{NO}_2$ site within the N-N spin-spin coupling network. These results are further supported by the overall similar $^{15}\text{N} T_1$ trend at the Earth’s magnetic field (ca. 10 $\mu$T in the basement of our lab at Detroit, MI, Table S1). Moreover, we find that each $^{15}\text{N}$ polarization build-up constant ($T_0$, Figure 2c and Figure 2d) at 0.4 $\mu$T is closely correlated with the corresponding $T_1$ value. In practice, this means that the increased relaxation rate caused by the presence of the quadrupolar $^{14}\text{N}$ spin in MNZ-$^{15}\text{N}_2$ (despite the peripheral position of the NO$_2$ group) allows for achieving the steady-state $\%P_{\text{SN}}$ faster on $^{15}\text{N}_3$ and $^{13}\text{N}_1$ sites—but at significantly lower levels. The correlation plot of $\%P_{\text{SN}}$ versus $T_1$ at 0.4 $\mu$T indeed exhibits a linear trend with R$^2$>0.99 (Figure 2instant). When the magnetic field is sufficiently high (e.g., 1.4 T, Figure 2g and Figure 2h), the increased frequency dispersion of the nuclear spins puts them in a weakly coupled regime with $^{15}\text{N}$ sites having significantly longer $T_1$ decay constants—on the order of many minutes.

On another note, the realization that scalar-coupled $^{14}\text{N}$ spins are highly deleterious in the context of SABRE-SHEATH suggests that if these quadrupolar effects would have been avoided, near-unity $P_{\text{SN}}$ would have been potentially achievable in the previous studies.\textsuperscript{18} It should be pointed out that substrate exchange of Ir-IMes catalyst may act as the potential source of additional undesirable $^{14}\text{N}$ relaxation (e.g., due to coupling effects of quadrupolar Ir nucleus and the chemical exchange process).\textsuperscript{13} Consequently, a series of control experiments were performed, where the catalyst concentration was systematically varied from 0.5 mM to 1 mM to 2 mM at a fixed concentration of metronidazole isotopologue (Figures S3a-d). The [catalyst] increase from 0.5 mM to 2 mM results in a stepwise decrease in $^{15}\text{N} T_1$ and $T_0$ by approximately 2-fold at 0.4 $\mu$T (Figure S3b and Figure S3c). However, because the interplay of $T_1$ relaxation and catalyst concentration is complex in the SABRE process,\textsuperscript{32, 33} these decreases in $^{15}\text{N} T_1$ and $T_0$ at 0.4 $\mu$T are offset by the
overall increased catalyst-to-substrate ratio (i.e., better substrate access to p-H$_2$ spin bath), resulting in somewhat greater $\%P_{\text{PSN}}$ in metronidazole-$^{15}$N$_2$ and similar $\%P_{\text{PSN}}$ in metronidazole-$^{15}$N$_2$ at higher [catalyst], Figure S3d. Of note, the Ir-IMes catalyst decreases $^{15}$N $T_1$ even at high magnetic fields (1.4 T, weakly coupled regime) for $^{15}$NO$_3$ (Figure S3a). Moreover, this observation clearly indicates a second reason (beyond agent purification) that SABRE catalyst removal is warranted as soon polarization build-up is completed to minimize $^{15}$N polarization losses prior to biomedical utilization.

**Conclusions**

In summary, the spin-relayed SABRE-SHEATH hyperpolarization approach allows efficient polarization of scalar coupled $^{15}$N-$^{15}$N spin networks. These networks may be created via two-bond $^{15}$N-$^{15}$N J-couplings at most. The presence of $^{14}$N spins in such networks must be avoided to prevent deleterious polarization losses due to quadrupolar relaxation effects (manifested on $^{15}$N as scalar relaxation of the second kind), in order to maximize the resulting $\%P_{\text{PSN}}$. Although the catalyst decreases the $^{15}$N spin-relaxation time constant, $T_1$, of metronidazole isotopologues in the microtesla regime in a concentration-dependent manner, the overall impact on the achievable $^{15}$N polarization level is relatively minor. On the other hand, the presence of a $^{14}$N nucleus in the scalar coupling network results in an approximately 3-fold decrease of microtesla $^{15}$N $T_1$ values for all $^{15}$N sites in the $^{15}$N$_2$-isotopologue versus the $^{15}$N$_2$-isotopologue over a wide range of catalyst concentrations. This $^{15}$N $T_1$ reduction results in a corresponding 3-fold decrease of $^{15}$N polarization levels. These findings have substantial translational relevance for the rational design of hyperpolarized MRI contrast agents comprising $^{15}$N- and $^{13}$C-labeled biomolecules—both in general, and in the specific case of SABRE-hyperpolarized metronidazole, an antibiotic that can be potentially employed for non-invasive hypoxia-sensing antibiotics, emerging cancer therapeutics such as...
evofosfamide and nimorazole radiosensitizers, etc. Feasibility of high resolution MRI of HP metronidazole is shown, which bodes well for potential biomedical applications.

Conflicts of interest
BMG, EYC declare stake ownership in XeUS Technologies, LTD.

Acknowledgements
This work was supported by the NSF under grants CHE-1904780 and CHE-1905341, NIH R21CA220137, DOD CDMRP W81XWH-12-1-0159/BC112431, W81XWH-15-1-0271 and W81XWH-15-1-0272. O.G.S. thanks the RFBR (#19-33-60045) for the support of mechanistic studies with the use of parahydrogen. N.V.C. and K.V.K. thank the Russian Ministry of Science and Higher Education (project AAAA-A16-116121501008-5) and the RFBR (#19-29-10003, #17-54-33037) for their support in the synthesis of 15N-labeled compounds. I.V.K. thanks the Russian Ministry of Science and Higher Education (project AAAA-A16-116121510087-5) and the RFBR (#19-33-60045) for the support of mechanistic studies with the use of parahydrogen.

Notes and References
10. ClinicalTrials.gov, Clinical trial #NCT01507467.
Presence of $^{14}\text{N}$ nucleus in the scalar coupling network causes deleterious effects in SABRE hyperpolarization in microtesla fields resulting in 3-fold decrease of $^{15}\text{N}$ $T_1$ and polarization values for all $^{15}\text{N}$ sites in $^{15}\text{N}_2$-isotopologue versus $^{15}\text{N}_3$-isotopologue.