



HyperBIRD: A Sensitivity-Enhanced Approach to Collecting Homonuclear-Decoupled Proton NMR Spectra**

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Dedicated to Prof. Alex Pines on the occasion of his 70th birthday

Abstract: Samples prepared following dissolution dynamic nuclear polarization (DNP) enable the detection of NMR spectra from low- γ nuclei with outstanding sensitivity, yet have limited use for the enhancement of abundant species like ^1H nuclei. Small- and intermediate-sized molecules, however, show strong heteronuclear cross-relaxation effects: spontaneous processes with an inherent isotopic selectivity, whereby only the ^{13}C -bonded protons receive a polarization enhancement. These effects are here combined with a recently developed method that delivers homonuclear-decoupled ^1H spectra in natural abundance samples based on heteronuclear couplings to these same, ^{13}C -bonded nuclei. This results in the HyperBIRD methodology; a single-shot combination of these two effects that can simultaneously simplify and resolve complex, congested ^1H NMR spectra with many overlapping spin multiplets, while achieving 50–100 times sensitivity enhancements over conventional thermal counterparts.

While dissolution dynamic nuclear polarization (DNP) can deliver sensitivity enhancements upwards of 10000 times for the NMR spectra of slowly relaxing species like ^{13}C and ^{15}N nuclei,^[1] there are still inherent limitations that prevent the extension of these methodological benefits to 1D ^1H NMR spectroscopy. In particular, the shorter longitudinal relaxation times T_1 of ^1H nuclei, challenge the times involved in the melting and shuttling of the sample from the polarizer to the NMR or MRI system. Only when using specialized hardware^[2,3] or when targeting special molecules such as water,^[4,5] has dissolution DNP been successfully applied to directly hyperpolarize and detect protons in solutions. Here we explore an alternative to bypass these limitations and

enable the acquisition of natural abundance ^1H NMR spectra enhanced by dissolution DNP, while enjoying the benefits of a simplified “one-site one-peak” format.

The first of the phenomena exploited to achieve this aim, is the spontaneous $^{13}\text{C} \rightarrow ^1\text{H}$ polarization transfer that can be induced upon hyperpolarizing lower- γ nuclei, and rapidly transferring the sample into an NMR tube at high field as per the standard dissolution DNP protocol. Once injected into the NMR spectrometer, the hyperpolarized nuclei will begin transferring their alignment to their directly bonded protons through a cross-relaxation process.^[6,7] When coupled to cross-correlation effects involving shielding and dipole–dipole anisotropies^[8,9] the ensuing proton NMR spectrum will display, for each chemical site, an asymmetric “triplet” where all components have similar intensities, despite their very different abundances. The middle of these components arises from protons bonded to the 99%-abundant, NMR-silent ^{12}C isotopes, whereas the external components arise from the 1% of the ^1H nuclei that are bonded to ^{13}C nuclei. The splitting of these external components derives from the heteronuclear J -coupling, while their 100-fold enhancement derives from the transfer of the carbon hyperpolarization to its directly-bonded protons. This enhancement of the so-called ^{13}C satellites reaches a maximum value about 5–10 seconds after injection, before gradually decaying back to its 0.5% equilibrium intensity value after $5 \cdot T_1^{\text{C}}$ times have elapsed after the injection of the sample. This sensitivity enhancement occurs spontaneously, and does not require any special sequence or pulsing.

This phenomenon is hereby exploited in combination with a second ^{13}C -associated feature, concerning a family of recently developed homonuclear ^1H – ^1H decoupling sequences,^[10,11] that use an isotopically selective detection of ^{13}C -bonded protons while suppressing the signals and the couplings arising from ^{12}C -bonded proton resonances.^[12] Homonuclear decoupling is achieved by these bilinear-rotation decoupling (BIRD) sequences^[13] using a windowed acquisition that operates synchronously with periodic inversions of the ^{12}C -bonded ^1H nuclei without altering the ^{13}C -bonded ones, thus effectively removing the ^1H – ^1H J -couplings over the course of a continuous ^1H signal acquisition.

An optimal matching is possible between these heteronuclear polarization-transfer and BIRD-based homonuclear decoupling protocols. This complementarity rests on the fact that both the transfer and the decoupling processes, rely on aspects of the spin dynamics that arise between a naturally abundant ^{13}C atom, and its directly bonded proton. One of these aspects is the aforementioned enhancement of the

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peak trajectories^[7] shows that the maximum intensity in the ¹³C-bonded proton signals as well as the asymmetry of the doublets will be linked to the molecular correlation time, the extent of ¹³C hyperpolarization, and the shielding anisotropy/dipolar fields contributing to the longitudinal relaxation time *T*₁. While it is unlikely that this set of parameters would be identical for any two inequivalent proton resonances, a suitable “average” delay can give significant enhancements on all ¹³C-bound protons in typical organic molecules; for the present study, this spontaneous transfer delay was set to 7 seconds. By this time, the one-shot dissolution process endows the ¹H spectrum arising from the ¹³C-bound protons in small- and medium-sized organic molecules, with a sensitivity enhancement of approximately 100 times vis-à-vis their thermal counterparts; this provides the ¹H-based experiment hereby considered with special sensitivity.

The sensitivity enhancement illustrated in Figure 2 for the ¹³C-bound proton resonances is further exploited in the experimental protocol shown in Figure 1, for the sake of enhancing spectral resolution by ¹H–¹H homonuclear decoupling in the post-dissolution phase. This procedure exploits the fact that, beyond an enhancement in sensitivity, the presence of a ¹³C atom can provide its directly bonded proton with a chance of distinguishing it from its surrounding neighbors. Therefore the incorporation of a bilinear rotation decoupling (“BIRD”) sequence^[13] based on a concatenated train of $(90)_y^H - 0.5/J_{CH} - (180)_x^H$ & $(180)_x^C - 0.5/J_{CH} - (90)_y^H$ pulses interleaved with periods of free evolution and data acquisition, can be used to achieve full decoupling. As further described in Ref. [11], these pulses can be used in a real-time fashion, to periodically impart 180° rotations on the ¹²C-bound protons while leaving unchanged the ¹³C-bound ones. One-dimensional signals collected from the latter protons while implementing this multiple-pulse procedure, leads to a robust single-shot experiment whereby ¹H nuclei appear as effectively homo- and hetero-decoupled.

A key to achieve such robust decoupling is, naturally, to have a sparse ¹³C enrichment to ensure the periodic rotation of all ¹²C-bound protons neighboring the targeted resonance. This brings about a number of limitations, including the sensitivity penalties normally associated with acquisitions on natural abundance samples. By selectively enhancing the ¹H resonances that are targeted by the homonuclear decoupling process, the hyperpolarization procedure illustrated in Figure 2 alleviates this, and provides an excellent complement to the BIRD-based decoupling. An example of this is illustrated by the DNP-enhanced, homonuclear-decoupled “HyperBIRD” ¹H spectrum shown in Figure 3. In the top trace of this figure a three-peak ¹H pyridine spectrum is displayed, showing similar signal intensity as a conventional thermal counterpart, but no ¹H–¹H *J* splittings. This single-shot spectrum enjoys the benefit of the 100 times signal enhancement derived from the ¹³C-derived DNP process, acting in unison with a ¹³C-based homonuclear decoupling that collapses the ¹H multiplet patterns into narrow singlet peaks. This can be appreciated from the BIRD-decoupled thermal counterpart in the center panel of Figure 3, which is inferior to its single-scan hyperpolarized counterpart even after 1024 averages (1 hour 40 minutes total acquisition time).

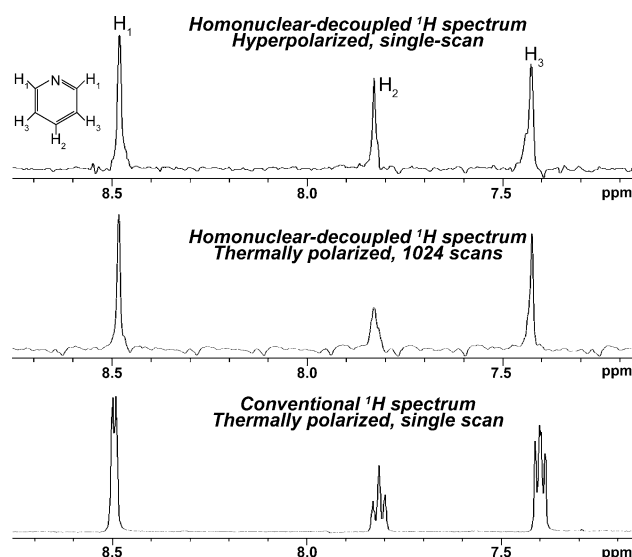


Figure 3. Demonstrating the ¹H-enhancing and decoupling features of HyperBIRD on a natural abundance pyridine sample. 5 μL of an equal part mixture of [D₆]DMSO and pyridine were hyperpolarized for this experiment. Dissolution was performed with 4 mL of [D₃]methanol giving a final sample concentration of about 7.8 mM. To appreciate the resolution and sensitivity enhancements of the HyperBIRD experiment this trace (top) is compared against a multi-scan BIRD spectrum collected after the sample returned to thermal equilibrium (center), as well as against a single-shot single-pulse *J*-coupled counterpart (bottom). The slightly larger artifacts shown by the thermally polarized BIRD spectrum reflect the challenge to completely suppress the (now much more intense) ¹²C-bonded proton resonances.

While the HyperBIRD experiment required a longer time than its thermal counterpart if including the sample pre-polarization period, its suppression of the natural-abundance ¹²C-bound background is also eased by the strong natural bias that the cross-relaxation effect does on behalf of the ¹³C-bound species. Finally, both of these spectra show signals that split into the expected multiplets in a single-pulse conventional acquisition (Figure 3, bottom).

The sensitivity and resolution enhancement brought about by this procedure is further exploited in Figure 4, which demonstrates the utility of HyperBIRD for analyzing an aromatic mixture containing many overlapping proton multiplets. The thermal equilibrium single-pulse spectrum of this quinoline/toluene/pyridine/indazole sample contains many spin multiplets, with spectrally congested regions where individual resonances are hard to identify and assign. In the hyperpolarized, homodecoupled spectrum all ¹³C-bound proton resonances are selectively enhanced and collapsed into single lines, providing an easier route to resolving the chemical shift of every site. When compared against literature values, this allows one to carry out an assignment of the origin of the chemical peaks.

Conclusions

The HyperBIRD technique as implemented here is still limited by a number of features, including an inability to decouple geminal protons and relatively lengthy sample pre-

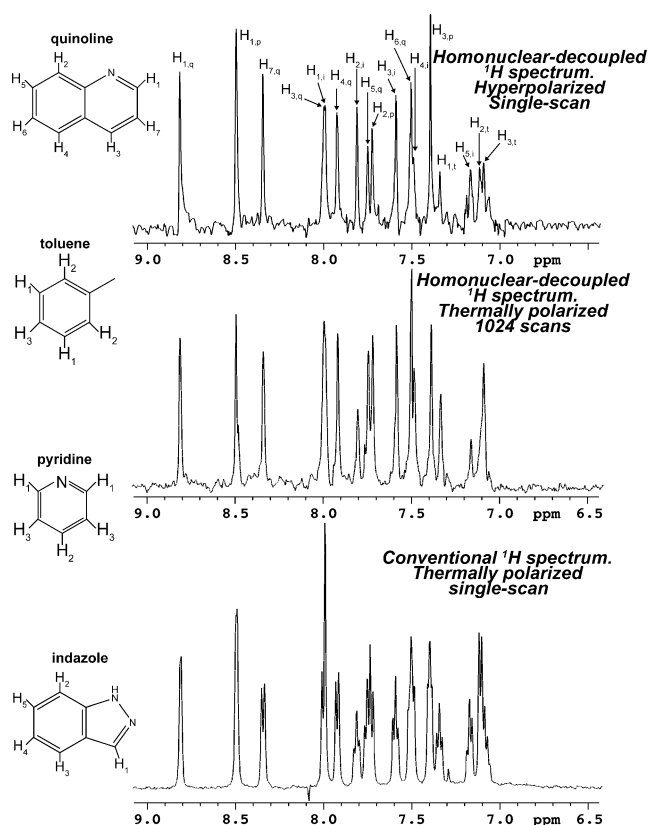


Figure 4. Application of the sequence introduced in Figure 1 to the analysis of a heterocyclic mixture with several overlapping multiplets. In the HyperBIRD spectrum (top) all individual ^1H resonances are resolved and tentatively assigned based on literature values; something clearly more difficult for the conventional proton spectrum (bottom). The fine residual coupling observable in the HyperBIRD spectrum around 7.1 ppm, is likely the result of insufficiently suppressed background (^{12}C - ^1H) signals. All spectra were recorded on the same sample, prepared by combining the indicated substrates in a 50/50 mixture of sulfolane and $[\text{D}_6]\text{DMSO}$. 5 μL of the ensuing mixture was hyperpolarized, and dissolved in 4 mL of $[\text{D}_3]\text{methanol}$ for the NMR observations. Final substrate concentrations were 2.53 mM for pyridine, quinoline, indazole, and 1.27 mM for toluene, all at natural abundance.

polarization times. Recent developments based on the use of homonuclear echoes^[14,15] could bypass the first of these limitations, while increasingly faster DNP processes have been demonstrated with the aid of cryogenic cross-polarization.^[16,17] With these and other emerging developments, the HyperBIRD technique could become increasingly promising for practical acquisitions of fully decoupled ^1H data. Moreover, while NMR experiments with enhanced ^1H sensitivity have also been demonstrated based on INEPT-based transfers of heteronuclear hyperpolarization,^[18–21] the cross-relaxation-based transfer demonstrated in this report offers a number of advantages. This method is simpler sequence-wise, as it does not need to adjust millisecond-long delays based on a particular J_{CH} value and/or multiplicity as is the case with a $^{13}\text{C} \rightarrow ^1\text{H}$ INEPT transfer; its spontaneous nature may also make it a preferable choice when considering heteronuclei that are more exotic than carbon-13. The cross-relaxation based polarization transfer is

also well suited to in vivo experiments, which tend to be performed in more hardware-limited scanners than their in vitro counterparts, and that inherently incorporate multi-second delays to allow the hyperpolarized substrate to reach the desired point of detection. Still, from an acquisition standpoint, the BIRD-based single-shot module used in this work should also be compatible with INEPT-based forms of hyperpolarization transfer. It is also interesting to consider the potential advantage of the 1D form of homodecoupled acquisition presented in this work, over single-shot 2D heteronuclear correlation experiments that starting from hyperpolarized ^{13}C or ^{15}N nuclei, have been demonstrated.^[18–20] The latter have naturally the prospect of providing an enhanced resolution and information vis-à-vis the 1D ^1H acquisitions hereby described, yet achieve this at the expense of more complex gradient-based manipulations and with a decreased signal-to-noise ratio. Still, given the advantages inherent to fast 2D NMR sampling schemes,^[22–24] it is interesting to further consider how heteronuclear cross-relaxation effects, hyperpolarization, and homonuclear BIRD decoupling concepts, could also be exploited in these kind of fast 2D experiments.

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