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Lighting up spin systems: enhancing characteristic ^{1}H signal patterns of fluorinated molecules†

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Fluorine is becoming increasingly prevalent in medicinal chemistry, both in drug molecules and in molecular probes. The presence of fluorine allows convenient monitoring of such molecules in complex environments by NMR spectroscopy. However, sensitivity is a persistent limitation of NMR, especially when molecules are present at low concentrations. Here, sensitivity issues with ^{1}H NMR are mitigated by sharing ^{19}F photochemically-induced dynamic nuclear polarisation with ^{1}H nuclei. Unlike direct ^{1}H enhancement, this method enhances ^{1}H signals without significantly distorting multiplet intensities, and has the potential to enable the use of suitable molecules as low-concentration probes.

Fluorinated molecules account for over 20% of pharmaceuticals currently available, and are commonly found in compound libraries used for drug screening.[1,2] Fluorinated species are also increasingly used as probes for studying biological processes, due to the high sensitivity of ^{19}F chemical shifts to the local environment, the absence of endogenous background signals, and 100% natural abundance.[3–7] However, structural information obtained by direct observation of sparse fluorine atoms is limited.

^{1}H NMR can provide much more information, by reporting multiple signals from the same molecule, and can be used to characterise the behaviour of a probe molecule in a complex system.[8,9] Unfortunately, ^{1}H spectra often suffer from signal overlap, especially in mixtures of protonated molecules, due to the limited chemical shift range and prominent signal multiplicity, making disentangling the spectra of individual species difficult. One strategy for mitigating the limitations of both nuclei is fluorine-edited selective TOCSY acquisition (FESTA), which exploits the exceptional chemical shift dispersion of ^{19}F spectra to acquire ^{1}H spectra containing only ^{1}H signals that are within the same spin system as a selected ^{19}F nucleus.[10] FESTA has been previously demonstrated to be particularly powerful in mixture analysis, allowing characteristic ^{1}H subspectra of fluorinated components to be obtained.[10–12] Unfortunately, the beneficial ability to observe the characteristic ^{1}H fingerprint patterns of particular fluorinated molecules comes with a sensitivity penalty, which may be critical if molecules are present at low concentrations. Here we introduce a method to address this.

Strategies to improve the sensitivity of NMR include using very high magnetic fields, cryoprobes,[13] parahydrogen-induced polarisation (PHIP),[14,15] and dynamic nuclear polarisation (DNP)[16–18] techniques. Unfortunately, these methods generally come at substantial cost and require additional complex hardware, such as gyrotrons and dissolution apparatus or parahydrogen generators. A potentially more convenient and relatively low-cost alternative to these approaches is photochemically-induced dynamic nuclear polarization (photo-CIDNP)[19–21] which, upon simple sample illumination, even using cheap LEDs, can lead to large signal enhancements in target molecules with low ionisation potentials, such as aromatic molecules. The relative enhancements obtained are larger for lower magnetic fields, so photo-CIDNP lends itself well to less expensive hardware, where the increase in sensitivity is at its most welcome.[22]

Illumination (historically with lasers, more recently with inexpensive LEDs)[23–25] of a photosensitiser, such as fluorescein or flavin, present in the sample leads to a non-Boltzmann distribution of nuclear spins in a target molecule *via* a radical pair mechanism.[26] A hyperpolarised signal arises because there is an overpopulation of nuclear spin states which experience faster intersystem crossing and less efficient paramagnetic relaxation.[27] However, hyperpolarisation only occurs for nuclei that have a significant hyperfine coupling to an unpaired electron. Other signals from the molecule are not enhanced and, disappointingly, signal intensities may even be reduced, as

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the polarisation produced by photo-CIDNP can be absorptive or emissive.[28] The net result is to distort the characteristic signal pattern of a probe molecule, potentially making the spectrum unrecognisable. These signal distortions and losses hinder the application of direct ^{1}H photo-CIDNP as a signal enhancement approach for molecular detection and monitoring in mixtures. It has been noted, however, that there are significant benefits to using heavier heteroatoms such as ^{19}F, ^{13}C or ^{15}N, which may result in greater hyperpolarization.[29–33] Given the ability to transfer magnetization from heteronuclei to protons, sharing such hyperpolarisation enables more sensitive heteronuclear correlation experiments, or can highlight through-space interactions between heteronuclei and neighbouring protons.[17,31,34]

In this study, we demonstrate how the benefits of FESTA[10] can be combined with those of photo-CIDNP to provide significant ^{1}H signal enhancements across a spin system that includes fluorine. This enables observation of the characteristic ^{1}H signals of a molecule with enhanced sensitivity. Using 6-fluoroindole (6FI), a common reagent in the preparation of fluorinated amino acids and antifungal and antibacterial agents,[35–37] as a model we show that the direct photo-CIDNP effect is here much greater for ^{19}F than for ^{1}H (Fig. 1d and c). Transferring the large ^{19}F hyperpolarisation to ^{1}H using FESTA demonstrates that multiple ^{1}H signals in the same spin system as fluorine can be greatly enhanced, with much less signal distortion than in direct ^{1}H photo-CIDNP (Fig. 1g and c). This approach also would allow subspectra for specific fluorinated molecules in complex mixtures to be obtained, using a frequency-selective shaped ^{19}F pulse for the initial excitation. The recently proposed NMRtorch approach[38] was used to illuminate a sample containing 1 mM 6FI and a flavin mononucleotide as a photosensitizer prior to data acquisition. Matching control spectra were recorded without illumination.

As 6FI is only present at 1 mM concentration, ^{1}H and FESTA NMR spectra recorded in the dark (Fig. 1a and e) suffer from limited signal-to-noise ratio. The ^{1}H NMR spectrum acquired with illumination (*i.e.*, direct ^{1}H photo-CIDNP) showed a 32-fold absorptive signal enhancement of the integral of H2 (Fig. 1c). However, other ^{1}H signals in the molecule showed only modest signal enhancements ($<$5-fold) and, importantly, the multiplet patterns of H4, H5 and H7 were all significantly distorted (Fig. S2, ESI†), complicating the recognition of this spectrum as belonging to 6FI. ^{19}F photo-CIDNP (Fig. 1d), on the other hand, resulted in 83-fold emissive signal enhancement compared to the corresponding dark reference spectrum (Fig. 1b).

As only certain 6FI ^{1}H signals are enhanced by direct ^{1}H photo-CIDNP, methods of sharing the benefits of hyperpolarisation amongst multiple ^{1}H signals were explored. Initially, ^{1}H selective TOCSY[39,40] (as previously proposed with photo-CIDNP by Goez *et al.*)[41] was trialled to share the hyperpolarisation of H2 around the coupled ^{1}H network. However, the signal enhancement was only efficiently shared between H2 and H1, and gave only $\sim$12-fold enhancement of each with the optimum mixing time (Fig. S3, ESI†). The distant aromatic protons of 6FI (H4, H5 and H7) showed no significant enhancement

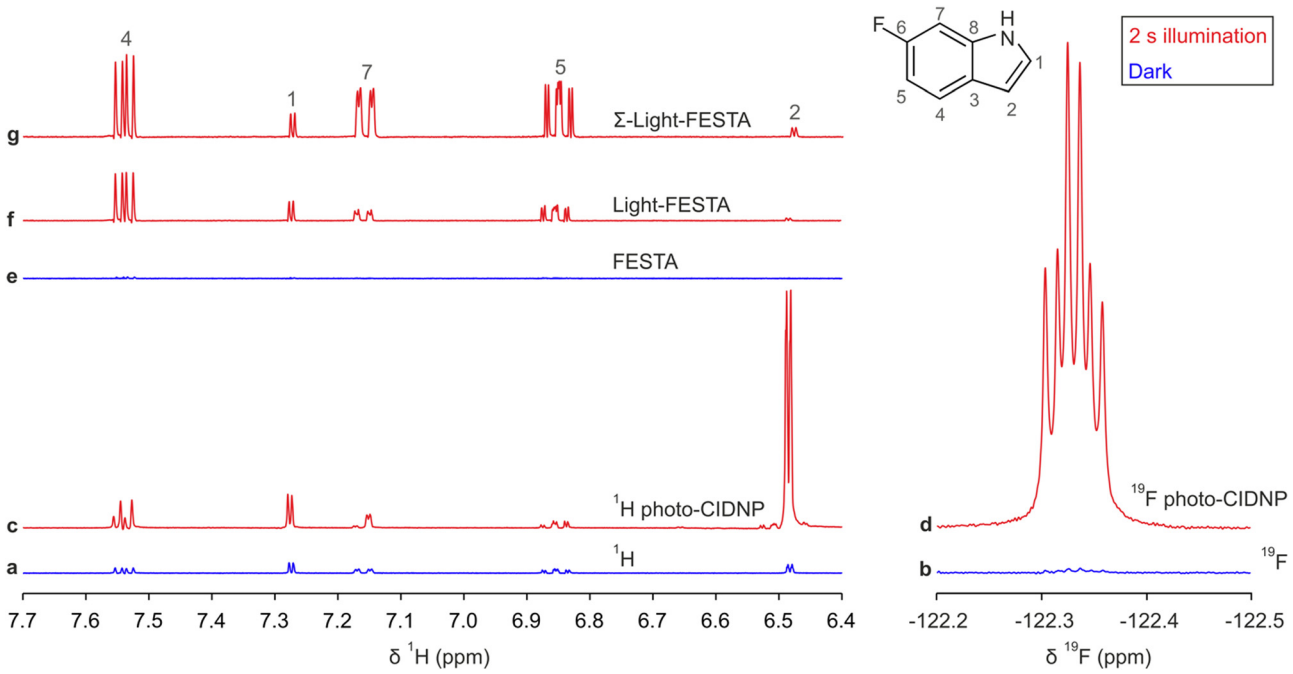

Fig. 1 (a) 500 MHz ^{1}H and (b) 470 MHz ^{19}F NMR spectra of 1 mM 6FI with 0.2 mM riboflavin 5′-monophosphate sodium salt in D_2O. (c) ^{1}H and (d) ^{19}F NMR spectra with 2 s illumination using a single LED with nominal 460 nm peak emission. Example ^{1}H FESTA spectra obtained (e) in the dark and (f) with 2 s illumination using the pulse sequence of Fig. 2 selectively exciting H7 (7.18 ppm). (g) Summation of three ^{1}H FESTA spectra where H4, H5 and H7 were selected (further information can be found in the ESI†). Spectra (d), (f) and (g) were emissive, and are shown inverted with respect to the dark spectra to facilitate comparison. All spectra are scaled consistently for a given nucleus.

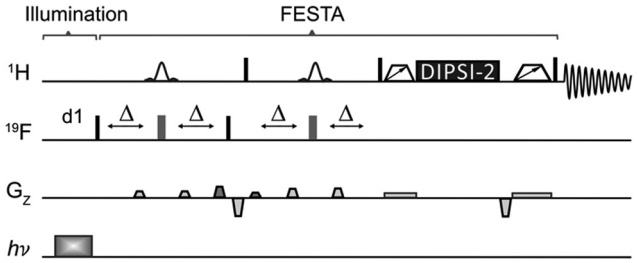

Fig. 2 Light-FESTA pulse sequence. The narrow black and wide grey rectangles represent hard 90° and 180° radiofrequency pulses, respectively. The shaped pulses represent selective 180° radiofrequency pulse applied to a chosen ^{1}H resonance coupled to ^{19}F. The trapezoids on either side of the isotropic mixing element (DIPSI-2) represent zero-quantum coherence suppression elements.[40] Field gradient pulses are employed to enforce the coherence transfer pathway. Illumination ($h\nu$) is applied during the recovery delay, d1. If more than one fluorine resonance is present, the first ^{19}F pulse should be frequency-selective. Further details are provided in the ESI.†

(Fig. S3, ESI†), because the small inter-ring couplings J_{HH} (Table S2, ESI†) severely limit the effectiveness of TOCSY transfer.

To obtain more uniform enhancement across multiple ^{1}H nuclei, the use of a new approach, Light-FESTA (Fig. 2), was investigated. This exploits the strong ^{19}F photo-CIDNP enhancement and the ability of FESTA to transfer that enhancement across a ^{1}H spin system with minimal multiplet distortion.[10] In Light-FESTA the selection of different ^{19}F-^{1}H coupling partners, *i.e.*, transferring F6 magnetisation to H4, H5 or H7, leads to preferential enhancement of different signals (Table 1). However, summing the spectra from these three separate Light-FESTA experiments into a single Σ-Light-FESTA spectrum (Fig. 1g) leads to a more even distribution of signal intensities for H4, H5 and H7. This makes the spectrum easily recognisable as that of 6FI and allows straightforward signal comparison with the reference^{1}H NMR spectrum (Fig. 1a). This contrasts with the direct ^{1}H photo-CIDNP spectrum (Fig. 1c), which is barely recognisable, with the signal intensities for H4, H5 and H7 being lower and their multiplet structures severely distorted.

Light-FESTA provides a significant signal enhancement (Fig. 1f) compared to standard FESTA (Fig. 1e), with minimal multiplet distortion. The background noise level was unaffected by illumination with NMRtorch.[38] The average sensitivity enhancement (defined as enhancement in the individual signal-to-noise ratios) was 34-fold for the H4–H5–H7 spin system (Table S3, ESI†), translating into a potential 1000-fold experimental time saving. Overall, Σ-Light-FESTA gives rise to >7 times more intense signals for the H4–H5–H7 spin system compared with a standard ^{1}H NMR spectrum recorded without illumination (Table 1) and is up to 49 times more sensitive than a standard FESTA experiment (Table S3, ESI†). Extra benefits include the ability to select a molecule of interest in complex mixtures, by applying selective ^{19}F and ^{1}H pulses at defined frequencies. As the transfer of magnetisation to other ^{1}H nuclei within a spin system is dependent on the isotropic mixing time (Fig. S4, ESI†), running several experiments with judicious choices of mixing times and summing the resulting spectra can lead to more even distribution of hyperpolarisation.

Finally, although 1D selective TOCSY did not yield satisfactory results for 6FI, in cases where there is an extensive scalar coupling network with appreciable J_{HH} values, a selective TOCSY experiment may be advantageous. Another interesting possibility, currently being explored, is to design a pulse sequence that pools the ^{1}H and the ^{19}F hyperpolarisation, sharing both among their neighbours.

In summary, we have shown that ^{19}F and direct ^{1}H photo-CIDNP enhancements can be shared amongst multiple ^{1}H nuclei within the same spin system, using FESTA or selective TOCSY, with the former being much more effective. Transferring the greater hyperpolarisation of ^{19}F to ^{1}H led to the enhancement of more signals, a more even distribution of signal intensities, and, in contrast to direct ^{1}H photo-CIDNP, preservation of normal multiplet structure. This makes the molecular spectral fingerprint easily recognisable. Light-FESTA not only eliminated the sensitivity penalty of the parent FESTA experiment, with a 34-fold enhancement, but actually increased the sensitivity of proton detection sevenfold compared to a standard ^{1}H NMR spectrum without illumination. Light-FESTA should therefore allow the analysis of molecules at low concentrations while retaining the selectivity advantages of FESTA. This approach will be particularly powerful when investigating fluorinated molecules known to show a photo-CIDNP effect. Such molecules could be used as probes to investigate complex system behaviour, or as constituents of compound libraries for drug screening.[42,43] Sample illumination here used the LED-based NMRtorch, a recent general tool for photo-NMR.[38] The approach presented in this paper can readily be extended to other spectral editing techniques, for example, to observe enhanced through-space interactions, determine molecular proximity, or quantify ligand–receptor interactions, as required in drug screening.[34,44] Although demonstrated using ^{19}F nuclei, Light-FESTA could be extended to achieve selective photo-CIDNP enhancement of ^{1}H using other heteronuclei such as ^{13}C or ^{15}N.[31,33]

The NMRtorch hardware was manufactured by the last author (APG). All authors contributed to the design of the experiments and pulse sequences, the analysis of the results, and the writing of the manuscript.

Table 1 Enhancements of signal integrals of 6-fluoroindole (1 mM) after 2 s illumination in the presence of riboflavin 5′-monophosphate sodium salt. Enhancement factors are provided for direct ^{1}H photo-CIDNP; three Light-FESTA experiments, selecting each in turn of the ^{1}H signals coupled to ^{19}F (H4, H5 and H7); and the result of summing all three Light-FESTA experiments (Σ-Light-FESTA). Enhancement factors were calculated using absolute integrals relative to the dark ^{1}H NMR spectrum

	H4	H1	H7	H5	H2
Direct ^{1}H photo-CIDNP	4.0	3.3	2.1	1.4	32.4
Light-FESTA H5 (6.86 ppm)	5.0	0.3	7.2	16.6	0.8
Light-FESTA H7 (7.16 ppm)	14.9	2.9	4.1	7.2	0.5
Light-FESTA H4 (7.54 ppm)	6.1	0.4	10.9	3.7	0.7
Σ-Light-FESTA	8.7	1.2	7.4	9.2	0.7

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Conflicts of interest

APG is the named author in patent application PCT/GB2021/051254, International Publication Number WO 2021/250372 A1. All other authors declare no competing interests.

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