ChemPubSoc Europe

DOI: 10.1002/cphc.201800570

CHEMPHYSCHEM Communications

Low-cost LED-based Photo-CIDNP Enables Biocompatible Hyperpolarization of ^{19}F for NMR and MRI at 7 T and 4.7 T

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Substrates containing ^{19}F can serve as background-free reporter molecules for NMR and MRI. However, in vivo applications are still limited due to the lower signal-to-noise ratio (SNR) when compared with ^{1}H NMR. Although hyperpolarization can increase the SNR, to date, only photo-chemically induced dynamic nuclear polarization (photo-CIDNP) allows for hyperpolarization without harmful metal catalysts. Photo-CIDNP was shown to significantly enhance ^{19}F NMR signals of 3-fluoro-DL-tyrosine in aqueous solution using flavins as photosensitizers. However, lasers were used for photoexcitation, which is expensive and requires appropriate protection procedures in a medical or lab environment. Herein, we report ^{19}F MR hyperpolarization at 4.7 T and 7 T with a biocompatible system using a low-cost and easy-to-handle LED-based set-up. First hyperpolarized ^{19}F MR images could be acquired, because photo-CIDNP enabled repetitive hyperpolarization without adding new substrates.

Fluorinated substrates are well established drugs and diagnostic pharmaceuticals in many medical applications, such as the antidepressant fluoxetine or the inhalational anesthetic desflurane.[1,2] Even their potential as contrast agents has already been demonstrated.[3–5] Due to the importance of these substrates in medicine, studies on the pharmacokinetics, metabolism, interaction with other biomolecules, or localization of the site of pharmacological action increasingly use MRS and MRI techniques because these enable non-invasive three-dimensional and time-resolved detection of structures and dynamics of NMR-detectable substrates, proteins, and biological tissue. Fluorinated compounds are especially well suited for biomedical imaging, given that under normal physiological conditions no ^{19}F-containing substrates are detectable in living organisms. Hence, fluorinated compounds may act as background-free reporter molecules.[5] But although ^{19}F NMR sensitivity is close to ^{1}H NMR sensitivity, the in vivo concentrations are often too low for detection of applied substances in a biomedically acceptable amount of time. To increase SNR sufficiently to enable detection, hyperpolarization methods such as parahydrogen-induced polarization (PHIP), dynamic nuclear polarization (DNP), chemically induced dynamic nuclear polarization (CIDNP) and photo-CIDNP are studied intensively.[6–8]

However, solvents used in these methods are usually organic, whereas catalysts as well as stable radicals are usually toxic and have to be extracted very quickly before hyperpolarized substances can be administered to a living organism. Although ^{19}F is a naturally abundant nucleus, until now, polarization transfer to ^{19}F in aqueous solutions could not be detected using e.g. parahydrogen-based hyperpolarization methods. Only when using acetone as solvent and Rh-based catalysts, ^{19}F PHIP-based hyperpolarization was sufficient to enable MRI.[9] The polarization factors decreased when increasing the water content.[10]

In order to apply hyperpolarization in biomedicine it would be best to perform the process of hyperpolarization in aqueous solutions with biocompatible non-toxic substrates. Furthermore, MR imaging protocols often utilize repetitive data acquisition to encode spatial information. This requires generating hyperpolarization repeatedly, ideally without adding new substrates.

Only one hyperpolarization technique – photo-CIDNP – enables hyperpolarization of ^{19}F directly in pure water.[2,11,12] The effect is based on irradiation of light (usually by a laser) and induces reversible photochemical reactions due to excitation of a photosensitizer, leading finally to hyperpolarized nuclei via hyperfine interactions. A well-studied system is 3-fluoro-DL-tyrosine, where Kuprov et al. found a ^{19}F 20- to 40-fold signal enhancement after irradiation with an argon laser.[13] This standard approach has the disadvantage that the high intensity of the laser light leads to significant heating of the sample (up to boiling) that may be harmful to biological tissue, thus limiting this otherwise promising technique for biomedical applications.

Recently, Feldmeier et al. presented an interesting alternative, showing that ^{1}H signal enhancement for flavin derivatives dissolved in CD_3CN or CD_3CN/D_2O-mixtures (1:1) can be achieved using a simple LED-based setup.[14,15] Nevertheless,

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/cphc.201800570

physiologically compatible solvents and substrates are indispensable for an in vivo application. Here, we present the first experiments using an LED-based assembly analogous to Feldmeier et al. (see the Supporting Information) for the repetitive generation of enhanced ^{19}F NMR signals of 3-fluoro-DL-tyrosine **1** in D_2O as well as in H_2O in presence of riboflavin 5′-monophosphate sodium salt hydrate **2** (see Figure 1 for

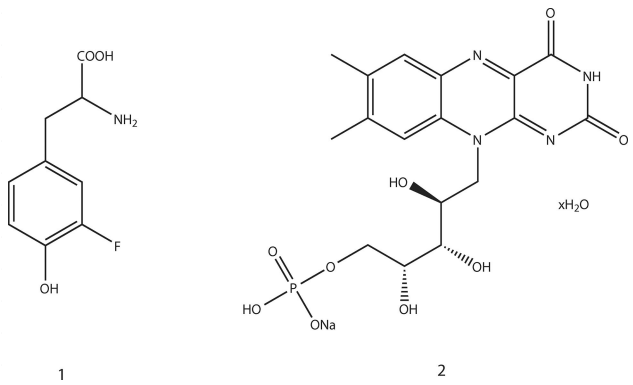

Figure 1. Molecular structures of 3-fluoro-DL-tyrosine **1** and riboflavin 5′-monophosphate sodium salt hydrate **2**.

structure of **1** and **2**). With the application of repetitive irradiation, the multiple enhanced signals could be averaged as in standard NMR/MRI experiments to achieve sufficient SNR.

Furthermore, in contrast to the set-up of Feldmeier et al.,[14] who performed their experiment at 600 MHz (14 T), we acquired hyperpolarized ^{19}F signals in a field of 7 T. The signal enhancement was sufficiently high to acquire hyperpolarized ^{19}F images in a standard preclinical MR scanner at 4.7 T typically used for animal experiments. This is important because photo-CIDNP is field dependent. Grosse et al. showed that higher signal enhancements are detectable in lower magnetic fields.[16]

The light was coupled into a glass fiber of about 5 m length that was inserted into the probe, which remained positioned in the bore during the complete experiment (7 T Bruker NMR spectrometer WB-300) (Figure S3 in the Supporting Information). The maximum output of the diode reached about 872 μW at the tip of the glass fiber). Two different concentrations of 3-fluoro-DL-tyrosine **1** were examined (for details of sample preparation see the Supporting Information).

As shown in Figure 2, the observable ^{1}H NMR signals of the aromatic protons of hyperpolarized 3-fluoro-DL-tyrosine **1** display strong phase dependencies, which can be controlled by irradiation time or concentration. Lower concentration of 3-fluoro-DL-tyrosine **1** increased the signal enhancement when using the same irradiation duration. This is demonstrated for the case of a 2 mM solution of 3-fluoro-DL-tyrosine **1** and 6 s irradiation time, where negative phase signals can be clearly observed. A doubling of the 3-fluoro-DL-tyrosine **1** concentration leads to these signals having a positive sign. With increasing irradiation, some of the signals exhibit a change in the sign of the phase (see Figure 2).

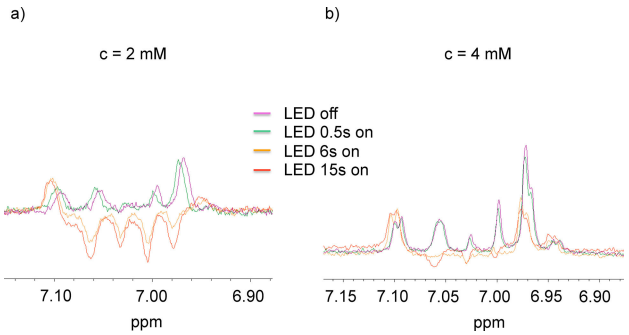

Figure 2. Section of the ^{1}H NMR spectra of a) 2 mM and b) 4 mM 3-fluoro-DL-tyrosine **1** and 0.21 mM riboflavin 5′-monophosphate sodium salt hydrate **2** dissolved in D_2O. For the measurements a 90° pulse was used. The displayed signals were assigned to 3-fluoro-DL-tyrosine **1** (see the Supporting Information).

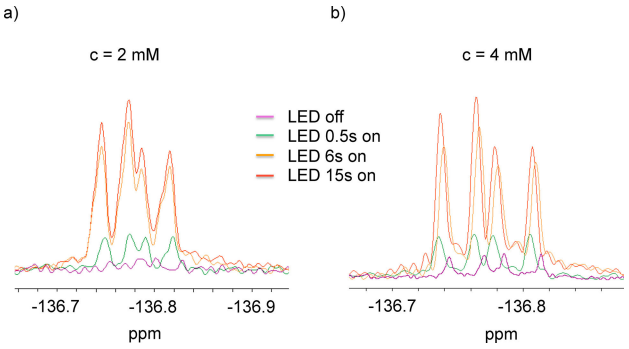

Figure 3. ^{19}F NMR spectra of a) 2 mM and b) 4 mM hyperpolarized 3-fluoro-DL-tyrosine **1** and 0.21 mM riboflavin 5′-monophosphate sodium salt hydrate **2** dissolved in D_2O. The thermic signal (violet, no hyperpolarization) serves as a reference for calculating the signal enhancement (8 for 2 mM and 4 for 4 mM).

In contrast to the ^{1}H signals, the ^{19}F signal ($\delta = -136.78$ ppm) exhibited quite strong signal enhancement (SE) of about 14-fold for a concentration 2 mM of 3-fluoro-DL-tyrosine **1** solution (Figure 3a) and an SE of about 7 for a concentration 4 mM of 3-fluoro-DL-tyrosine **1** (Figure 3b). The phases of the signals were always positive. To determine the maximum SE the irradiation time was varied between 0.5 s and 15 s. With our experimental set-up the SE was almost in saturation after an irradiation time of 6 s.

In addition to 3-fluoro-DL-tyrosine, the effect of light irradiation on the ^{19}F NMR signal of 2-fluoro-DL-tyrosine was investigated. As shown in the supporting information, no ^{19}F MR signal enhancement can be detected. Rather, a reduction of the signal intensity is observed with increasing light irradiation when fluorine is in ortho-position.

Hyperpolarization of ^{19}F in 3-fluoro-DL-tyrosine **1** was previously investigated by Kuprov et al.[13] and by Güden-Silber et al.[17] Using an argon laser with maximum output power of 25 W Kuprov found an SE of up to 40-fold for ^{19}F.[13] The authors also observed ^{1}H hyperpolarization of the nucleus ortho-standing to fluorine. The ^{1}H signal exhibited positive and negative changes in the phase as a function of irradiation time. The signals after about 6 s irradiation time (Figure 2A in Ref. [13])

agree well with our results. Güden-Silber et al. showed that air-cooled laser diode (10 W) generated photo-CIDNP of 7 mM 3-fluoro-DL-tyrosine with 0.2 mM FMN in water even allowed for spatially resolved MRI using a multi chemical shift-selective imaging (mCSSI) sequence.[17]

Both these results and our own successful spectroscopic studies motivated us to test whether the measured signal enhancement of ^{19}F would allow for imaging of the hyperpolarized 3-fluoro-DL-tyrosine **1** at a field strength of 4.7 T (200 MHz) using the same concentrations and the same LED-based set-up as in the spectroscopic study (for experimental details see the Supporting Information). To improve biocompatibility, D_2O was replaced with a physiologic salt solution with H_2O as solvent. First, ^{1}H MR images (Figure 4a) were acquired to locate

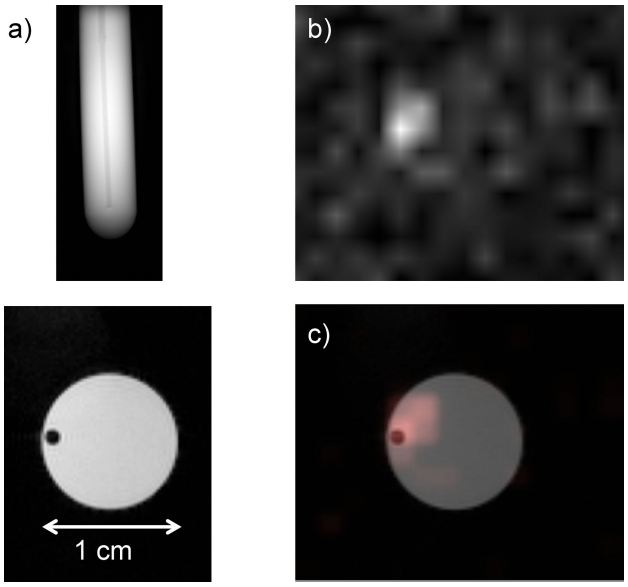

Figure 4. Representative ^{1}H and ^{19}F MR images of a physiologic salt solution containing 2 mM 3-fluoro-DL-tyrosine **1** and 0.21 mM riboflavin 5′-monophosphate sodium salt hydrate **2** were acquired with a multi-spin echo sequence (RARE) at 4.7 T (200 MHz, Bruker animal scanner; 1 average, TE = 14 ms, TR = 5000 ms, field of view = 50 × 50 mm, matrix = 256 × 256, slice thickness: 2 mm (axial) or 20 mm (sagittal), RARE factor = 8): a) sagittal view with of the sample filled in a 10 mm NMR tube. The optical fiber is visible in both images. b) ^{19}F image of the same sample after hyperpolarizing the 3-fluoro-DL-tyrosine (continuous irradiation during experiment; measurement at ^{19}F tyrosine signal of 188.5330369 MHz using a RARE sequence, 256 averages, TE = 14 ms, TR = 1000 ms, field of view = 50 × 50 mm, matrix = 32 × 32, slice thickness = 20 mm, RARE factor = 8). c) ^{19}F image (b) color-encoded and overlaid onto ^{1}H image (a). No MRI signal was detectable without irradiation (same conditions).

the optical fiber used for irradiation within the probe and to finally allow for matching the ^{19}F images to the spatially higher resolved ^{1}H images. As expected, no ^{1}H signal enhancement was detectable, irrespective of irradiation time, while the ^{19}F was sufficiently enhanced so that a ^{19}F image could be acquired (see Figure 4b and 4c). The ^{19}F signal in the thermal polarized sample falls below the detection limit under the according conditions.

Summarizing, we showed in our study that the ^{19}F hyperpolarization is strongly dependent from the position of the substituent (ortho/meta). Furthermore, it is shown that a low-cost LED set-up can be used to increase the ^{19}F NMR signal of 3-fluoro-DL-tyrosine in an isotonic salt solution in presents of a photosensitizer. This is very helpful for potential future medical applications because the light provided by the LED is much safer to apply than laser light (e.g. no detectable heating of the sample, lower safety level). Additionally, the sample is not heated up as when using laser irradiation.

The lower signal enhancement when compared with DNP or PHIP hyperpolarization techniques is counter-balanced by two advantages: (1) In contrast to DNP and PHIP, where nuclei with long T_1 relaxation times are often hyperpolarized, the sample mixture used in the current study is biocompatible and no further purification is necessary. In case of DNP examinations a free radical is used and the sample has to cool down to $\approx$1.1 K.[18,19] Even if commercial machines are available that heat the DNP sample and remove the harmful solvent and radical within a very short time, there is still a period of time between hyperpolarization and measurement and that is too long for ^{19}F signal enhancements. Nevertheless, a first clinical study demonstrated the potential of DNP for ^{13}C.[20] (2) More critically, DNP and PHIP require adding new substances in order to repeat the hyperpolarization step. In contrast, photo-CIDNP is a cyclic process that can be repeated many times without adding new substrates, limited solely by the transfer products or photo bleaching that ultimately renders the hyperpolarization process inefficient. Thus, standard NMR and MRI experiments based on repetitive excitation and data acquisition steps can be much more easily applied in photo-CIDNP than in PHIP and DNP. Photo-CIDNP also allows a fast and direct spatial encoding by illuminating different parts of the sample.[21] It was recently shown that ^{19}F photo-CIDNP allows for the detection of a diluted p-fluorophenol sample containing only 0.8 pmol/μL of the fluorinated substrate.[22] CIDNP is field dependent[23] but it was shown that hyperpolarization can be achieved over a wide range covering the typical field strengths of clinical MRI scanners between 1.5 and 3 T.[24–26] Recently, 7 T MRI were introduced into clinical routine, and first ^{19}F signals were acquired from whole body 7 T scanners.[27] Our results therefore support the expectation that LED-based hyper-polarization may serve as a new innovative research and diagnostic technique in biomedical applications.

Experimental Section

Experimental details are described in the Supporting Information.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG BE 1824/12-1).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: fluorine · 3-fluoro-DL-tyrosine · hyperpolarization · NMR spectroscopy · photo-CIDNP

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Manuscript received: June 17, 2018
Accepted Article published: June 26, 2018
Version of record online: ■■, ■■■■