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Dissolution DNP for *in vivo* preclinical studies

Arnaud Comment *

[a] General Electric Healthcare, Pollards Wood, Nightingales Lane, Chalfont St Giles, Buckinghamshire HP8 4SP, United Kingdom
[b] Institute of Physics of Biological Systems, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland

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ABSTRACT

The tremendous polarization enhancement afforded by dissolution dynamic nuclear polarization (DNP) can be taken advantage of to perform preclinical *in vivo* molecular and metabolic imaging. Following the injection of molecules that are hyperpolarized via dissolution DNP, real-time measurements of their biodistribution and metabolic conversion can be recorded. This technology therefore provides a unique and invaluable tool for probing cellular metabolism *in vivo* in animal models in a noninvasive manner. It gives the opportunity to follow and evaluate disease progression and treatment response without requiring *ex vivo* destructive tissue assays. Although its considerable potential has now been widely recognized, hyperpolarized magnetic resonance by dissolution DNP remains a challenging method to implement for routine *in vivo* preclinical measurements. The aim of this article is to provide an overview of the current state-of-the-art technology for preclinical applications and the challenges that need to be addressed to promote it and allow its wider dissemination in the near future.

1. Introduction

New technologies often create two clashing waves of over-optimism and skepticism. Hyperpolarized magnetic resonance (MR) was clearly no exception to this rule. Many among the early pessimists have now changed their mind and some of the overenthusiastic medical and biomedical researchers were somewhat disappointed by the unanticipated difficulties. However, the preclinical studies that are ongoing at the time I am writing this manuscript are expected to further demonstrate that it is possible to noninvasively detect biological processes *in vivo* in a way that only *true believers* had anticipated.

Today, it therefore seems quite straightforward to answer the question raised at the 2008 annual congress of the European Society for Magnetic Resonance in Medicine and Biology (ESMRMB, Valencia, Spain) during the roundtable discussion, namely "*Hyperpolarization or hype and polarization?*". Hopefully, this perspective article will convince the outstanding pessimists that they should consider investing in this technology, in particular in dissolution dynamic nuclear polarization (DNP) for preclinical applications.

2. Hyperpolarization by dissolution DNP in a nutshell

Although this is most likely the section you may want to skip if you are already familiar with this technology, I will attempt to describe what hyperpolarization is as briefly as possible and review part of the jargon that has been established in the field.

The term *hyperpolarization* was originally introduced in the MR community by researchers working on noble gas MRI [1], notably ^{3}He and ^{129}Xe. When it relates to liquid-state NMR, *hyperpolarized* refers to a nuclear spin state that is obtained after either (1) a rapid and large temperature jump through dissolution DNP [2] or the *brute force* method, which has also been proposed for hyperpolarizing noble gases [3], or (2) spin order transfer from parahydrogen through a method called parahydrogen-induced nuclear polarization (PHIP) [4–6]. While the brute force method only relies on the Boltzmann polarization that is obtained after reaching thermal equilibrium at low temperature and high magnetic field, dissolution DNP makes use of electron spins to dynamically boost the nuclear polarization up to a very high level without having to reach temperatures below about 1 K. The PHIP method also relies on low temperature to favor a specific spin state of dihydrogen, namely the parahydrogen singlet state, but the target molecule that needs to be polarized for the MR experiments does not experience any temperature jump. Instead, a room-temperature chemical reaction transfers spin order from parahydrogen to the molecule of interest and the insensitive nuclei (typically ^{13}C) located in the molecule are subsequently polarized via a low-field polarization transfer [7].

* Address: General Electric Healthcare, Pollards Wood, Nightingales Lane, Chalfont St Giles, Buckinghamshire HP8 4SP, United Kingdom.
E-mail addresses: arnaud.comment@ge.com, arnaud.comment@epfl.ch

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The highest liquid-state polarizations that have been reported so far were obtained using dissolution DNP [8,9]. The principle of DNP is to make use of the much larger gyromagnetic ratio of electron spins to enhance the nuclear spin polarization through cross-polarization driven by a slightly off-resonance irradiation of the ESR line. Two fundamental physical parameters make it possible to obtain nuclear polarizations close to unity in a field of 3 T or above and at a temperature of about 1 K: (a) the electron spin polarization is essentially 1 (Fig. 1), and (b) the electron spin relaxation is long enough to allow the saturation of the ESR and hence promote cross-polarization with adjacent nuclear spins (Fig. 2). The unpaired electron spins, which are also referred to as *polarizing agents* in the context of dissolution DNP, are usually introduced in the form of persistent radicals into the sample prior to cooling and solidifying the sample in a glassy form. To date, the most efficient polarizing agents are trityl or 1,3-bisdiphenylene-2-phenylallyl (BDPA) radicals [2,10], although nitroxyl radicals have also been used for *in vivo* preclinical work [11–13], and a new type of non-persistent photo-induced radicals show promising features [14,15].

The original hyperpolarizer was operating at 3.35 T [2], but it was later shown that larger polarizations can be obtained in a field of 5 T [16,17] or 7 T [18]. As a rule of thumb, at least for samples containing wide-ESR-line-width polarizing agents such as nitroxyl radicals, the maximum polarization that can be achieved at a working temperature of about 1 K is inversely proportional to the temperature [19]. Therefore, within the parameter space explored so far by the researchers working on dissolution DNP, increasing the field and lowering the temperature will lead to larger polarizations. Note that the operating field and temperature also strongly affect the dynamics of DNP and the polarization build-up times will also increase with increasing field and decreasing temperature.

3. What unique information can be gathered using *in vivo* hyperpolarized MR

Before going further into some of the technical aspects of the dissolution DNP method, I would like to give a succinct overview of the assets of hyperpolarized MR in the context of *in vivo* preclinical applications.

Hyperpolarized MR provides a unique way to measure metabolism in real time. No other technique allows probing metabolic transformations *in vivo* with the time resolution of one second.

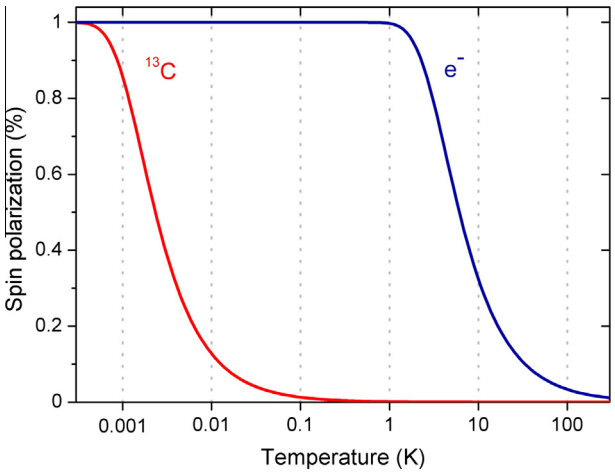

Fig. 1. Theoretical ^{13}C (red curve) and electron (blue curve) spin polarization at 5 T as a function of temperature. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

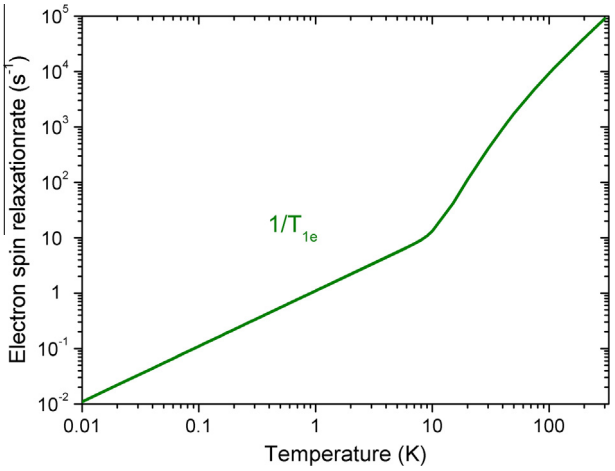

Fig. 2. Electron spin longitudinal relaxation rate ($1/T_{1e}$) for nitroxyl radicals at 0.35 T calculated as a function of temperature from the expression given in [74]. Note that T_{1e} is expected to have a similar temperature dependence at higher magnetic field and for other types of radicals [75,76].

The essential point is that the largely enhanced nuclear polarization affords sufficient sensitivity to acquire, in addition to the spectral information that allows distinguishing the various metabolites, high-spatial and high-temporal resolution data. Therefore, metabolic pools of relatively small size can be detected in specific areas of the body under investigation with high temporal resolution. It must be underlined that the spatial and temporal resolutions are nevertheless both restricted by the available signal-to-noise ratio (SNR): the higher the spatial resolution, the lower the temporal resolution, and vice versa.

Several recent reviews have covered the various preclinical applications performed to date with the most prominent metabolic precursors [20,21], in particular in the context of cancer research [22,23], and I refer the readers to these articles if they wish to have a complete view of the current state-of-the-art biomedical applications. In the present perspective article, I will focus on one of the most interesting yet challenging substrate, namely ^{13}C-glucose. It will be taken as case study to highlight the shortcomings of dissolution DNP and discuss how to address them.

Only one *in vivo* metabolic study with hyperpolarized ^{13}C-glucose has been so far published [24], but it is certainly not because of a lack of interest or relevance. Glucose is a substrate of major interest for at least two main reasons: first, because it is an essential fuel for mammals, in particular for their brain; second, because upregulated glycolysis is a hallmark of most cancer cells. In fact, the latter partially explains why the first *in vivo* results were obtained in a cancer model [24]. The first *in vitro* experiments have already been performed several years ago in *Escherichia coli* and yeast [25,26], and more recently in cancer cells [27,28]. However, in order to achieve the necessary sensitivity to generally observe glucose metabolites *in vivo* in rodents, several challenges must be overcome.

Positron emission tomography (PET) following the administration of the ^{18}F-fluorodeoxyglucose glucose analog (FDG) is currently considered as one of the most established modalities to detect tumors and to evaluate their progression and the impact of treatments. FDG-PET provides information on glucose uptake, but it does not allow measuring the fate of glucose once it has entered the cells and undergone the phosphorylation process catalyzed by hexokinase. Hyperpolarized MR therefore brings a unique way of performing *true* metabolic imaging since it will report on glycolysis well beyond cellular uptake and yield spatiotemporal information on the metabolites deriving from the pre-