Appendix E1

In this appendix, we include a glossary of terms and a more complete introduction to Overhauser DNP. We also give further details about our equipment.

Glossary

DNP: Dynamic nuclear polarization; a method of increasing the MR signal intensity of nuclei by transferring the higher polarization of an unpaired electron or free radical to the nuclei.

Electron spin resonance: Spectroscopy on unpaired electrons.

Enhancement: The amount of MR signal intensity increase observed after the application of a hyperpolarization procedure.

Free radical: An unpaired electron on an organic molecule.

Fringe field: The magnetic field outside of the homogeneous location of the magnetic field used for imaging or spectroscopy.

Hyperpolarization: The use of methods to artificially increase the polarization of MR-active nuclei beyond what is available with thermal polarization.

Magnitude image: Combination of the real and imaginary parts of a complex MR image, removing phase information, the default format for anatomical imaging.

Overhauser DNP: DNP that occurs by means of the Overhauser effect.

Overhauser effect: Transfer of polarization from a species with a higher gyromagnetic ratio to one with a lower gyromagnetic ratio by means of relaxation processes involving both species, mediated by rapid molecular motion. Examples include DNP (electron to ^{1}H) and the nuclear Overhauser effect (nuclear Overhauser enhancement, ^{1}H to ^{13}C).

Polarization: The difference in nuclear or electron populations between the two allowable Zeeman energy states when the nuclei or electrons are placed in an external magnetic field (assuming that they are spin 1/2 particles). Greater polarization leads to larger MR signal intensity.

Processed image: A term used to refer to our custom method of creating phase-sensitive images from the time domain raw data, in which the enhanced, inverted signal is shown in color and the unenhanced signal is shown in grayscale.

RELIC: Remotely enhanced liquids for imaging contrast. The technique in which a liquid (usually water) is hyperpolarized outside of the MR imaging location and then transferred or injected into the sample or subject during MR imaging to provide contrast between the inflowing liquid and the bulk liquid signal already present.

Spin-density image: An image that is not T1 or T2 weighted, in which the intensity of the image represents the relative number of nuclei in the voxel.

Thermal polarization: The polarization of a spin system at equilibrium at a given magnetic field and temperature (ie, steady-state polarization of a system at equilibrium before any hyperpolarization techniques are applied). Thermal polarization (P) increases linearly with

increasing magnetic field and decreases with increasing temperature according to the following equation:

$$P = \frac{\hbar \gamma B_0}{2k_B T}$$

where $\hbar$ is the reduced Planck constant, γ is the gyromagnetic ratio of the nuclei, B_0 is the magnetic field, k_B is the Boltzmann factor, and T is the temperature of the system.

Unpaired electron: An electron that is unpaired in an atomic or molecular orbital. Unpaired electrons have a net magnetic moment and can be used for electron spin resonance spectroscopy or DNP. Unpaired electrons can be found on transition metal ions (eg, gadolinium ion [Gd^{3+}]), on specially prepared organic spin labels (eg, nitroxides) or other organic molecules.

Overhauser DNP Introduction

Magnetic resonance is a versatile group of methods used in a wide range of disciplines, from the physical sciences to medicine. However, magnetic resonance is inherently insensitive, which leads researchers to develop hyperpolarization methods to increase the sensitivity of an MR experiment.

MR spectroscopy and imaging rely on information from the nuclear spin, which is a property intrinsic to many nuclei that causes them to behave like an atomic magnet. For spin 1/2 nuclei (hydrogen 1 [^{1}H], carbon 13 [^{13}C], phosphorus 31 [^{31}P]) placed in an external magnetic field, the nuclear spins will align either with or against the magnetic field, creating a two-state system (this visualization is an approximation, but is sufficient for our purposes here). The energy difference between these two spin states determines the precession frequency of the nuclei, which is measured in a magnetic resonance experiment.

To conduct an MR experiment, there must be some difference in the populations of the nuclear spin states, because nuclei need to move between the two spin states during an MR measurement. However, because the energy difference between these two spin states is much smaller than the substantial thermal energy available at room temperature, these two spin states are almost equally populated (ie, almost as many nuclear spins are aligned with the field as are aligned against it). In fact, only approximately one of every million nuclear spins is in excess in the lower energy state at room temperature and conventional magnetic fields, (ie, only one nucleus out of every million nuclei in the sample contributes to the observed MR signal). The population difference between the two energy levels is known as the *polarization* of the system, and because the population difference depends on temperature, it is known as *thermal polarization*. The methods that increase the sensitivity of NMR and MR imaging are known as *hyperpolarization* methods, because they increase the polarization of the system beyond what is conventionally available. Because greater polarization leads to greater observed MR signal intensity, this has many obvious and important advantages.

DNP is a hyperpolarization method that relies on the interaction between the nuclei of interest and an unpaired electron (free radical) species. Electrons are spin 1/2 particles, and the subject of their own magnetic resonance spectroscopy known as electron spin resonance or electron paramagnetic resonance. Because the gyromagnetic ratio of an electron is 658 times larger than that of ^{1}H, the energy difference between the two electron spin states and the population difference is much larger, leading to increased polarization. The goal of DNP is to

transfer the much greater polarization of free radicals to nearby nuclei of interest, thus dramatically increasing the polarization and MR signal intensity of the target nuclei. The term DNP refers to any of the methods for hyperpolarization in which the interaction between unpaired electron spins and nuclei are used, while Overhauser DNP specifically refers to DNP that proceeds via the mutual (cross) relaxation between the electron and nuclear spins that is facilitated by molecular or particle motion. The cross relaxation mechanism behind Overhauser DNP also functions between two nuclei; in this instance it is known has the nuclear Overhauser effect and has applications for enhancing ^{13}C MR imaging signal intensity via nearby ^{1}H nuclei.

To perform hyperpolarization via Overhauser DNP, the free radicals and nuclei of interest must be brought in close contact, usually by dissolving the radicals in solution with the nuclei of interest. Then, the electron spin resonance transition of the free radical is saturated by continually applying electromagnetic radiation at the resonance frequency of the electron. This causes the population of the two electron spin states to equalize. If the target nuclei are in close contact with the radical, a through-space cross relaxation occurs, which means that both a nuclear and electron spin simultaneously change spin states, slightly changing the population of all spin states involved. Because the electron spins are under continuous saturation, the electron populations again equalize, but the nuclear spin state remains slightly altered. This process repeats many times, leading to a nonequilibrium buildup of nuclear polarization, known as hyperpolarization, which is observed as dramatically increased MR signal intensity. The decay of the hyperpolarized nuclear spin state occurs on the T_{1n} timescale, which is much slower than the cross relaxation processes, allowing for the buildup of enhanced nuclear polarization. When electrons and ^{1}H are involved in the Overhauser DNP process, the enhanced nuclear polarization is opposite to the thermal polarization, giving rise to a higher population of nuclear spins in the higher energy state. This leads to observed MR signal of opposite phase (sign) from the unenhanced (thermally polarized) signal. For Overhauser DNP, the polarization buildup rate is dictated by the proton T_{1n} of the radical solution, so that hyperpolarization is achieved in less than 1 second for the specific radical system used here. Further mechanistic details and examples of Overhauser DNP are available in a recent review (27).

Hyperpolarization Equipment

We perform hyperpolarization at 0.35 T for two reasons. First, Overhauser DNP produces maximum water polarization between 0.35 and 0.9 T (see Fig 1b of reference 24). Second, the instrumentation to achieve the required electron spin resonance saturation at 0.35 T, and thus, efficient DNP, is relatively available and easy to work with, owing to the development of radar devices at these frequencies. Because it is desirable to minimize transit times between hyperpolarization and delivery to the subject, the 0.35-T fringe field location of the MR imaging magnet was used for hyperpolarization, because this was the closest possible location to the subject in the center of the MR imaging magnet.

At 0.35 T, the electron transition frequency is 9.8 GHz (in the microwave region of the electromagnetic spectrum), and this is the frequency at which the water and immobilized radical mixture needs to be irradiated with high power. Electromagnetic waves at 9.8 GHz are not easily transmitted through coaxial cable and antennas without substantial loss, thus metal waveguides and resonant microwave cavities are required instead. In our system, microwave radiation was generated by a yttrium iron garnet synthesizer (MLSL-1178; Microlambda Wireless, Fremont, Calif) and amplified with a custom-built amplifier (33) to produce approximately 23 watts of

power. This power level was nearly optimal for our cavity design, because higher power levels boil the water in the microwave cavity. The microwaves from the amplifier traveled through a WR90 waveguide to a custom-built, fixed-frequency TE_{102} resonant microwave cavity located in the 0.35-T fringe field position of the MR imaging magnet. Here the microwave radiation is transmitted to the water and immobilized radical mixture to perform DNP. Because the field homogeneity requirements for DNP are much lower than those for imaging, a simple linear gradient coil (1.7-ampere current, approximately 1 T/m) was used as a shim system around the microwave cavity, providing ^{1}H line widths of less than 70 kHz in the fringe-field location; these ^{1}H line widths are sufficiently narrow to not limit the DNP performance.

In our experiment, we enhanced the ^{1}H MR signal of liquid water by using nitroxide radicals that had been immobilized to a solid support (agarose beads). The dipolar (physical) contact between the water and radical and their molecular dynamics were not substantially altered by the immobilization process, allowing for efficient Overhauser DNP enhancement approaching the enhancement levels of a solution of radicals. The radicals were immobilized to agarose beads, which were held in the hyperpolarization location (0.35-T fringe field) while water was continuously passed through and over the immobilized radical beads, allowing for the production of radical-free hyperpolarized water. This continuously-produced hyperpolarized water was injected into the subject located in the center of the MR imaging magnet (1.5 T) to produce unique flow perfusion contrast on an MR image. The immobilized nitroxide radicals used in this work were produced by means of a custom synthesis process, as described previously (24). This formulation of immobilized radicals is the first to provide Overhauser DNP enhancement levels of approximately 100-fold at 0.35 T, comparable to levels achieved with nitroxide radicals freely dissolved in solution.

The flow system consisted of low-pressure high performance liquid chromatography tubing and connectors (VICI, Houston, Texas or Upchurch Scientific/Idex, Oak Harbor, Wash), and the water flow was driven by a high performance liquid chromatography pump (Pharmacia P-500, now GE Life Sciences/GE Healthcare, Piscataway, NJ). The immobilized radicals were held in a custom-built polymeric cell inside the microwave cavity, which contained a filter to hold the immobilized radicals inside the cavity while allowing water to flow through. The connection from the hyperpolarization to the imaging location was made with 1/8-inch outside diameter, 0.006-inch inside diameter polymeric tubing, where water traveled approximately 1.5 m from the hyperpolarization to the imaging location in approximately 1.3 sec at a flow rate of 1.5 mL/min. The water leaving the microwave cavity was heated with coiled resistive heating wire (Omega, Stamford, Conn), which warmed the water to 37°C at injection and increased the relaxation time constant T_1 during transit. Degassed deionized water was used for all experiments. A homebuilt MR spectroscopic coil and portable MR spectrometer (Kea; Magritek, Wellington, New Zealand) were used for cavity positioning.

Reference

33. Armstrong BD, Lingwood MD, McCarney ER, Brown ER, Blümler P, Han S. Portable X-band system for solution state dynamic nuclear polarization. J Magn Reson 2008;191(2):273–281.