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pCEST: Positive contrast using Chemical Exchange Saturation Transfer

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ABSTRACT

Chemical Exchange Saturation Transfer (CEST) contrast utilizes selective pre-saturation of a small pool of exchanging protons and subsequent detection of the decrease in bulk water signal. The CEST contrast is negative and requires detection of small signal change in the presence of a strong background signal. Here we develop a Positive CEST (pCEST) detection scheme utilizing the analogous nature of the CEST and off-resonance $T_{1\rho}$ experiments and exploring increased apparent relaxation rates in the presence of the selective pre-saturation. pCEST leads to the positive contrast, i.e., increased signal intensity as the result of the presence of the agent and RF pre-saturation. Simultaneously substantial background suppression is achieved. The contrast can be switched "ON" and "OFF", similar to the original CEST.

1. Introduction

Chemical Exchange Saturation Transfer (CEST) can be used to modify MR image contrast [1–4]. This approach is based on saturating a small pool of exchanging protons, endogenous or exogenous, and subsequent observation of the reduction of the water signal due to the exchange of the saturated spins with water. In a typical CEST experiment using small organic molecules, slowly exchanging —NH or —OH groups are pre-saturated via the application of selective RF irradiation, CW or pulsed. The CEST approach offers a variety of attractive features: it can be switched on and off at the operator's discretion; and, it can provide amplification so that some metabolites can be detected even when present at concentrations that are not usually observable in MRI [5]. Since the RF irradiation is applied at the characteristic frequency of an exchanging proton, multiple functional groups can be imaged simultaneously or sequentially [6]. Finally, the microenvironment of the functional group can affect CEST contrast. This has been exploited to image differences in pH or the presence of local metabolites [1,3–5,7–23].

There are two categories of CEST agents: (i) endogenous or exogenous diamagnetic molecules containing functional groups with exchanging protons such as —OH or —NH, in mobile proteins, peptides or small organic molecules – DIACEST [5,8,10–15,23]; and (ii) exogenous paramagnetic lanthanide (III) complexes containing exchanging —NH group or water molecules that exchange from the coordination sphere of the lanthanide – PARACEST[16,24–26]. PARACEST agents have a number of potential advantages. Because of larger frequency shifts between the exchanging resonance(s) and the water resonance, PARACEST agents can exhibit a wide range of exchange lifetimes (from μs–ms) and remain in the slow-to-intermediate exchange regime on the NMR time scale [25,27–31]. PARACEST agents with rapid exchange rates theoretically allow detection of much lower concentrations than typical for DIACEST agents. PARACEST agents have also been designed to report important biological indices, including: pH [16,17,32], temperature [18,33], metal ions [34,35], lactate [26] and glucose concentrations [19], and enzyme activity [20,21]. They have also been used for protein [36] and cell labeling [22]. The generation of the contrast effect may, however, require higher RF powers for PARACEST than DIACEST, potentially leading to SAR limitations in *in vivo* studies.

CEST contrast is negative, i.e., the water proton signal *decreases* in tissue areas of interest. Typical changes in intensity that must be detected are small, on the order of 5%. When such small signal changes must be detected, background noise and background artifacts can strongly influence the observed effect. Background suppression techniques are widely used in MRI. In particular, Arterial Spin Labeling (ASL) employs background suppression to improve image quality [37–39]. ASL is of particular relevance to CEST since both techniques need to detect small signal changes and both employ pair-wise image subtraction: "on"/"off" images in the CEST case and "tagged"/"nontagged" in the ASL case. It was shown in the ASL case, that despite the background signal subtraction, instabilities in the background signal can still add substantial noise to the difference image [37]. These artifacts can be reduced by the suppression of the background signal. Such suppression in CEST studies will allow better utilization of the dynamic range to collect only a "useful" signal, i.e., the small signal originating from the

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contrast agent of interest. In ASL, background suppression is achieved by the application of multiple inversion pulses [37–39], a technique that is not directly applicable to CEST. Since CEST contrast is negative, it cannot be detected if the overall signal is suppressed. Positive CEST contrast is essential to employ simultaneous background suppression.

There are several examples where contrast agents or techniques originally developed to create negative contrast were re-designed to create positive contrast. One example is superparamagnetic iron oxide (SPIO) particles [40–44]. Originally, these particles were imaged using negative contrast generating sequences, but more recently, techniques generating positive contrast were created and are now used widely [40–44].

Here, we describe a general approach to create Positive CEST signal (pCEST). The method utilizes a modification of the off -resonance spin-lock experiment. It uses saturation-transfer induced changes in relaxation rates to generate positive contrast. This concept was briefly mentioned by Ward et al. [3], but, to our knowledge, has never been realized in practice. The method employs an inversion pulse followed by a saturation pulse and leads to a substantial reduction of the background signal. In this paper, the method is presented in detail and demonstrated *in vitro* with PARACEST agents.

2. Theoretical background

In this section we will overview concepts and theoretical background relevant to the proposed detection scheme. The chemical exchange of magnetization between two pools, A and B, can be described by the Bloch–McConnell equations. In the presence of RF irradiation, the equations in the RF rotating frame are given by [45]:

$$\begin{aligned}
\frac{d}{dt}M_x^A &= -\rho_{2A}M_x^A - \Delta_A M_y^A + k_B M_x^B \\
\frac{d}{dt}M_y^A &= \Delta_A M_x^A - \rho_{2A}M_y^A + \omega_1 M_z^A + k_B M_y^B \\
\frac{d}{dt}M_z^A &= -\omega_1 M_y^A - \rho_{1A}M_z^A + k_B M_z^B + R_{1A}M_0^A \\
\frac{d}{dt}M_x^B &= -\rho_{2B}M_x^B - \Delta_B M_y^B + k_A M_x^A \\
\frac{d}{dt}M_y^B &= \Delta_B M_y^B - \rho_{2B}M_y^B + \omega_1 M_z^B + k_A M_y^A \\
\frac{d}{dt}M_z^B &= -\omega_1 M_y^B - \rho_{1B}M_Z^B + k_A M_x^B + R_{1B}M_0^B
\end{aligned} \tag{1}$$

where

$$\begin{aligned}
&\rho_{1A} = R_{1A} + k_A, \rho_{2A} = R_{2A} + k_A \\
&\rho_{1B} = R_{1B} + k_B, \rho_{2B} = R_{2B} + k_B \\
&\Delta_{A,B} = \omega_{A,B} - \omega_{RF}
\end{aligned}$$

In these equations, ω_{RF} and ω_1 are the frequency, and amplitude of the RF irradiation (in rad units), respectively; Δ_A is the chemical shift offset from RF frequency for pool A, R_{1A} $(=1/T_{1A})$ is the spin lattice relaxation rate of pool A; $R_{2A}(=1/T_{2A})$ is the transverse relaxation rate of pool A; τ_A is the mean lifetime of a proton in A; and, $k_A = 1/\tau_A$ is the transition rate of a nuclei leaving pool A. Similar definitions apply to B. In the following we will designate the free water as pool A and the agent-bound water as pool B. The value of k_A is defined by the detailed–balance relationship:

$$\begin{aligned}
k_A &= \left(\frac{M_0^B}{M_0^A}\right)k_B = p_B k_{ex} \\
k_B &= \left(\frac{M_0^A}{M_0^B}\right)k_A = p_A k_{ex} \\
k_{ex} &= k_A + k_B
\end{aligned} \tag{2}$$

M_0^A and M_0^B are the equilibrium magnetizations and p_A and p_B are the fractional populations of pools A and B, respectively (with p_A=M_0^A/(M_0^A+M_0^B) and p_B=M_0^B/(M_0^A+M_0^B)). The chemical shift difference between the two pools is $\Delta\omega = \omega_B - \omega_A$. Our representation is in the RF rotating frame; hence, when the RF frequency is applied on -resonance with pool B: $\omega_{RF} = \omega_B$ and $\Delta_B = 0$. We designate this case (Fig. 1, panel a) by "RF ON". "RF OFF" will designate the case when the RF is applied at the frequency $-\omega_B$ with respect to water (i.e., $\omega_{RF} = \omega_A - \Delta\omega$, $\Delta_A = \Delta\omega$ and $\Delta_B = 2\Delta\omega$).

The solution of Eq. (1) yields magnetization as a function of the experimental RF saturation parameters: off-resonance values (Δ_A, Δ_B), intensity (ω_1) and the RF pulse length (T_{pw}). In a typical CEST experiment, a detection RF pulse is applied after the saturating RF pulse and the Z-magnetization is measured (Fig. 1, panel b). The intensity, I, measured in the experiment is dependent on the three above mentioned RF parameters and is normalized by the signal intensity at the absence of RF preparation, I_0. The display of I/I_0 as a function of RF frequency with respect to water ($\Delta_{RF} = \omega_{RF} - \omega_A$), at fixed ω_1 and T_{pw}, is called the Z-spectrum. These saturation transfer experiments can be transient, i.e., with $T_{pw} < T_{1a}$, T_{1b}, or steady-state, with $T_{pw} \gg T_{1a}$, T_{1b}. Before translation to imaging, these experiments were widely known and used extensively in dynamic studies of solutions of various small molecules and proteins (e.g., see Ref. [46–52]).

In an off-resonance spin-lock experiment, the RF is applied and the relaxation times are measured parallel ($T_{1\rho} = 1/R_{1\rho}$ or perpendicular ($T_{2\rho} = 1/R_{2\rho}$) to the effective field [53–55]. The dynamics of magnetization, which are described by Eq. (1), are analogous for CEST and off-resonance $T_{1\rho}$ or $T_{2\rho}$ experiments. The experiments differ in the initial condition, observable operator and whether the observation is transient or in steady-state. In a CEST experiment, the initial magnetization is always parallel to the Z-axis while in the $T_{1\rho}$ ($T_{2\rho}$) experiment it is parallel (perpendicular) to the effective field. For CEST, I/I_0 is typically measured at a single fixed T_{pw}, while in the $T_{1\rho}$ ($T_{2\rho}$) experiment the measurement is performed as a function of T_{pw} in order to calculate $T_{1\rho}$ ($T_{2\rho}$). Yet, the time constants governing the experimental dynamics are the same and can be derived by solving Eq. (1). Thus, the analytical expressions derived and used in connection with $T_{1\rho}$ ($T_{2\rho}$) experiments can be applied to CEST as well. Such solutions will be used to qualitatively understand the experimental results presented below. The similarities between the spin-lock and CEST experiments were recently explored in greater detail by Jin et al. [56].

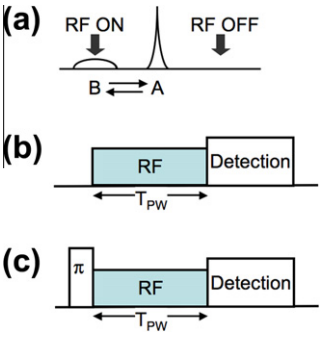

Fig. 1. Panel a: A schematic of an NMR spectrum of two pool system consisting of a larger population A (e.g., bulk or 'free' water) in exchange with a smaller population B (e.g., water bound to the PARACEST agent). The bold arrows mark application of RF on resonance with the exchanging pool B (*RF ON*) or the control experiment, with RF moved an equal frequency to the opposite side of population A (*RF OFF*). Panel b: A schematic of a pulse sequence utilized for saturation transfer experiments (T_{pw} is the RF pulse length). Panel c: A schematic of a pulse sequence utilized for apparent relaxation time measurements and for the creation of the Positive CEST effect (pCEST); π is an inversion pulse.