Multivalent Imaging with a Cocktail of PARACEST Agents: Utility of BIRDS for CEST Imaging

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INTRODUCTION PARACEST agents, which exploit proton exchange mechanisms (e.g., between exchangeable/bound water protons and bulk water protons) have opened a new avenue for smart MRI contrast agents allowing various environmental parameters (e.g., temperature and pH) to be imaged **[1]**. While combination of two or more PARACEST agents, each sensitive to an independent parameter, is believed to allow multi-parametric detection, results suggest that the multiple-pool exchange situation is not a simple linear combination of two-pool exchange models **[2]**. One solution to this problem involves introduction of a second PARACEST agent that can serve as an "internal control" for the first agent **[3-4]**. On one hand the cocktail of two PARACEST agents provide advantages for quantitative CEST imaging, but on the other hand the CEST effect of the first agent can also be affected by the introduction of the second PARACEST agent, which can complicate the quantitative analysis **[2]**. Interestingly, PARACEST agents also contain nonexchangeable protons that can provide molecular readout with ultrafast chemical shift imaging, as detected by a novel technique called Biosensor Imaging Redundant Deviation in Shifts (BIRDS) **[5-6]**. BIRDS detection with PARACEST agents has shown its biosensing potential, such as readouts of temperatures and/or pH **[7]**. Here, we test the hypothesis that the BIRDS properties in the cocktail of two PARACEST agents will not only be unaffected but also enhance precision by adding redundancy for quantifying the CEST contrast.

MATERIALS AND METHODS CEST spectra for phantoms of Tm-DOTAGly$_4^-$, Eu-DOTAGly$_4^-$, and the cocktail of the two agents were acquired with continuous wave saturation at 16.7 μT for 1 sec in the frequency range from -100 to 100 ppm with 250 Hz per step. NMR proton spectra of these three phantoms at various pH (6.7-7.9) and temperatures (25 – 40 °C) were acquired for BIRDS calibrations. All MR spectroscopy is acquired using 11.7-T Bruker vertical-bore spectrometer.

RESULTS The CEST peaks of the amide protons in TmDOTAGly$_4^-$ (-52.5 ppm, Tm-NH) and the bound water proton in EuDOTAGly$_4^-$ (51 ppm, Eu-H_2O) do not overlap with those in the cocktail of the two agents (**Fig. 1A**). The CEST effects in the mixture are attenuated for both exchange sites. The subtraction of CEST spectrum of each single agent from that of the cocktail of two agents reveals that the attenuation in the CEST effects are different for each agent, i.e., 5.5% for Eu-H_2O and 2.4% for Tm-NH (**Fig. 1B**). The ^{1}H spectra of the three phantoms are shown in **Fig. 2**. The chemical shifts of the proton resonances do not change in the cocktail phantom demonstrating that no interference between each agent (**Fig. 2C**). Furthermore, the temperature and pH dependencies of H1 of TmDOTAGly$_4^-$ and H4 of EuDOTAGly$_4^-$ are retained in the cocktail phantoms indicating that BIRDS properties are unaffected in the cocktail of two PARACEST agents.

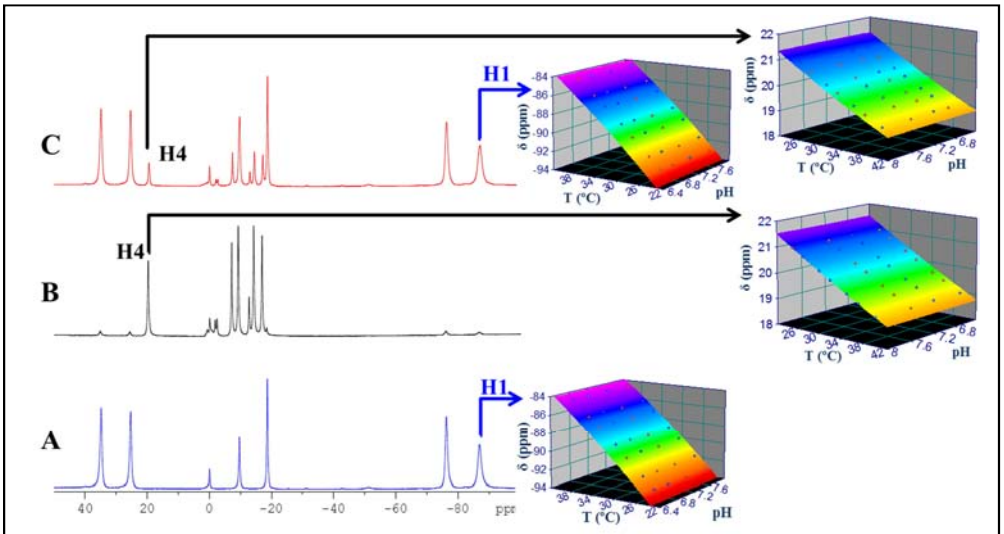

Figure 2. BIRDS characterization of PARACEST agents at pH 7.4 and 35 °C. Proton spectra of A) Tm-DOTAGly$_4^-$, B) Eu-DOTAGly$_4^-$, and C) the cocktail of the two agents. The proton resonances were assigned to Tm-DOTAGly$_4^-$ (Blue) and Eu-DOTAGly$_4^-$ (Black).

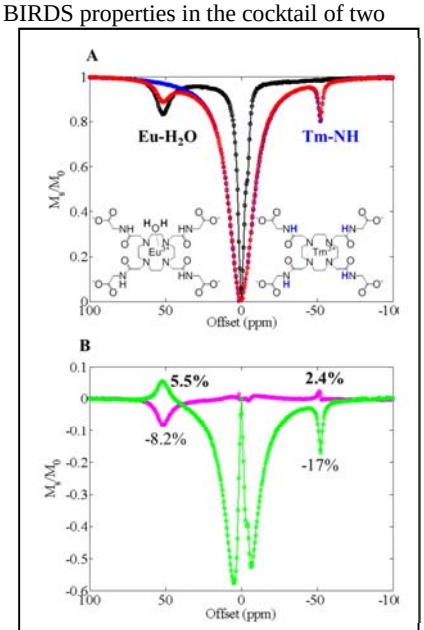

Figure 1. A) CEST spectra of 10mM TmDOTAGly$_4^-$ (Blue), 10mM EuDOTAGly$_4^-$ (Black), and the cocktail of the two agents (Red) at pH7.4 and 35 °C. B) Subtraction of CEST spectra of TmDOTAGly$_4^-$ (Magenta) and EuDOTAGly$_4^-$ (Green) from that of the cocktail, respectively.

DISCUSSION These results demonstrate that BIRDS signals are retained in the cocktail of the two PARACEST agents. Moreover, signals from different PARACEST agents can be considered as additional redundancies for BIRDS to improve the precision. BIRDS detection using two PARACEST agents show great potential for in vivo molecular imaging by itself or it can be used as complimentary to PARACEST to offer an additional caliber for quantitative CEST measurements such as ratiometric PARCEST **[8]**.

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