Towards Receptor Targeted 13C Hyperpolarized MR Contrast Agents

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Introduction Dynamic Nuclear Polarization (DNP) [1] and Parahydrogen And Synthesis Allow Dramatically Enhanced Nuclear Alignment (PASADENA) [2,3,4,5,6] increase nuclear spin polarization to order unity approaching the conditions, when nearly all nuclear magnetic moments give rise to the MR signal. Both methods provide MR sensitivity enhancement by 10^4-10^6 fold. So far, the focus of the majority of in vitro and in vivo work was limited to the studies of metabolic events governing biochemical pathways. When compared to Positron Emission Tomography (PET), hyperpolarized MR, by exploiting species-specific and site-specific chemical shifts, potentially provides significantly more metabolic information rather than just the molecular uptake by the tissues.

Purpose To design ^{13}C hyperpolarized molecular agents capable of specific interactions with receptors (Fig. 1A).

Methods We utilized PASADENA to hyperpolarize 2,2,3,3-tetrafluoropropyl 1-^{13}C-propionate (TFPP) using the double bonded molecular precursor 2,2,3,3-tetrafluoropropyl 1-^{13}C-acrylate (Fig. 1B) [7]. PASADENA hyperpolarization of TFPP yields aqueous solutions of polarization similar to that of succinate [5] and 2-hydroxyethyl propionate [4, 6] routinely reaching polarization of 15-20% in our laboratory.

Results A ^{13}C spin lattice relaxation time of 45 s for hyperpolarized TFPP in aqueous solutions was measured by small angle excitation pulses (Fig. 2A). We investigate interactions of ^{13}C hyperpolarized TFPP with synthetic 1,2-dimyristoylphosphatidylcholine (DMPC) (Avanti Polar Lipids, Inc., Alabaster, AL) membranes by mixing lipid membranes with TFPP hyperpolarized solutions (Fig. 2B). We find that the longitudinal decay time of ^{13}C is reduced to 20 seconds. Moreover, a second resonance 3 ppm away from main solution resonance is detected, which we attribute to slow exchange with DMPC membranes based on our previous ^{19}F studies of TFPA [7]. Nearly identical ^{13}C decay times for both resonances also support this hypothesis. Since the long-term goal of these studies is to utilize TFPP in vivo by means of I-V injection, TFPP solution was mixed with human serum albumin (Fig. 2C). While ^{13}C longitudinal decay is decreased to 27 seconds, no second resonance is observe indicating that the slow-exchange signature of TFPP is specific for lipids, but not for hydrophobic proteins such as albumin. Moreover, our competition test (Fig. 2D) in which hyperpolarized TFPP was mixed with both albumin and lipid membranes yielded resolved a spectrum similar to Fig. 2A, except that the decay time is further reduced to 14 -17 seconds.

Discussion We demonstrated that it is possible to design a molecular ^{13}C hyperpolarized agent with receptor interaction specificity and detect these events by NMR. We also conclude that ^{13}C T_1 is sufficiently long to permit *in vivo* injection followed by fast NMR detection using MRI, MRS or CSI (experiments in progress in our laboratory).

Acknowledgements We thank the following for funding: Tobacco Related Disease Research Program 16KT-0044, NIH/NCI R01 CA 122513, 1R21 CA118509, 1K99CA134749-01, Rudi Schulte Research Institute, James G. Boswell Fellowship, American Heart Association, American Brain Tumor Association, Beckman Institute Pilot Program: "Spin Polarized Molecules for Structural and Systems Biology", Prevent Cancer Foundation.

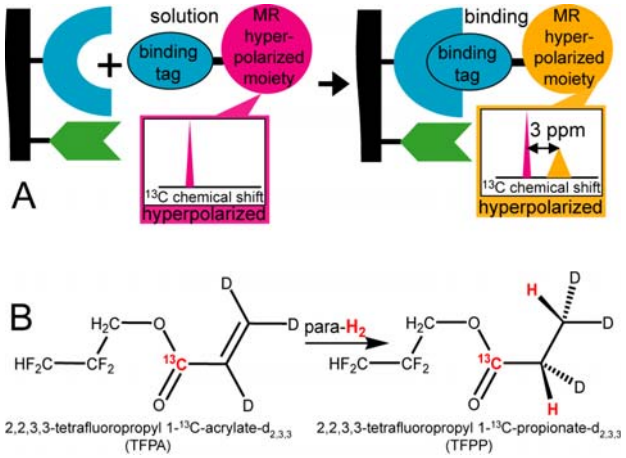

Figure 1. A) Diagram of receptor targeted molecular binding of ^{13}C hyperpolarized TFPP molecular reagent, B) Molecular cis addition of parahydrogen to TFPA to produce TFPP. The catalytic reaction was carried out at 62°C.

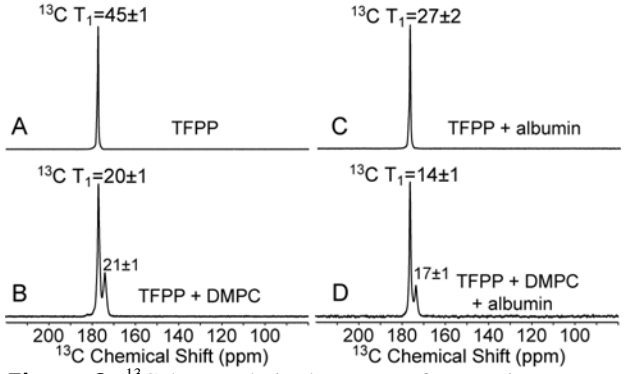

Figure 2. ^{13}C hyperpolarized spectra of TFPP in aqueous solutions acquired with small angle excitation pulse at 35-40°C: A) 6 mM TFPP, 4.5 M EtOH, B) 2 mM TFPP, 1.5 M EtOH, 20 mM DMPC, C) 6 mM TFPP, 1% human serum albumin, 4.5M EtOH, D) 4 mM TFPP, 1.5 M EtOH, 20 mM DMPC, 1% human serum albumin. ^{13}C T_1 shown for each resonance was measured by recording ^{13}C hyperpolarized signal decay with small angle excitation pulses.

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