HOW LOW CAN WE GO? LIMITS OF DETECTION IN PASADENA 13C HYPERPOLARIZATION

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Objective: Fast ^{13}C imaging at safe, physiological concentrations of water-soluble, non-toxic, hyperpolarized ^{13}C contrast agents.

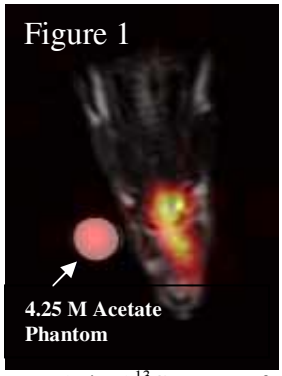
Background: *In vivo* ^{13}C MRS of human brain defines concentrations of important fuels and neurotransmitters between 1-10 mM and reaction rates of 1-5 µmoles/min/gram. Two novel methods of hyperpolarization of ^{13}C, dynamic nuclear polarization (DNP) and Parahydrogen and Synthesis Allow Dramatically Enhanced Nuclear Alignment (PASADENA); provide signal enhancement for ^{13}C in excess of 10,000. Both techniques have shown utility in *in vivo* fast ^{13}C imaging and spectroscopy and DNP is undergoing clinical trials. However, even after removal of toxic constituents and sterilization, the concentration of ^{13}C pyruvate contrast agent employed (0.3M) will be 3000 times higher than physiological,presenting unacceptable biochemical and osmotic stress. Similarly, PASADENA (a.k.a. parahydrogen induced polarization: PHIP) ^{13}C imaging, which was demonstrated initially with acetone-solutions, and later with aqueous solutions of a ^{13}C reagent, employed a concentration (0.3M) 250 times higher than the known lethal dose (LD50 = 1.3mM). Last year, we demonstrate with PASADENA, that ^{13}C imaging can be achieved as concentrations as low as 0.5 mM *in vitro* and 9 mM *in vivo*. This year, we have improved on the polarization of the molecules to go as low as 10µM *in vitro* and 100 µM (0.1 mM) *in vivo*. With the now achievable ^{13}C signal enhancement of 100,000 fold, *in vivo* images of ^{13}C metabolites comparable to those available from proton of water (80M) should permit contrast agent concentrations to be reduced to 1mM.

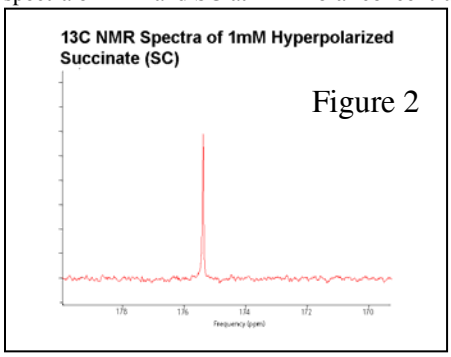
Materials and Methods: To facilitate routine PASADENA trials, GE Healthcare, Malmo has developed an automated polarizer for the initial steps of synthesis and polarization transfer, presently installed at the Huntington Medical Research Institutes, Pasadena. The polarizer was individually optimized for the production of two hyperpolarized water soluble molecules; a: 1- ^{13}C hydroxyethylpropionate (HEP), b: 1-^{13}C sodium succinate (SC) was located at distinct positions to each MR instrument: (i) high resolution vertical bore Varian Mercury 7T NMR spectrometer, (ii) horizontal bore Bruker Avance 4.7T animal scanner, and (iii) 1.5T GE Signa clinical scanner, all equipped with ^{13}C detection capability. Hyperpolarized ^{13}C signal intensity was quantified with comparison to an internal ^{13}C standard containing known concentration of 1-^{13}C sodium acetate.

Results: Polarization of over 40% was obtained for HEP, while ~20% polarization was obtained for SC. Signal enhancement ranged between 18,000 and 100,000 (Table 1). Intensity of ^{13}C images/spectra was linear and proportional to concentration. *In vitro* ^{13}C spectra were readily produced at ^{13}C concentrations as low as 10µM. Figure 1 shows an *in vivo* 3D FIESTA brain image of rat on injection of 25 mM solution of hyperpolarized ^{13}C succinate (SC) via carotid artery. Figure 2 & 3 shows *in vitro* ^{13}C NMR spectra of HEP and SC at millimolar concentrations.

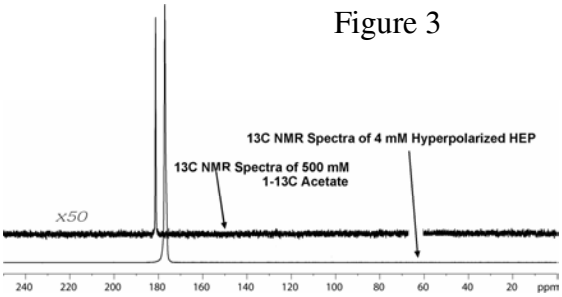
System	%STD	N	Signal Gain
Bruker Avance 4.7T	14%	4	50,000 fold
GE Signa 1.5T	9%	2	101,000 fold
Varian Mercury 7T	46%	8	18,100 fold

Table 1: Reproducibility of hyperpolarization with HEP





Discussion: PASADENA lends itself to rapid generation of hyperpolarized ^{13}C reagents for studies necessary to establish dose-response curves *in vitro* and *in vivo*. As was theoretically anticipated, the signal enhancement achieved by routine hyperpolarization was sufficient to permit detection hyperpolarized ^{13}C reagents for studies necessary to establish dose-response curves *in vitro* and *in vivo*. The signal enhancement achieved by routine hyperpolarization was sufficient to permit detection of ^{13}C concentrations as low as 0.01 mM. Somewhat higher ^{13}C concentration, 0.1 mM, necessary for *in vivo* imaging are in part a function of the additional time taken for injection. Allowing a dilution factor of 4 – 12 fold when contrast agent is mixed with circulating blood, results *in vivo* and *vitro* were comparable. Our previous estimate of the limit of detection (9mM) was far above the relevant physiological concentration of most biological compounds. We can now estimate the "limit" to be an order of magnitude lower and within the physiological concentrations of most biochemical processes.



Conclusion: *In vivo* PASADENA ^{13}C imaging with hyperpolarized contrast agents can be achieved using near/under physiological concentrations of naturally occurring metabolites and relevant unsaturated bio-molecules.

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References: a) P. Bhattacharya, K. Harris, A.P. Lin, M. Mansson, V.A. Norton, W.H. Perman, D.P. Weitekamp, andB.D. Ross, *MAGMA* 18 (2005) 245-256. b) Bhattacharya, P. K.; Chekmenev, E. Y.; Harris, K.; Weitekamp, D. P.; Tan, C. T.; Ross, B. D. *JMR* (2006) submitted.