Development of Hyperpolarized 13C-MRS Probes for Oxidative Stress measurement

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Purpose: Oxidative stress (OS) is a state of imbalance arising from overproduction of reactive oxygen species (ROS) within normal cells. ROS are products of normal metabolisms and they can be beneficial or harmful to cells and tissues based on their biological concentrations. At physiological low levels, ROS function as messengers in intracellular signaling and regulation, whereas excess ROS induce oxidative modification of biological macromolecules that affects many protein functions. Indeed, OS has been implicated in various diseases such as atherosclerosis, diabetes, neurodegeneration and cancer.[1] This study sought to develop new hyperpolarized (hp) ^{13}C MRS substrates to image OS in vivo. Specifically, we explored exploiting the redox interaction between the sulfur-based cysteine (Cys), N-acetyl cysteine (NAC), and Mercaptopyruvate (MCP) and their oxidized forms

Methods: All chemicals unless otherwise stated were purchased from Sigma Aldrich. [U-$^{13}C_3$-$^{2}H_3$-^{15}N]L-Cys and [1-^{13}C]L-Cys were purchased from Cambridge Isotope Laboratories and hyperpolarized using a dynamic nuclear polarizer. [1-^{13}C]L-Cys were dissolved in 1:1 (v/v) water/glycerol (6M) or 2:3 (v/v) water/DMA solution (2M), mixed with trityl radical OX063 (15 mM) and a 1:50 Dotarem solution (0.1 v/v), and polarized at ~94 GHz in a field of 3 T. Similarly, [U-$^{13}C_3$-$^{2}H_3$-^{15}N]L-Cys were copolarized with unlabeled pyruvic acid (neat, 1M). MCP was synthesized from bromopyruvic acid and potassium thioacetate, and the resulting product was deacetylated using NaOH/MeOH. Oxidation of Cys/MCP/NAC was performed by reacting the substrate with 1 equimolar H_2O_2 (30% solution) and 0.01 equimolar NaI in water (pH~7.4) for 24 hr. ^{13}C-NMR studies were performed in D_2O on an 11.7 T Varian spectrometer (125 MHz 13C, Varian Instruments) using an inverse triple resonance ($^{1}H/^{13}C/^{15}N$) 5 mm probe. T1 of a hyperpolarized [1-^{13}C]L-Cys was measured in the MR scanner using a small-flip-angle pulse-and-acquire sequence with a TR of 3 s. The liquid-state polarization level was estimated from the MR measurements of hyperpolarized and thermal-state signals, considering the T1 decay and the signal loss from radiofrequency (RF) excitations.

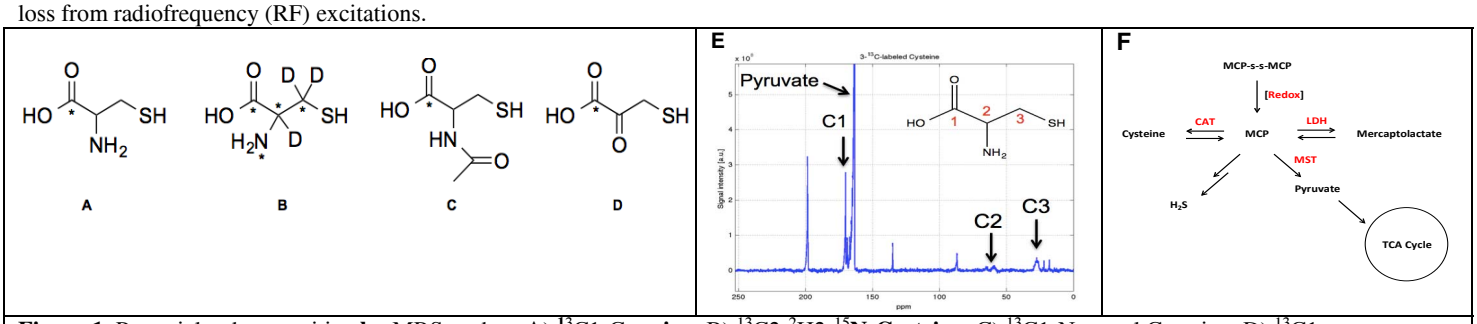

Figure 1. Potential redox-sensitive **hp** MRS probes. **A**) $^{13}C1$-Cyste**ine**; B) $^{13}C3$-$^{2}H3$-^{15}**N-Cysteine**; C) $^{13}C1$-N-acetyl Cysteine; D) $^{13}C1$-Mercaptopyruvic Acid. **E) Hyperpolarization of $^{13}C3$-$^{2}H3$-^{15}N-Cysteine. F)** Mercaptopyruvate disulfide (MCP-s-s-MCP) undergoes cellular reduction to mercaptopyruvate (MCP). MCP is converted to cysteine or mercaptolactate, metabolized to pyruvate and hydrogen sulfide (H_2S). CAT = cysteine amino transferase; LDH = lactate dehydrogenase; MST = mercaptosulfur transferase.

Results and Discussion: Polarization of [1-^{13}C]L-Cys in water/glycerol or water/DMA mixture yielded liquid-state polarization levels of 2-3% with a T_1 relaxation time of 22 sec. Given this relatively low polarization level, we measured hyperpolarization at different carbon position using [U-$^{13}C_3$-$^{2}H_3$-^{15}N]L-Cys to identify whether different carbon ^{13}C-labeling gave more desirable polarization. Our results indicated that $^{13}C1$ exhibited the highest level of polarization and the longest T_1 relaxation time among the different cysteine carbons (Fig 1C). We also investigated other potential hp MRS probes and examined NAC because of its structural similarity with L-Cys. We reasoned that the polarization level of NAC would have been greatest at the C1 position while the biggest chemical shift due to any redox changes would have been at the C3 position. As ^{13}C-labeled NAC is not commercially available, we tested our hypothesis with high-res ^{13}C NMR and compared the chemical shift differences between its reduced and oxidized species based on their isotopic natural abundance. In particular, we observed minimal 0.4 and 1.2 ppm changes *in vitro* at the C1 carbon of NAC and L-Cys respectively (Fig 2A and B), further limiting their application for *in vivo* detection. However, the chemical shift difference between oxidized and reduced MCP species was much larger with a 3.3 ppm change at the C1 carbon (Fig 2C). These results, coupled with the potentially larger polarization level (based on its close analog ^{13}C-pyruvic acid), suggest that MCP could be exploited for measuring cellular redox state *in vivo*. However, to date, we have not found a stable enough synthesis process to generate the quantities of MCP needed for *in vivo* experiments.

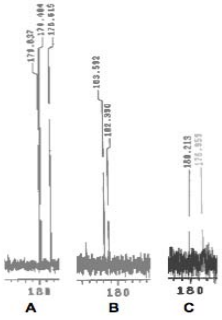

Fig.2 High-resolution ^{13}C-NMR of (A) NAC and NAC Disulfide (B) Cys and CySS (C) MCP and MCP Disulfide

Conclusion: We have identified MCP to be a viable substrate for *in vitro* OS assessment and are currently developing large-scale MCP synthesis for testing the potential of this substrate for *in vivo* OS measurements.

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References: **1.** Halliwel B, Gutteridge J. Free radicals in Biology and medicine. New york: Oxford University Press; 1995.