A genetically encoded hyperpolarized MRI reporter for in vivo cell tracking

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We have been developing the first genetically engineered (GE) reporter system for cell tracking using hyperpolarized (HP) magnetic resonance spectroscopic imaging (MRSI) to address an urgent need for the quantitative tracking of cellular therapies. This GE probe would allow for longitudinal studies by identifying the presence and viability of cellular therapies *in vivo* with high sensitivity, as HP-MRSI can increase signal 10^6 over traditional MRI methods while enabling the longitudinal detection of enzyme function. We have developed a MR-compatible, highly specific enzyme-substrate pair incorporating porcine liver esterase (PLE) and a 1-methyl-cyclopropylester protecting group. This system is robust *in vitro*, adapted from a published fluorescent imaging system with a highly specific enzyme-substrate pair using porcine liver esterase (PLE) and a 1-methyl-cyclopropylester protecting group and derivatives with very low background hydrolysis endogenously. Several substrates (Figure 1A-B) were designed to have favorable characteristics for enabling dynamic nuclear polarization magnetic resonance spectroscopic imaging (DNP-MRSI) including long T_1 relaxation (T_{1r}) times, sufficient metabolism with purified PLE, low toxicity, and rapid conversion observable as 2-3 ppm chemical shifts. Additionally, PLE was able to be stably transfected into both standard cell lines and hepatocellular carcinoma (HCC) cell lines, as well as grown as flank tumors, without phenotypic differences.

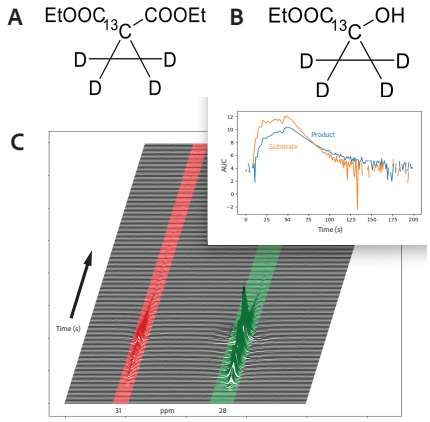

Figure 1 (A, B) Singly ^{13}C-labelled [A] or [B[with deuterated ring. **(C)** Hollow fiber experiment containing 10^8 SNU449 cells stably expressing PLE and dTomato. Singly lableled [A] was HP and injected over 90s into flow chamber with pump off. Spectra were taken every second using a commercail dual tuned ^{13}C/^{1}H coil on a MR Solution 7T scanner. **(C Inset)** Change in AUC of each respective peak over time as substrate (orange) converts to product (blue).

We have demonstrated rapid cellular update and conversion using HP-NMR and isotopically-labelled substrates (Figure 1C). ^{13}C-lableled substrates with deuterated cyclopropyl rings did not have any splitting in their spectra, and both had T_{1r} times of greater than 60s. To test metabolism *en cellulo* using DNP-NMR spectroscopy, a cellular perfusion system using a hollow fiber bioreactor made to fit into a horizontal bore MRI. Cartridges were seeded with 10^8 PLE-expressing adherent HCC cells then transferred to the magnet bore with media continuously pumping. HP ^{13}C isotopically-labelled substrate is injected through a port immediately before the hollow fiber cartridge before acquisition. Spectra were acquired every second for 200 seconds. We saw complete hydrolysis of substrate, as shown by the shift from 28ppm to 31ppm. The T_{1r} time was about 60s. This experiment demonstrated cells can uptake substrate and for PLE can hydrolyze it *in vivo* in time frames compatible with HP-MRSI.

Initial *in vivo* experiments are being conducted in BALB/c mice with HCC tumors stably expressing PLE injected by tail vein with HP ^{13}C substrate. Spectra were acquired every 2 seconds for 2 minutes. Preliminary data shows poor signal-to-noise (SNR) and longevity of the signal for substrate A, with signal being too low to observe product formation, most likely. Tests of substrate A in mouse plasma shown dramatically shortened T_{1r} in comparison with buffer, or effects on substrate B (30s, 60s and 60s, respectively). Substrate B shows good SNR and better signal longevity, with an apparent *in vivo* T_{1r} of 25s. However, no product formation was observed in this first attempt. Substrate B has increased water solubility and less interaction with plasma, improving its *in vivo* compatibility, but cellular uptake needs to be improved.

Our next experiments are to test other cell types, such as CAR-T cells engineered to express PLE, for uptake of both substrates. Then, we will either work to improve reduce plasma interactions or cellular uptake, by modifying substrates A and B, respectively, should these cells not metabolize them quickly. Future directions include the tracking of cellular therapies and development of the system for the deprotection of pro-drugs as a therapeutic or suicide gene using *in vivo* gene therapy systems.