Pyruvate Decarboxylase as a Reporter Gene for Magnetic Resonance Spectroscopic Imaging (MRSI)

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Target audience Researchers using MRS to study the activity of transgenic promoters *in vivo.*

Purpose To combine the advantages of MRI – spatial and temporal resolution and penetration depth sufficient for *in vivo* imaging and the over 10000 fold increase in sensitivity of ^{13}C-NMR experiment afforded by Dynamic Nuclear Polarisation, we used inducible expression of a bacterial gene encoding the enzyme, pyruvate decarboxylase (PDC), as a marker for DNP magnetic resonance spectroscopic imaging. The ^{13}C NMR signals of PDC's substrate, [1-^{13}C] pyruvate, and of its product, H$^{13}CO_3^-$ (Fig1), are resolved by their chemical shifts, which allows measurement of PDC activity in vivo with the use of DNP ^{13}C NMRS with hyperpolarized [1-^{13}C] pyruvate.

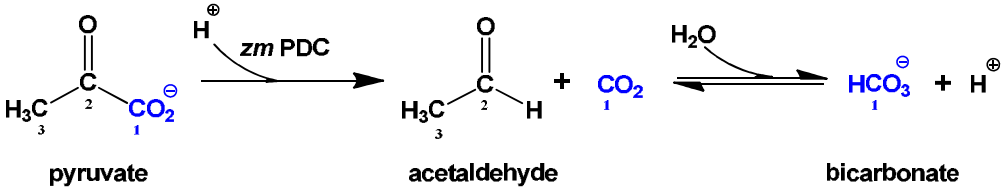
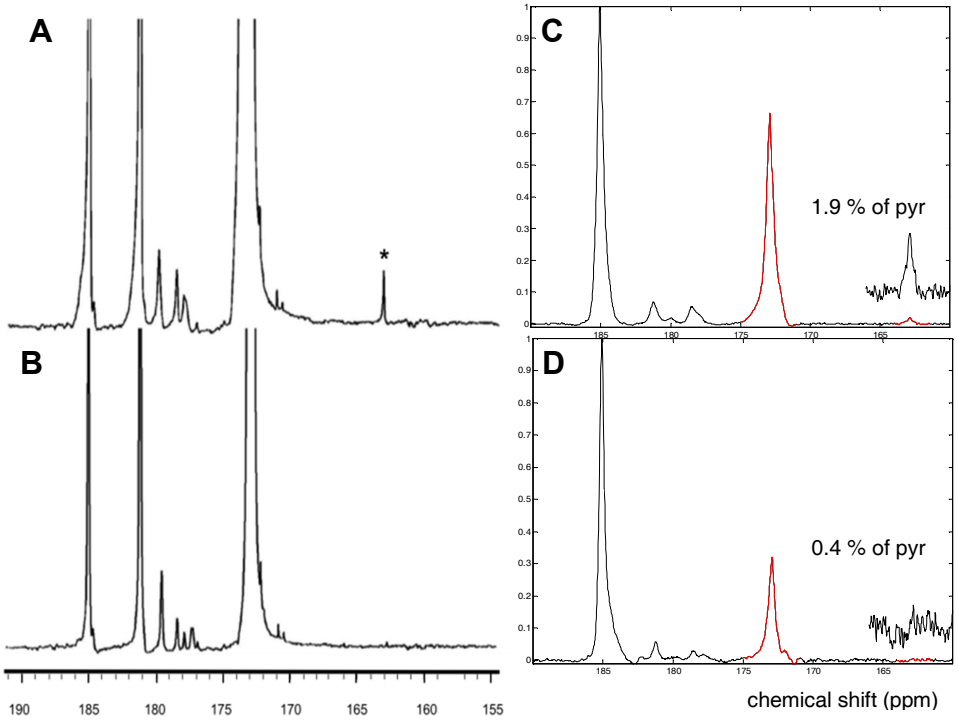


Figure 1 The reaction catalysed by PDC.

Methods Both constitutively and doxycycline (tet-on)-inducible PDC-GFP-V5-expressing HEK293T cells were used for *in vitro* ^{13}C-MRS experiments, while only inducible cells were used *in vivo*. PDC expression was induced *in vivo*, 21-25 days after the cells were implanted into the flanks of SCID mice, by adding doxycycline to the drinking water for 96 hours prior to ^{13}C-MRS experiments.

Results A prominent ^{13}C-bicarbonate signal was observed *in vitro* after injection of hyperpolarized [1-^{13}C] pyruvate into a suspension of PDC expressing cells at 9.4 T (Fig 2 A, B). Similarly, *in vivo* ^{13}C MRS measurements at 7 T with hyperpolarised [1-^{13}C] pyruvate showed a resonance from H$^{13}CO_3^-$ at 162 ppm in tumours in doxycycline-treated animals; spectra of tumours from untreated animals did not show this signal (Fig 2 C,D).



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HCO_3^- / Lactate ratio

	mean	SD	n
No DOX	0.0065	0.0048	10
DOX 96 hr	0.0123	0.0042	8

p= 0.0148 (2-tailed t-test)

Figure 2 (A) ^{13}C spectrum of PDC-expressing cell suspensions **(B)** Spectrum collected from non-expressing cells. **(C,D,E)** Example of ^{13}C NMR spectra acquired from a 6 mm thick slice through a tumour from a doxycycline- treated and untreated animal, respectively. **(E)** Summary of the bicarbonate signal normalized to lactate peak before and 96 hours post doxycycline treatment.

Conclusions We have demonstrated the feasibility of using PDC as a reporter gene for DNP ^{13}C MRS.