## 14 substrates are "ready-to-go" for *in vivo* DNP hyperpolarization studies

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**Introduction:** *In-vivo* metabolism of small $^{13}$C labelled molecules can be studied with the DNP-MR technique (1, 2, 3). However, such studies require substrate concentrations and experimental time windows that deviate significantly from studies using conventional MR techniques. To obtain high enough S/N ratios of metabolites the injected substrates need to be highly polarized at the moment of injection. This requires a high solid state polarization of the substrate and an efficient dissolution process but also a composition of the injected solution that does not affect the liquid state $T_1$ negatively. In addition the injected solutions need to be physiologically acceptable with respect to pH, ion strength and temperature. Here we show the result of an *in vivo* screen of the spin–lattice relaxation time constants ($T_1$) in healthy mouse with 14 selected compounds.This result allows for new biochemistry to be studied and provides a good basis for the development of pre-clinical and clinical metabolic substrates.

**Methods:** All substrates have been polarized in a prototype DNP polarizer (GE Healthcare design) operating at 3.35 T and 1.2 K. The substrates were dissolved in 40 mM phosphate buffer added EDTA. The dissolved samples were divided into two parts. One part was placed in a 9.4 T magnet connected to an Inova Varian spectrometer for determination of the liquid state polarization and determination of $T_1$ at 37 °C. Another part was injected into the c57BL/6 mice (*i.v.*, 175 µl, ~ 50 mM, 6s). The in vivo experiments were performed on a 2.35T Bruker Biospec Avance II system. The mice were anaesthetized with isoflurane. ECG, breathing rate and temperature was monitored (SA instruments, USA). A series of slice selective $^{13}$C spectra were acquired with low flip angle (10 deg.) in a whole body rat coil.

**Results and discussion:** 14 compounds were prepared for *in vivo* hyperpolarization with high solid state concentrations ranging from 3-9 M. Liquid state polarizations varied between 15-40%, with polarization build-up times of no longer than 1 hour. A comparison between $T_1$ at 9.4 T and $T_1$ measured *in vivo* in healthy mice at 2.35 T is represented in Figure 1. For most of the compounds there is only a small difference between the *in vitro* and *in vivo* $T_1$ considering the differences in field strength, solution viscosity and protein binding. Three of the tested substrates show a significantly shorter relaxation time *in vivo* compared to *in vitro*. This is due to a combined effect of metabolism and relaxation in the blood. None of the substrates showed any effects on ECG or respiration in the animals during the experiments.

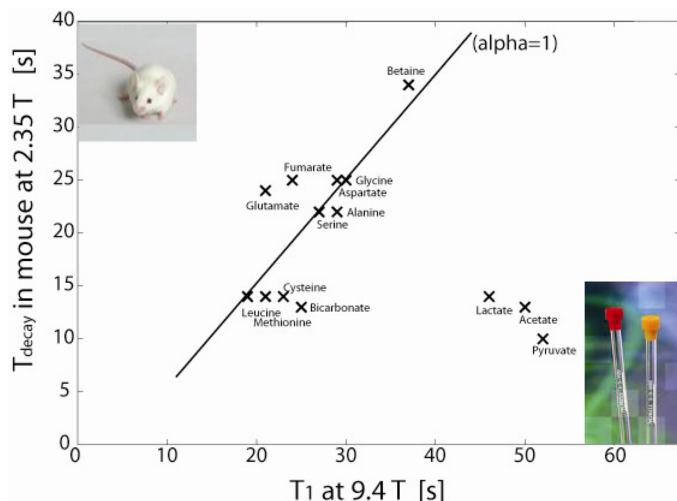


*Figure 1. Relaxation time constants of 14 substrates measured in healthy mouse compared to $T_1$ in vitro. $T_{decay}$ refer to relaxation due to both $T_1$ and possible metabolism.*

| Substrate | Polarization liquid state | Substrate | Polarization liquid state |
|---|---|---|---|
| Acetate | 20 % | Glutamate | 17 % |
| Alanine | 30 % | Glycine | 15 % |
| Aspartate | 17 % | Lactate | 23 % |
| Betaine | 40 % | Leucine | 29 % |
| Bicarbonate | 15 % | Methionine | 26 % |
| Cysteine | 17 % | Pyruvate | 27 % |
| Fumarate | 19 % | Serine | 28 % |

*Table 1. Liquid state polarization measured 20 s post dissolution, at the time of injection into the animal. All polarizations obtained with polarization build-up times of less than 1 hour.*

**Conclusion:** 14 substrates have been prepared and tested for *in vivo* studies. All substrates showed good tolerance in the mouse, and all substrates polarize to high levels within an hour in the polarizer. We here demonstrate a large extension of substrates available for hyperpolarization studies *in vivo*.

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**References:** [1] Ardenkjaer-Larsen et al. PNAS 100:10158,2003 [2] Albers et al., Cancer res. 68 (20): 8607-15, 2008 [3] Gallagher et al., Nature 453 (7197): 940-3, 2008.