Imaging pH *in vivo* using hyperpolarized ^{13}C-labeled bicarbonate

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Abstract
Alterations in pH underlie many pathological processes and therefore there is a pressing need for techniques that could be used to measure tissue pH in the clinic. We show here that tissue pH can be imaged *in vivo* from the ratio of the signal intensities of hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ following injection of hyperpolarized $H^{13}CO_3^-$. The technique has been demonstrated by showing that the average pH of a mouse tumor was significantly lower than the surrounding tissue in keeping with an acidic extracellular environment.

Introduction
Tissue acid-base balance is tightly controlled with the main buffer being bicarbonate (HCO_3^-), which resists changes in pH through interconversion with carbon dioxide (CO_2) in the reaction catalyzed by the enzyme carbonic anhydrase[1]. Many pathological states are associated with a disruption of this acid-base balance: for example tumors have an acidic extracellular pH[2]. Although pH is a fundamental physiological parameter, it has proven to be difficult to measure *in vivo* and there is currently no clinical tool available to provide a non-invasive spatial distribution of pH within humans. Dynamic Nuclear Polarization (DNP) has emerged recently as a technique for radically increasing the sensitivity of ^{13}C-MRS[3]. We show here that $H^{13}CO_3^-$ can be hyperpolarized using this method and can then be used to image tissue pH *in vivo*.

Methods
^{13}C-labeled cesium bicarbonate was produced from $^{13}CO_2$ and was hyperpolarized in a prototype polarizer (GE-Healthcare). The cesium was removed using an ion-exchange column after dissolution. pH was calculated using the Henderson-Hasselbalch equation from the relative concentrations of $H^{13}CO_3^-$ and $^{13}CO_2$ seen in each spectrum or voxel. Animal experiments were performed by tail vein injection of 0.2 ml into mice bearing murine lymphoma tumors and the ^{13}C images were acquired using a surface coil. For some experiments, the tumors were acidified by administration of 0.8 mmol NH_4Cl via a nasogastric tube 4 h prior to the imaging experiment and were made alkaline by giving the animals 0.2 M $NaHCO_3$ to drink for 5-7 d prior to imaging. The *in vivo* pH was validated using a ^{31}P-MRS probe (3-aminopropylphosphonate; 3-APP) which has a pH-dependent chemical shift and which was administered intraperitoneally.

Results
The pH of the lymphoma tumors *in vivo* was calculated as 6.7 ± 0.1 (n = 12). The average non-slice selective pH, which includes a weighted mean of the tumor pH and that of surrounding normal tissue, was significantly higher at 7.1 ± 0.1 (n = 8; $p < 0.01$). Tumor pH was modified by changing systemic pH. The pHs measured using hyperpolarized $H^{13}CO_3^-$ showed good agreement with the pHs calculated using 3-APP (Fig. 1). Finally, the spatial distribution of pH within the animal, determined using chemical shift imaging (CSI), confirmed a low pH within the tumor (Fig. 2).

Discussion
We have demonstrated that pH can be imaged *in vivo* using hyperpolarized bicarbonate. The latter has been validated using a well characterized ^{31}P-MRS probe. The technique has two major advantages: firstly, unlike most other pH probes this measurement of pH is ratiometric and therefore independent of the probe concentration; secondly, because bicarbonate is endogenous and present at high concentration in humans it is unlikely to be toxic. Given the wide spectrum of diseases in which pH is disrupted, this method presents the possibility of a potentially powerful imaging tool in humans. We have termed this new method to measure pH: *Carbonic Anhydrase Mediated Bicarbonate Ratiometric Imaging using DNP-Generated Enhancement* (**CAMBRIDGE**).

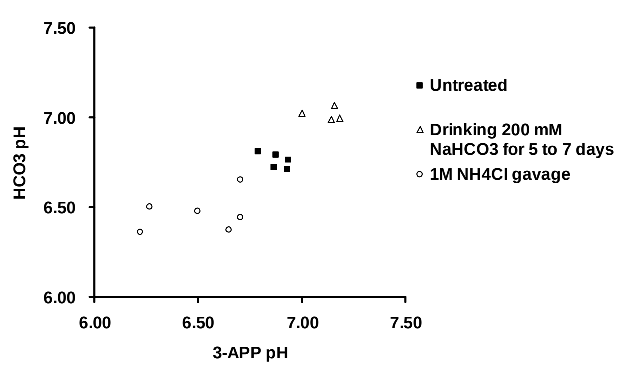

Fig. 1. Comparison of *in vivo* tumor pHs calculated with both hyperpolarized $H^{13}CO_3$ and 3-APP.

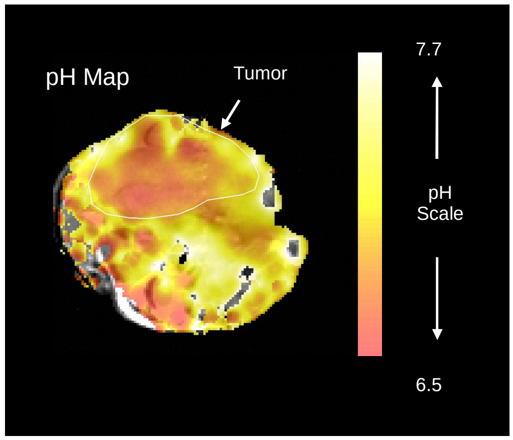

Fig. 2. *In vivo* pH map calculated from $H^{13}CO_3^-$ and $^{13}CO_2$ peak intensities following injection of hyperpolarized HCO_3^-.

References
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