

A Hydrogen Peroxide-Responsive Hyperpolarized ^{13}C MRI Contrast Agent

Alexander R. Lippert,[†] Kayvan R. Keshari,[§] John Kurhanewicz,[§] and Christopher J. Chang^{*,†,‡}

[†]Department of Chemistry and [‡]Howard Hughes Medical Institute, University of California, Berkeley, California 94720, United States

[§]Department of Radiology and Biomedical Imaging, University of California, San Francisco, California 94158, United States

 Supporting Information

ABSTRACT: We report a new reaction-based approach for the detection of hydrogen peroxide (H_2O_2) using hyperpolarized ^{13}C magnetic resonance imaging (^{13}C MRI) and the H_2O_2 -mediated oxidation of α -ketoacids to carboxylic acids. ^{13}C -Benzoylformic acid reacts selectively with H_2O_2 over other reactive oxygen species to generate ^{13}C -benzoic acid and can be hyperpolarized using dynamic nuclear polarization, providing a method for dual-frequency detection of H_2O_2 . Phantom images collected using frequency-specific imaging sequences demonstrate the efficacy of this responsive contrast agent to monitor H_2O_2 at pre-clinical field strengths. The combination of reaction-based detection chemistry and hyperpolarized ^{13}C MRI provides a potentially powerful new methodology for non-invasive multi-analyte imaging in living systems.

Reactive oxygen species (ROS) are intimately involved in the genesis and progression of numerous ailments¹ ranging from cancer² to neurodegeneration³ to diabetes.⁴ The relative stability and diffusibility of H_2O_2 poise this ROS to act as a potential diagnostic marker for the presence and progression of a wide range of pathological states. Indeed, the measurement of H_2O_2 in exhaled breath condensates has been used as a clinical marker for illnesses associated with lung inflammation,⁵ including asthma,⁶ chronic obstructive pulmonary diseases,⁷ and cystic fibrosis.⁸ The clinical use of this direct marker for oxidative stress and inflammation in deep tissues is hampered in large part by a lack of methods to non-invasively detect H_2O_2 in thicker, non-transparent specimens.⁹ As part of a larger program in our laboratory to develop novel ways to study the chemistry and biology of H_2O_2 in living cells and animals by molecular imaging,¹⁰ we now introduce a new type of reaction-based hyperpolarized ^{13}C MRI contrast agent for the detection of H_2O_2 .

MRI offers a promising approach for precise non-invasive molecular imaging of deep tissues in real time. However, its full potential as an imaging modality has yet to be realized because of a low sensitivity¹¹ that restricts most MRI experiments to imaging highly abundant endogenous protons in water and lipids. To overcome this limitation, hyperpolarization methods¹² such as optical pumping,¹³ para- H_2 -induced polarization,¹⁴ and dynamic nuclear polarization (DNP)¹⁵ can drastically enhance an MRI signal by manipulating spin states to produce samples with large non-equilibrium spin populations, leading to highly

magnetized samples and signal enhancements approaching 10^5 -fold. The development of rapid dissolution procedures¹⁶ following DNP has allowed the use of hyperpolarized [$1\text{-}^{13}\text{C}$]-pyruvate,¹⁷ [$2\text{-}^{13}\text{C}$]-fructose,¹⁸ [$5\text{-}^{13}\text{C}$]-glutamine,¹⁹ ^{13}C - HCO_3 ,²⁰ [$1,4\text{-}^{13}\text{C}_2$]-fumarate,²¹ and ^{15}N -choline,²² alone and in combination,²³ as metabolic probes.²⁴ These hyperpolarized probes ultimately report on the flux of specific enzymes by monitoring the conversion of isotopically labeled natural substrates to their metabolic products. On the other hand, our interest in the detection of molecular species such as H_2O_2 , which can simultaneously originate from multiple cellular sources, requires the use of probes that are not prone to rapid metabolism, so as to avoid false positives. In addition to being metabolically inert and non-toxic, an ideal ^{13}C MRI probe of this type should also possess favorable physical properties for DNP, produce an observable chemical shift upon rapid and selective reaction with the analyte of interest, and provide a system where both reactants and products have ^{13}C -labeled nuclei with long spin–lattice relaxation times (T_1).

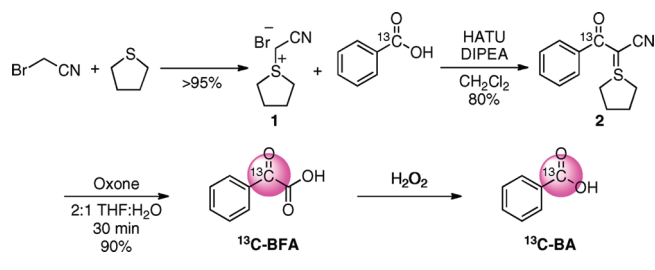
To this end, observations of the rapid oxidative decarboxylations of α -ketoacids²⁵ led us to investigate these species as hyperpolarizable ^{13}C MRI contrast agents using a reaction-based scheme for H_2O_2 detection. Initial studies focused on benzoylformic acid (BFA) because this α -ketoacid is non-toxic and undergoes minimal metabolism in both cells²⁶ and animals.²⁷ Kinetics measurements reveal that the reaction between BFA and H_2O_2 proceeds rapidly to produce benzoic acid (BA) within minutes with a second-order rate constant of $10.31 \pm 0.26 \text{ M}^{-1} \text{ min}^{-1}$ (Supporting Information, Figure S1). Moreover, the ^{13}C ketone nucleus of BFA and the ^{13}C carbonyl nucleus of BA are expected to have long T_1 values (on the order of tens of seconds), and the chemical shift difference between this carbon atom upon conversion of BFA to BA, the product of oxidative decarboxylation, is ca. 20 ppm. Encouraged by these initial findings, we synthesized BFA with a ^{13}C -label on the ketone carbon (^{13}C -BFA) using sulfur ylide acylation and oxidation chemistry (Scheme 1).²⁵ Reaction of neat bromoacetonitrile with tetrahydrothiophene provided the cyanosulfonium bromide **1**. The cyanosulfur ylide formed *in situ* was then coupled with ^{13}C -benzoic acid (^{13}C -BA) using HATU to yield the ^{13}C -cyanosulfur ylide **2**. Finally, oxone oxidation afforded ^{13}C -BFA after HPLC purification.

With this compound in hand, we proceeded to test its ability to undergo DNP under clinically relevant conditions. In these standard protocols, DNP results from the transfer of polarization from a stable organic radical species to a spin $S = 1/2$ nucleus by

Received: December 23, 2010

Published: March 02, 2011

Scheme 1. Design of a Hyperpolarized ^{13}C MRI Contrast Agent for Detection of H_2O_2 through H_2O_2 -Mediated α -Ketoacid Oxidative Decarboxylation and Synthesis of ^{13}C -BFA and Its H_2O_2 -Mediated Conversion to ^{13}C -BA



microwave irradiation at 1–2 K in a solid-state glass.¹⁵ Efficient transfer of polarization from the free radical to the nuclei of interest relies heavily on the formation of a uniform glass, which is typically achieved with highly concentrated solutions; for biological use, water is an appropriate solvent so that no residual toxic organic solvents are present after rapid dissolution of the hyperpolarized sample. We found that ^{13}C -BFA is extremely soluble in water at concentrations >5 M, with solutions reproducibly forming a glassy solid upon flash-freezing in liquid N_2 . Representative preparations utilize 6.0 M solutions of ^{13}C -BFA and 15 mM OX063 radical in water and were hyperpolarized at 1.33 K, irradiating at 94.094 GHz, using a HyperSense instrument (Oxford Instruments). Under these unoptimized conditions, ^{13}C -BFA polarizes with a build-up time constant of 899 s, providing 5.2% polarization and a ~ 5500 -fold signal enhancement. The T_1 values of the labeled $^{13}\text{C}2$ ketone were measured to be 24.4 ± 0.4 s at 11.7 T and 18.6 ± 0.3 s at 14.1 T (Figure S3).

We then evaluated hyperpolarized samples of ^{13}C -BFA for spectroscopic detection of H_2O_2 (Figure 1). After hyperpolarization, the sample was rapidly dissolved in 100 mM phosphate buffered to pH 7.8 with 0.3 mM EDTA to a final concentration of 5 mM and reacted with various concentrations of H_2O_2 . Spectra were acquired with a single scan in the presence of H_2O_2 concentrations ranging from 10 to 1000 μM . Figure 1a shows a set of spectra 21 s after rapid dissolution and reaction with H_2O_2 . The labeled carboxylate $^{13}\text{C}1$ resonance of the ^{13}C -BA product exhibits a chemical shift of 176 ppm, and the unlabeled carboxylate C1 carbon of the starting ^{13}C -BFA appears as a doublet at 173.5 ppm; the ratio of the integrated ^{13}C -BA peak to the unlabeled ^{13}C -BFA peak displayed a maximum at 21 s and a good linear correlation with increasing concentrations of H_2O_2 (Figure 1b), indicating that hyperpolarized ^{13}C -BFA can readily detect concentrations of H_2O_2 at high micromolar levels *in vitro* that are within the range implicated in states of oxidative stress that lead to cellular senescence,²⁸ despite trace amounts of ^{13}C -benzoic acid ($<0.1\%$) produced during synthetic manipulation and rapid dissolution procedures that can reduce the accuracy of such measurements. Although we were able to detect these levels of H_2O_2 using this first-generation probe, ongoing efforts are geared toward improving sensitivity to lower physiological levels that might be encountered *in vivo* through newer probes and optimized hyperpolarization protocols.

The oxidative decarboxylation of BFA is relatively selective for H_2O_2 over other biologically relevant ROS as monitored using analytical HPLC. After reacting BFA for 20 min at room temperature, only samples treated with H_2O_2 show significant conversion to benzoic acid (Figure 2). Only upon addition of

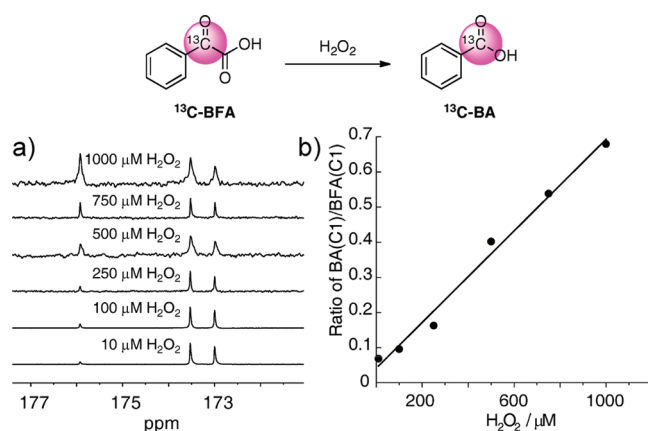


Figure 1. (a) ^{13}C NMR spectra of hyperpolarized ^{13}C -BFA after 21 s of reaction with 10, 100, 250, 500, 750, and 1000 μM H_2O_2 at 11.7 T. Spectra were acquired with a single scan every 3 s with a 5° pulse, except for 10 and 100 μM , which were acquired after 21 s with a 90° pulse. (b) Linear correlation of the ratio of integrated peak intensities of the C1 (carboxylate carbon) of ^{13}C -BA to the C1 (carboxylate carbon) of ^{13}C -BFA versus the concentration of H_2O_2 ; $R^2 = 0.988$.

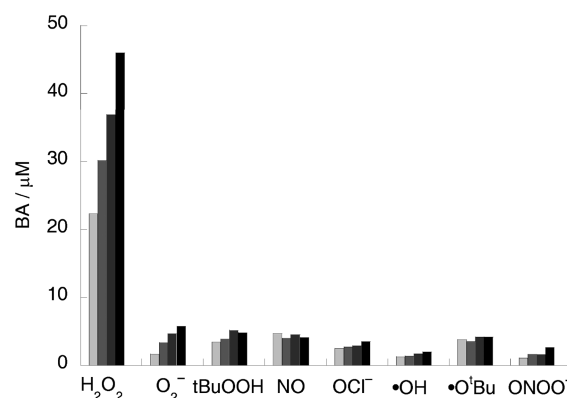


Figure 2. Response of 50 μM BFA to various ROS. All ROS were added at 5 mM, except for O_2^- , which was generated enzymatically at a rate of 24 $\mu\text{mol}/\text{min}$ for 120 min (2.9 mM total). Concentrations of benzoic acid were measured by HPLC after 0, 5, 10, 15, and 20 min of reaction except for that with O_2^- , which was measured after 0, 30, 60, 90, and 120 min of reaction.

exceedingly high, non-physiological concentrations does OCl^- or ONOO^- (500 mM) show reactivity with BFA (Figure S2), producing 20% and 70% NMR conversions, respectively. We speculate that the selectivity for H_2O_2 over OCl^- arises from an increased nucleophilicity of the former, whereas the selectivity over ONOO^- is most likely due to the short lifetime of the latter under physiological conditions.²⁹

Finally, we established the ability of ^{13}C -BFA to image H_2O_2 at pre-clinical field strengths. Specifically, we attained phantom images using a 14.1 T micro-imager equipped with 100 G/cm gradients and millipede ^1H and quadrature birdcage ^{13}C RF coils. Figure 3 displays phantoms of samples containing 20 mM hyperpolarized ^{13}C -BFA with 0, 25, 50, 100, and 200 mM added H_2O_2 , thermally polarized 5 M ^{13}C -BA in dimethyl acetamide (DMA), and thermally polarized 5 M ^{13}C -BFA in H_2O . First, we acquired ^1H spin echo images of all the tubes to indicate tube placement (Figure 3a). Next, we acquired ^{13}C MRI images using frequency-specific excitation pulses to obtain images of thermally

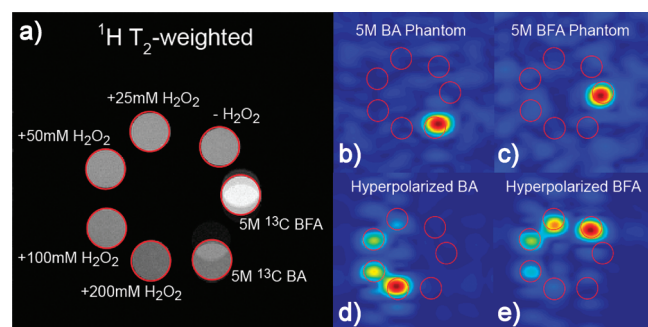


Figure 3. Phantom images of 5 M thermally polarized ^{13}C -BFA in H_2O , 5 M thermally polarized ^{13}C -BA in DMA, and 20 mM hyperpolarized ^{13}C -BFA in 100 mM phosphate, 0.3 mM EDTA buffered at pH 7.8 with 0, 25, 50, 100, and 200 mM H_2O_2 . (a, left side) ^1H spin echo image. (b–e, right) Frequency-specific images with selective excitation of the resonance of (b, top left) 5 M ^{13}C -BA in DMA, (c, top right) 5 M ^{13}C -BFA in H_2O , (d, bottom left) ^{13}C -BA buffered at pH 7.8, and (e, bottom right) ^{13}C -BFA buffered at pH 7.8. Images (b)–(e) were acquired after ~ 37 s of reaction with H_2O_2 with a $T_R = 150$ ms, FOV $40 \times 40 \times 40$ mm, $16 \times 12 \times 12$ matrix, and zero-filled to a final resolution of 1.25 mm isotropic.

polarized ^{13}C -BA in DMA (Figure 3b), thermally polarized ^{13}C -BFA in H_2O (Figure 3c), hyperpolarized ^{13}C -BA produced from the H_2O_2 -mediated conversion of hyperpolarized ^{13}C -BFA in 100 mM phosphate buffer, 0.3 mM EDTA at pH 7.8 (Figure 3d), and hyperpolarized ^{13}C -BFA in 100 mM phosphate buffer, 0.3 mM EDTA at pH 7.8 (Figure 3e). DMA was necessary to dissolve the thermally polarized 5 M ^{13}C -BA due to its low aqueous solubility, and an unbuffered system was used for the thermally polarized 5 M ^{13}C -BFA, causing a 2–3 ppm change in the chemical shifts for these species and requiring adjustment of the corresponding frequency-specific pulses for these images. ^{13}C images were acquired using a frequency-specific 90° pulse and GRASE-type readout for each resonance.³⁰ The overall acquisition time for the ^{13}C images was ~ 150 ms, revealing the dramatic reduction in acquisition time gained by using hyperpolarized probes. Moreover, the thermally polarized 5 M phantoms in Figure 3b,c are scaled by 10-fold compared to the hyperpolarized 20 mM phantoms in Figure 3d,e, indicating the marked signal enhancement for the hyperpolarized samples of several orders of magnitude. A clear increase in the intensity of the ^{13}C -BA images with a concomitant decrease in ^{13}C -BFA signals can be observed with increasing H_2O_2 concentrations, demonstrating the power of using rapid and selective reaction-based hyperpolarizable probes to image H_2O_2 levels via ^{13}C MRI.

In summary, we have described a hyperpolarized reaction-based ^{13}C MRI probe strategy for the selective detection and imaging of H_2O_2 . This work expands the applications of hyperpolarized ^{13}C MRI for imaging non-enzymatic species by utilizing non-endogenous and biocompatible hyperpolarized probes. This first-generation ^{13}C -BFA probe is a viable candidate for detecting H_2O_2 at oxidative stress levels, and we are currently developing H_2O_2 probes with increased sensitivity, as well as expanding the scope of other ^{13}C -labeled compounds as *de novo* MRI contrast agents.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic and experimental detail, including procedures for the synthesis of compounds,

selectivity assays, polarization procedures, spectroscopy, and imaging. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author
chrischang@berkeley.edu

■ ACKNOWLEDGMENT

This work has been supported by the National Institutes of Health (GM 79465), Amgen, Astra Zeneca, Novartis, and the Packard and Sloan Foundations. C.J.C. is an Investigator of the Howard Hughes Medical Institute. We gratefully acknowledge Dr. Hikari Yoshihara, Mr. Martin Ipu, and Mr. Bertram Koelsch for experimental assistance.

■ REFERENCES

- (1) (a) Rhee, S. G. *Science* **2006**, *312*, 1882–1883. (b) Stone, J. R.; Yang, S. *Antioxid. Redox Signal* **2006**, *8*, 243–270. (c) Miller, E. W.; Chang, C. J. *Curr. Opin. Chem. Biol.* **2007**, *11*, 620–625. (d) Winterbourn, C. C. *Nat. Chem. Biol.* **2008**, *4*, 278–286. (e) Poole, L. B.; Nelson, K. J. *Curr. Opin. Cell. Biol.* **2008**, *12*, 18–24. (f) Paulsen, C. E.; Carroll, K. S. *ACS Chem. Biol.* **2010**, *5*, 47–62.
- (2) (a) Fruehauf, J. P.; Meyskens, F. L., Jr. *Clin. Cancer Res.* **2007**, *13*, 789–794. (b) Finkel, T.; Serrano, M.; Blasco, M. A. *Nature* **2007**, *448*, 767–774. (c) Ishikawa, K.; Takenaga, K.; Akimoto, M.; Koshikawa, N.; Yamaguchi, A.; Imanishi, H.; Nakada, K.; Honma, Y.; Hayashi, J. *Science* **2008**, *320*, 661–664.
- (3) (a) Mattson, M. P. *Nature* **2004**, *430*, 631–639. (b) Lin, M. T.; Beal, M. F. *Nature* **2006**, *443*, 787–795. (c) DiMauro, S.; Schon, E. A. *Annu. Rev. Neurosci.* **2008**, *31*, 91–123.
- (4) (a) Houstis, N.; Rosen, E. D.; Lander, E. S. *Nature* **2006**, *440*, 944–948. (b) Pop-Busui, R.; Sima, A.; Stevens, M. *Diabetes-Metab. Res. Rev.* **2006**, *22*, 257–273. (c) Jay, D.; Hitomi, H.; Griendling, K. K. *Free Radical Biol. Med.* **2006**, *40*, 183–192.
- (5) Halliwell, B.; Whiteman, M. *Br. J. Pharmacol.* **2004**, *142*, 231–255.
- (6) Emelyanov, A.; Fedoseev, G.; Abulimity, A.; Rudinski, K.; Fedoulov, A.; Karabanov, A.; Barnes, P. J. *Chest* **2001**, *120*, 1136–1139.
- (7) Mercken, E. M.; Hageman, G. J.; Schols, A. M.; Akkermans, M. A.; Bast, A.; Wouters, E. F. *Am. J. Respir. Crit. Care Med.* **2005**, *172*, 994–1001.
- (8) Robroeks, C. M. H. T.; Rosias, P. P. R.; van Vliet, D.; Jöbsis, Q.; Yntema, J.-B. L.; Brackel, H. J. L.; Damoiseaux, G. M. C.; den Hartog, G. M.; Wodzig, W. K. W. H.; Dompeling, E. *Free Radical Biol. Med.* **2008**, *19*, 652–659.
- (9) (a) Lee, D.; Khaja, S.; Velasquez-Castano, J. C.; Dasari, M.; Sun, C.; Petros, J.; Taylor, W. R.; Murthy, N. *Nat. Mater.* **2007**, *6*, 765–769. (b) Van de Bittner, G. C.; Dubikovskaya, E. A.; Bertozzi, C. R.; Chang, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 21316–21321.
- (10) (a) Chang, M. C. Y.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 15392–15393. (b) Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652–16659. (c) Albers, A. E.; Okreglak, V. S.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 9640–9641. (d) Miller, E. W.; Bian, S. X.; Chang, C. J. *J. Am. Chem. Soc.* **2007**, *129*, 3458–3459. (e) Miller, E. W.; Tulyathan, O.; Isacoff, E. Y.; Chang, C. J. *Nat. Chem. Biol.* **2007**, *3*, 263–267. (f) Srikun, D.; Miller, E. W.; Domaille, D. W.; Chang, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 4596–4597. (g) Dickinson, B. C.; Chang, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 9638–9639. (h) Albers, A. E.; Dickinson, B. C.; Miller, E. W.; Chang, C. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5948–5950. (i) Srikun, D.; Albers, A. E.; Nam, C. I.; Iavarone, A. T.; Chang, C. J. *J. Am. Chem. Soc.* **2010**, *132*, 4455–4465. (j) Dickinson, B. C.; Huynh, C.; Chang, C. J. *J. Am. Chem. Soc.* **2010**,

- 132, 5906–5915. (k) Miller, E. W.; Dickinson, B. C.; Chang, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 15681–15686. (l) Lippert, A. R.; Gschneidtnr, T.; Chang, C. J. *Chem. Commun.* **2010**, 46, 7510–7512. (m) Dickinson, B. C.; Peltier, J.; Stone, D.; Schaffer, D. V.; Chang, C. J. *Nat. Chem. Biol.* **2011**, *7*, 106–112.
- (11) Terreno, E.; Castelli, D. D.; Viale, A.; Aime, S. *Chem. Rev.* **2010**, *110*, 3019–3042.
- (12) (a) Viale, A.; Aime, S. *Curr. Opin. Chem. Biol.* **2010**, *14*, 90–96. (b) Taratula, O.; Dmochowski, I. J. *Curr. Opin. Chem. Biol.* **2010**, *14*, 97–104.
- (13) (a) Spence, M. M.; Rubin, S. M.; Dimitrov, I. E.; Ruiz, E. J.; Wemmer, D. E.; Pines, A.; Yao, S. Q.; Tian, F.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 10654–10657. (b) Spence, M. M.; Ruiz, E. J.; Rubin, S. M.; Lowery, T. J.; Winssinger, N.; Schultz, P. G.; Wemmer, D. E.; Pines, A. *J. Am. Chem. Soc.* **2004**, *126*, 15287–15294. (c) Wei, Q.; Seward, G. K.; Hill, P. A.; Patton, B.; Dimitrov, I. E.; Kuzma, N. N.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2006**, *128*, 13274–13283. (d) Lowery, T. J.; Garcia, S.; Chavez, L.; Ruiz, E. J.; Wu, T.; Brotin, T.; Dutasta, J.-P.; King, D. S.; Schultz, P. G.; Pines, A.; Wemmer, D. E. *ChemBioChem* **2006**, *7*, 65–73. (e) Schröder, L.; Lowery, T. J.; Hilty, C.; Wemmer, D. E.; Pines, A. *Science* **2006**, *314*, 446–449.
- (14) (a) Bowers, C. R.; Weitekamp, D. P. *Phys. Rev. Lett.* **1986**, *57*, 2645–2648. (b) Bowers, C. R.; Weitekamp, D. P. *J. Am. Chem. Soc.* **1987**, *109*, 5541–5542. (c) Golman, K.; Axelsson, O.; Jóhannesson, H.; Månsson, S.; Olofsson, C.; Petersson, J. S. *Magn. Reson. Med.* **2001**, *46*, 1–5. (d) Bhattacharya, P.; Chekmenev, E. Y.; Perman, W. H.; Harris, K. C.; Lin, A. P.; Norton, V. A.; Tan, C. T.; Ross, B. D.; Weitekamp, D. P. *J. Magn. Reson.* **2007**, *186*, 150–155. (e) Reineri, F.; Viale, A.; Giovenzana, G.; Santelia, D.; Dastu, W.; Gobetto, R.; Aime, S. *J. Am. Chem. Soc.* **2008**, *130*, 15047–15053. (f) Reineri, F.; Santelia, D.; Viale, A.; Cerutti, E.; Poggi, L.; Tichy, T.; Premkumar, S. S. D.; Gobetto, R.; Aime, S. *J. Am. Chem. Soc.* **2010**, *132*, 7186–7193.
- (15) (a) Borghini, M. *Phys. Rev. Lett.* **1968**, *20*, 419–421. (b) Abragam, A.; Goldman, M. *Rep. Prog. Phys.* **1978**, *41*, 395–463. (c) Comment, A.; Van Den Brandt, B.; Uffman, K.; Kurdzesau, F.; Jannin, S.; Konter, J. A.; Hautle, P.; Wenckebach, W. T.; Gruetter, R.; Van Der Klink, J. J. *Concepts Magn. Reson., Part B* **2007**, *31B*, 255–269.
- (16) (a) Ardenkjaer-Larsen, J. H.; Fridlund, B.; Gram, A.; Hansson, G.; Hansson, L.; Lerche, M. H.; Servin, R.; Thaning, M.; Golman, K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10158–10163. (b) Golman, K.; Ardenkjaer-Larsen, J. H.; Petersson, S.; Månsson, L.; Leunbach, I. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10435–10439.
- (17) (a) Golman, K.; in 't Zandt, R.; Thaning, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 11270–11275. (b) Golman, K.; in 't Zandt, R.; Lerche, M.; Pehrson, R.; Ardenkjaer-Larsen, J. H. *Cancer Res.* **2006**, *66*, 10855–10859. (c) Kohler, S. J.; Yen, Y.; Wolber, J.; Chen, A. P.; Albers, M. J.; Bok, R.; Zhang, V.; Tropp, J.; Nelson, S.; Vigneron, D. B.; Kurhanewicz, J.; Hurd, R. E. *Magn. Reson. Med.* **2007**, *58*, 65–69. (d) Albers, M. J.; Bok, R.; Chen, A. P.; Cunningham, C. H.; Zierhut, M. L.; Zhang, V. Y.; Kohler, S. J.; Tropp, J.; Hurd, R. E.; Yen, Y.-F.; Nelson, S. J.; Vigneron, D. B.; Kurhanewicz, J. *Cancer Res.* **2008**, *68*, 8607–8610. (e) Marjańska, M.; Iltis, I.; Shestov, A. A.; Deelchand, D. K.; Nelson, C.; Uğurbil, K.; Henry, P.-G. *J. Magn. Reson.* **2010**, *206*, 210–218.
- (18) Keshari, K. R.; Wilson, D. M.; Chen, A. P.; Bok, R.; Larson, P. E. Z.; Hu, S.; Criekeing, M. V.; Macdonald, J. M.; Vigneron, D. B.; Kurhanewicz, J. *J. Am. Chem. Soc.* **2009**, *106*, 5503–5507.
- (19) Gallagher, F. A.; Kettunen, M. I.; Day, S. E.; Lerche, M.; Brindle, K. M. *Magn. Reson. Med.* **2008**, *60*, 253–257.
- (20) Gallagher, F. A.; Kettunen, M. I.; Day, S. E.; Hu, D.-E.; Ardenkjaer-Larsen, J. H.; in 't Zandt, R.; Jensen, P. R.; Karlsson, M.; Golman, K.; Lerche, M. H.; Brindle, K. M. *Nature* **2008**, *453*, 940–943.
- (21) Gallagher, F. A.; Kettunen, M. I.; Hu, D.-E.; Jensen, P. R.; in 't Zandt, R.; Karlsson, M.; Gisselsson, A.; Nelson, S. K.; Witney, T. H.; Bihndiek, S. E.; Hansson, G.; Peitersen, T.; Lerche, M. H.; Brindle, K. M. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 19801–19806.
- (22) Sarkar, R.; Comment, A.; Vasos, P. R.; Jannin, S.; Gruetter, R.; Bodenhausen, G.; Hall, H.; Kirik, D.; Denisov, V. P. *J. Am. Chem. Soc.* **2009**, *131*, 16014–16015.
- (23) Wilson, D. M.; Keshari, K. R.; Larson, P. E. Z.; Chen, A. P.; Hu, S.; Criekeing, M. V.; Bok, R.; Nelson, S. J.; Macdonald, J. M.; Vigneron, D. B.; Kurhanewicz, J. *J. Magn. Reson.* **2010**, *205*, 141–147.
- (24) For an example of a non-endogenous hyperpolarized ^{89}Y complex used as a pH probe, see: Jindal, A. K.; Merritt, M. E.; Suh, E. H.; Malloy, C. R.; Sherry, A. D.; Kovács, Z. *J. Am. Chem. Soc.* **2010**, *132*, 1784–1785.
- (25) Ju, L.; Lippert, A. R.; Bode, J. W. *J. Am. Chem. Soc.* **2008**, *130*, 4253–4255.
- (26) Wang, J.-Z.; Lu, X.-Y.; Zhao, N.-P.; Cheng, Y.-Y.; Zeng, S. *Biomed. Chromatogr.* **2007**, *21*, 497–501.
- (27) (a) Amin, Y. M.; Nagwekar, J. B. *J. Pharm. Sci.* **1975**, *64*, 1804–1812. (b) Amin, Y. M.; Nagwekar, J. B. *J. Pharm. Sci.* **1975**, *64*, 1813–1818. (c) Amin, Y. M.; Nagwekar, J. B. *J. Pharm. Sci.* **1976**, *65*, 1341–1345.
- (28) Giorgio, M.; Trinei, M.; Miglaccio, E.; Pelicci, P. G. *Nature Rev. Mol. Cell. Biol.* **2007**, *8*, 722–728.
- (29) (a) For the preparation of ONOO⁻, see: Uppu, R. M. *Anal. Biochem.* **2006**, *354*, 165–168. (b) Ferrer-Sueta, G.; Radi, R. *ACS Chem. Biol.* **2009**, *4*, 161–177.
- (30) Oshio, K.; Feinberg, D. A. *Magn. Reson. Med.* **1991**, *20*, 344–349.