

Supporting Information

Real-Time *In Vivo* Detection of H₂O₂ using Hyperpolarized ¹³C- Thiourea

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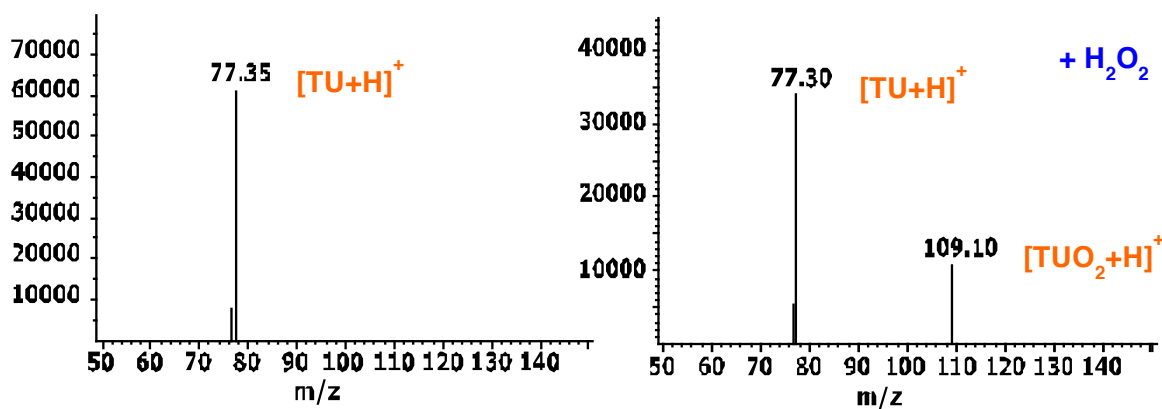


Figure S1. MS analysis of thiourea (TU) oxidation by H₂O₂ as reported in positive ion mode. TUO₂ was detected after addition of H₂O₂

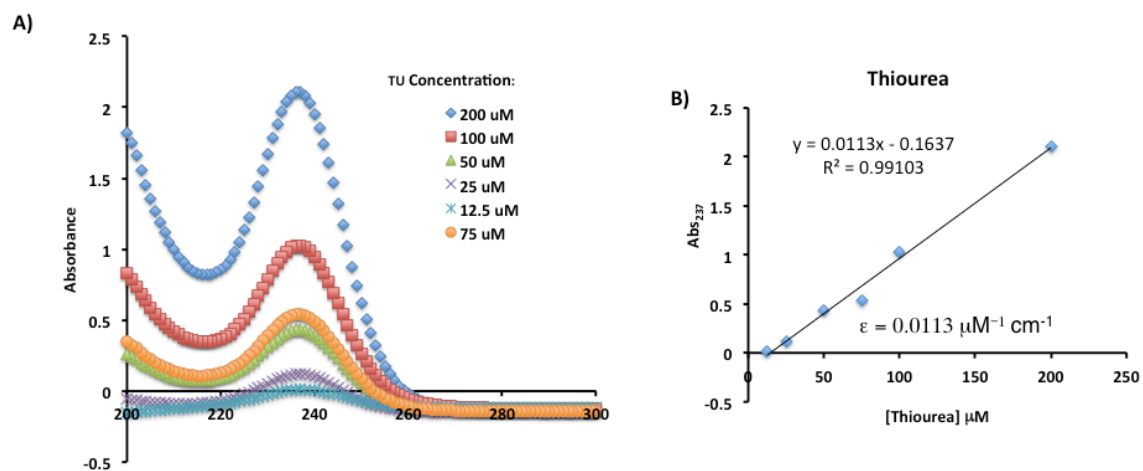
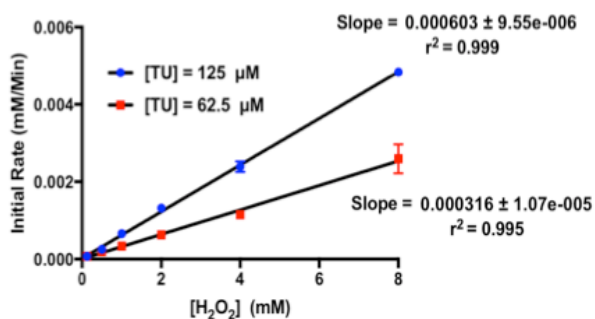


Figure S2 A) UV/Vis spectroscopic analysis of different thiourea concentrations (200-300 nm). Thiourea has a specific absorbance at 237 nm. B) Extinction coefficient for thiourea is estimated at $0.0113 \mu\text{M}^{-1} \text{cm}^{-1}$ (path length = 1 cm).



$$\text{Rate} = k_2 [\text{TU}]^x [\text{H}_2\text{O}_2]^y$$

Initial Rate is linearly proportional to $[\text{H}_2\text{O}_2]$
 $\Rightarrow y = 1$

Slope = $k_2 [\text{TU}]^x$
 \Rightarrow slope is linearly proportional to $[\text{TU}]$
 $\Rightarrow x = 1$

$$k_2 = 0.082 \pm 0.002 \text{ M}^{-1} \text{ s}^{-1}$$

$$= 4.94 \pm 0.08 \text{ M}^{-1} \text{ min}^{-1}$$

Figure S3. Rate of thiourea oxidation by H_2O_2 . Initial rate of oxidation was calculated from the first 500 s of reaction, and plotted against H_2O_2 concentration. The second order rate constant was estimated from the slope of the graph; an average value (k_2) was calculated from two different thiourea concentrations.

	Hyper Sense (3.35 T, operating 1.2K)	SpinLab (5 T, operating 0.8K)
T_1 (s)	30.91	57.50
Initial % Polarization	3.1	11.5
Scanner % Polarization	1.1	6.5
Time delay from dissolution to scanner (s)	32	33

Figure S4. Spin-lattice relaxation time (T_1) and polarization level of hyperpolarized ^{13}C -TU as measured in 3T Scanner.

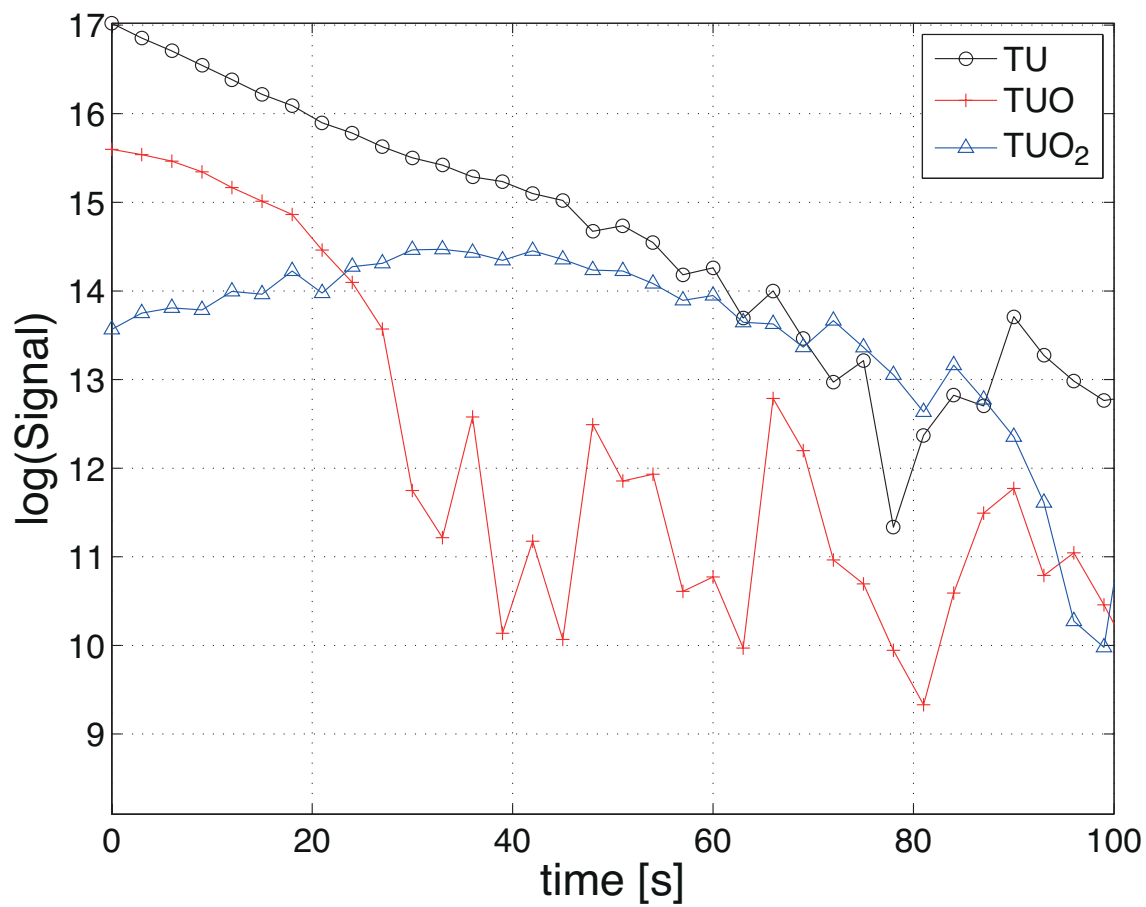


Figure S5. *In vitro* signal decays of hyperpolarized ^{13}C -thiourea and oxidation products (TUO and TUO_2) at 3T when mixed with H_2O_2

General Methods

All commercially obtained reagents were used as received unless otherwise noted. OX063 radicals were obtained from Oxford Instruments (UK), and AH11151 radicals were obtained from GE Healthcare (USA). ^{13}C -thiourea was from Cambridge Isotope (Andover, MA). NaONOO was from Calbiochem (Billerica, MA). TBHP was from Alfa Aesar (Tewksbury, MA). All other reagents were from Sigma-Aldrich (St. Louis, MO). ^{13}C NMR spectra were recorded on a Varian Inova 500 (125 MHz) spectrometer and are reported in terms of chemical shift. All chemical shifts are reported in parts per million (δ) relative to DMSO-d₆ or TMS (0 ppm) in D₂O. ESI LC-MS analyses were performed on Shimadzu LCMS2020 with a Synergi Hydro-RP 80, 4 μM column (30x2mm). A binary gradient of acetonitrile (with 0.1% formic acid) in water (with 0.1% formic acid) was used at a flow rate of 0.6 mL min⁻¹.

Reaction of Thiourea with Different ROS

Various ROS (15 mM) were reacted with thiourea (10 mM) in D₂O for 10 min before spectral measurement by ^{13}C -NMR. The ROS were prepared as follows: Hydrogen peroxide (H₂O₂), tert-butyl hydroperoxide (TBHP), peroxyxynitrite (NaONOO) and hypochlorite (NaOCl) were diluted to a 30 mM stock solution in D₂O from commercially available reagents before being delivered to an equal volume of 20 mM thiourea solution. Superoxide (KO₂) was prepared similarly as described above in DMSO and reacted with thiourea to give a final concentration of 12 mM O₂^{•-}. Hydroxyl radicals ($\bullet\text{OH}$) were generated by Fenton reaction using 30 mM H₂O₂ and 300 mM FeSO₄ prior to addition of equal volume of 20 mM thiourea. To determine the extent of thiourea oxidation by H₂O₂, 10 μM ^{13}C -thiourea was reacted with different H₂O₂ concentrations (2-27 μM) in D₂O for 10 min, and the accumulation of oxidized product was monitored by ^{13}C -NMR. The ratios of integrated peak intensity of the oxidized products (^{13}C -TUO₂) to the total signal intensities (^{13}C -TU + ^{13}C -TUO₂) were plotted against H₂O₂ concentration.

Kinetic Measurement of Thiourea Oxidation with H₂O₂

The extinction coefficient for thiourea was calculated according to Beer-Lambert law by monitoring UV absorbance at 237 nm (A_{237}) across different thiourea concentrations (12.5-200 μM). The initial rate of thiourea oxidation was measured at 237 nm (thiourea consumption) as a function of time with a LAMBDA 25 UV-vis spectrophotometer (Perkin-Elmer). Thiourea oxidation is initiated by the addition of freshly prepared H₂O₂ (0.125-8 mM) into thiourea solution (0.0625 and 0.125 mM) in PBS, pH 7.4. Reactions were monitored as A_{237} change, and the initial rate of oxidation (mM/Min) was calculated for the first 500 s and plotted against H₂O₂ concentration. The second order rate constant (k_2) was calculated from the slope of the graph (Fig S2) and averaged across two different thiourea concentrations. All reaction mixtures had a minimal volume of 100 μL and their readings were taken in a UVette cuvette (Eppendorf).

Polarization Method and T₁ Measurement *in vitro*

A HyperSense DNP system (Oxford Instruments Molecular Biotoools, Oxford, UK) and a SPINlab polarizer (GE Healthcare, Waukesha, WI) were used for the hyperpolarization of [1- ^{13}C]-thiourea. For the HyperSense, 4 M ^{13}C -thiourea and 15 mM OX063 (Oxford

instruments) were polarized in 60% (v/v) glycerol by irradiating at 139.88 GHz at 1.2 K. The frozen sample was rapidly dissolved in 100 mM Trizma buffer at pH 7.6 (dissolution media) to give a 50 mM final thiourea concentration before transferring to a clinical 3T MR Scanner for spectral measurement. For *in vitro* detection of thiourea oxidation, hyperpolarized ^{13}C -thiourea was rapidly dissolved in the dissolution media and mixed with H_2O_2 (final concentration = 50 μM) for ~ 30 s before spectral acquisition. For the SPINlab, 4 M ^{13}C -thiourea and 15 mM AH111501 (GE Healthcare) were placed in the sample vial with 16 mL of dissolution media in the research fluid path (GE Healthcare) and polarized in 60% (v/v) glycerol by irradiating at 139.88 GHz at 0.8 K. Approximately 7 mL of final solution of 50 mM thiourea was collected and scanned using a clinical 3T MR Scanner. Liquid-state polarization and longitudinal relaxation time (T_1) were estimated by comparing the hyperpolarized thiourea signal and the thermal polarization signal using an *in vitro* sample. A non-selective pulse-and-acquire sequence with a constant flip angle of 5.625° , 5-kHz spectral width and 2,048 points, and temporal resolution of 3 s for 4 min was used for the hyperpolarized sample. Resonance frequencies of TU and the oxidized products are assigned based on the resonance of a 1-M ^{13}C -bicarbonate phantom measured prior to each hyperpolarized scan. The T_1 was estimated after correcting the signal loss due to the radiofrequency (RF) excitations. After the hyperpolarized magnetization is fully relaxed, the thiourea solution was doped with gadolinium to shorten the T_1 (30 $\mu\text{L}/\text{mL}$) and the thermal polarization was measured 400 times and averaged with 90° flip-angles and 10 s of repetition time. A $^{13}\text{C}/^1\text{H}$ dual-tuned quadrature volume coil (diameter = 60 mm) was used for both RF transmit and data acquisitions.

***In Vivo* Detection of ^{13}C -Thiourea with Magnetic Resonance Spectroscopy Imaging**

The rats and mice were anesthetized with 1.5-3 % of isoflurane in oxygen (1.5 L/min) for tail vein catheterization and imaging experiments. Respiration, temperature, heart rate and oxygen saturation of the animals were monitored throughout the experiments. Body temperature was regulated at $\sim 36\text{-}37^\circ\text{C}$ using a warm water blanket placed underneath the animals, and respiration was maintained at ~ 60 breaths/min. ^{13}C -thiourea was polarized using the SPINlab and rapidly dissolved as previously described. Hyperpolarized ^{13}C -thiourea solution was injected into a rat through a tail vein catheter at a rate of approximately 0.25 mL/s for imaging experiments (total injection volume = 4 ml). For *in vivo* detection of thiourea oxidation, hyperpolarized ^{13}C -thiourea was rapidly dissolved in dissolution media, mixed with H_2O_2 (final concentration = 30 μM) and injected into the intraperitoneal cavity of a mouse at a rate of approximately 30 $\mu\text{L}/\text{s}$ (total injection volume = 1 ml). The time delay from dissolution to start of injection was ~ 20 s. All animal procedures were approved by the local Institutional Animal Care and Use Committee.

Animal experiments were performed on a clinical 3T MR scanner (GE Healthcare, Waukesha, WI). Single-shot fast spin-echo MR images in the axial, sagittal and coronal planes with 2-mm slice thickness were acquired as anatomical references for prescribing the ^{13}C MR spectroscopy experiments. For ^{13}C MRSI studies in rats, the $^{13}\text{C}/^1\text{H}$ dual-tuned quadrature transmit/receive RF volume coil was used. A single time-point ^{13}C imaging was acquired using a free induction decay chemical shift imaging sequence over

an oblique slice that contains heart, liver, and kidney (field of view = $80 \times 80 \text{ mm}^2$, matrix size = 16×16 , spectral width = 5 kHz, spectral point = 256, slice thickness = 1.5 cm, repetition time = 75 ms). For ^{13}C MRS studies in mice, a custom-built ^{13}C transmit/receive surface coil (diameter = 28 mm) was placed over the liver with mouse supine and a quadrature volume ^1H coil (diameter = 70 mm) was used for anatomical localization and to confirm the position of the ^{13}C coil with respect to the liver. A non-selective pulse-and-acquire sequence with flip-angles of 10° , 5-kHz spectral width and 2,048 points was used to acquire ^{13}C spectra every 3 s for 4 min immediately after the ^{13}C -thiourea injection.