Supporting Information

Detection and quantification of hydrogen peroxide in aqueous solutions using chemical exchange saturation transfer (CEST)

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S1. Effect of H_2O_2 on water relaxation times.

Method: Longitudinal (T_1) relaxation times of the samples were assessed using a RARE-based saturation recovery sequence with eight T_R times ranging between 200 ms to 15,000 ms. T_1 relaxation times were estimated by fitting the ROI values to Equation S4 using Matlab,

$$S(T_R) = M_0 \times [1 - exp\left(-\frac{T_R}{T_1}\right)] \qquad \text{Eq. S1}$$

where $S(T_R)$ are the MRI signal at each T_R time, and the theoretical maximal MRI signal S_0 and T_1 time are the parameters to be estimated.

T_2 relaxation times were acquired using a Carr-Purcell-Meiboom-Gill (CPMG) method[1]. Briefly, a T_2 preparation module was added in the front of a fast spin-echo imaging readout, i.e., Acquisition with Relaxation Enhancement (RARE) pulse sequence. The T_2 preparation period consisted of an element of CPMG pulse train with t_{CPMG} =10 ms. We used a total 16 CPMG loop number from 2 to 1024, making the echo times from 20 ms to 10.24 sec. The imaging parameters were: T_R/T_E=25000/4.3 ms, RARE factor=16, a 64x64 acquisition matrix with a spatial resolution of *c.a.* 250x250 μm^2, and slice thickness of 1 mm. The acquisition time for each T_2-weighted image was 1 min 40 s. To obtain r_{2ex} of the compound, T_2 relaxation times of the compound at different concentrations, i.e., 1, 2, 5 and 10 mM, were measured and fitted to Equation S1.

$$R_2 = R_2^0 + r_2 \times [C] \qquad \text{Eq. S2}$$

Where R_2^0 is the inherent R_2 relaxation rate of the solutions and [C] is the concentration of the agent.

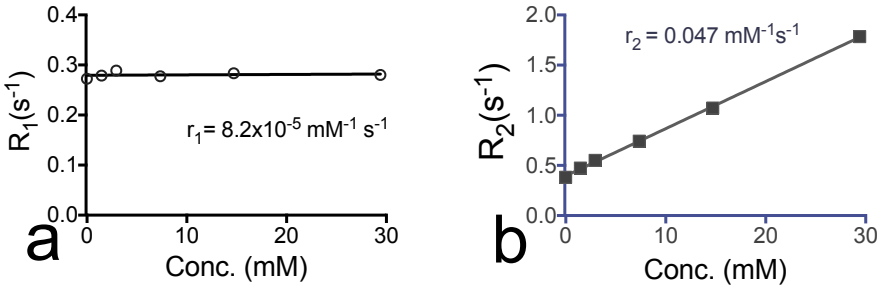

Figure S-1. Longitudinal (**a**) and transverse (**b**) relaxation rates of H_2O_2 solution of different concentrations.

S2. Saturation time (T_{sat}) and saturation power(B_1) dependence of the CEST signal of H_2O_2

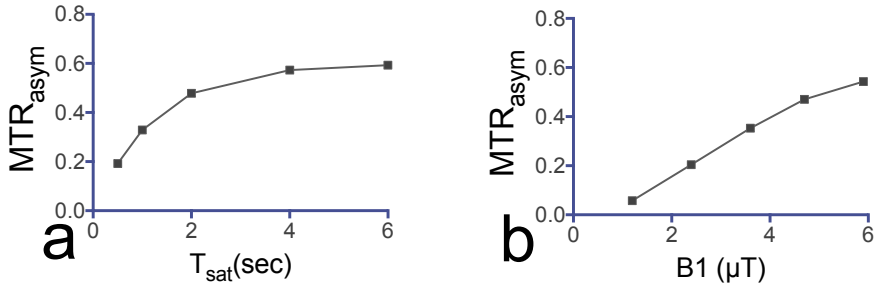

Figure S-2. Saturation parameter dependence of the CEST signal of 1% H_2O_2. (**a**) CEST signal with saturation time (t_{sat}) of 1, 2, 3, 4 and 6 sec, and a fixed saturation field strength (B_1) of 4.7 μT; and (**b**) CEST signal with B_1 of 1.2, 2.4, 3.6, 4.7 and 5.9 μT and a fixed t_{sat} of 4 sec. All data were corrected for B_0 inhomogeneity using the WASSR method[2,3].

S3. Estimation of exchange rate of H_2O_2 using the QUEST method

Method: The exchange rate of exchangeable protons of H_2O_2 (6.2 ppm) at pH 6.0 was measured using the QUantifying Exchange using Saturation Time (QUEST) method[4]. In brief, the CEST contrast for samples containing 1% H_2O_2 (60 mM of exchangeable protons) at pH 6.0 was measured with saturation delays of 0.5, 1, 2, 4, and 6 sec, using

a saturation field strength of 5.9 μT (250Hz) and the repetition time (TR) set to 10 sec, using the RARE imaging sequence. The calculated MTR_{asym} values were then fit using numerical solutions to the Bloch equations with exchange rate (k_{ex}) and water T_{2W} being the free parameter. The fixed model parameters were water R_{1w}=0.283 s^{-1}, solute R_{1s}= R_{1w}=0.283 s^{-1} and solute R_{2s}= 66 s^{-1}.

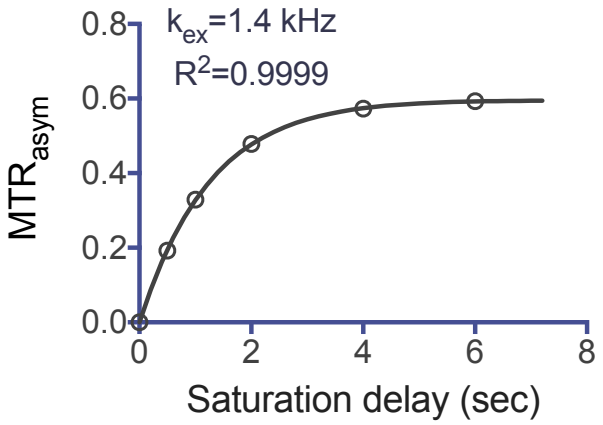

Figure S-3: Estimation of exchange rate of 1% H_2O_2 at 6.2 ppm at pH=6.0 and 37°C using the QUEST method.

S4. pH dependence of the NMR and CEST signal of H_2O_2

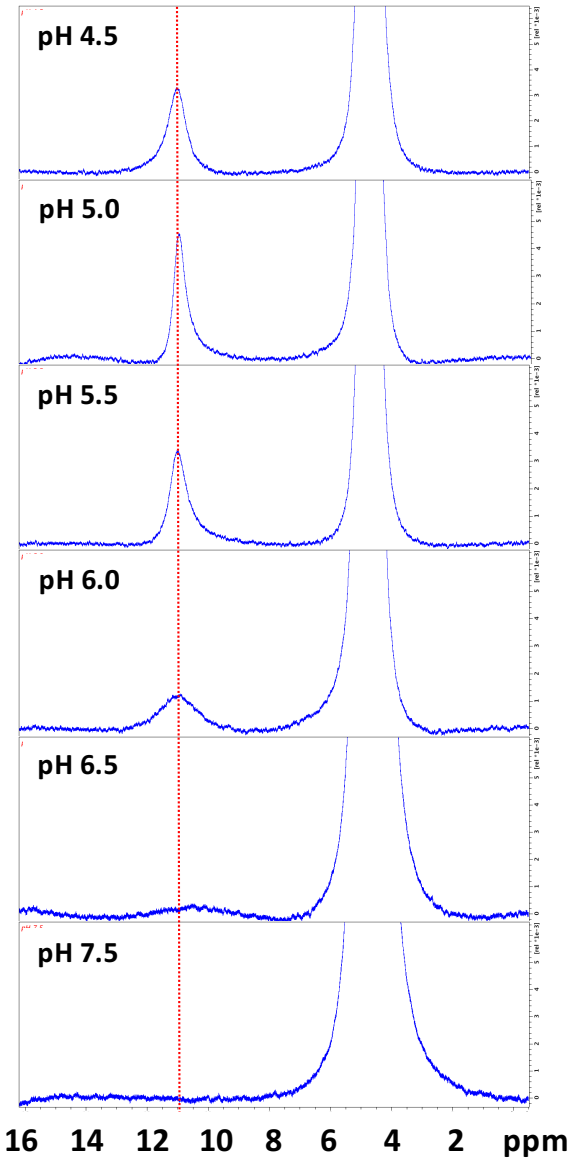

Figure S-4. NMR ^{1}H spectra of 1% H_2O_2 solutions at different pHs. The solvent used in is 90% H_2O +10% D_2O. Red dotted line indicates the chemical shift of 11 pm (or 6.3 ppm apart from water). The measurement was conducted using a standard 1D NMR pulse sequence at room temperature on a Bruker Avance III 500 MHz NMR spectrometer. The number of average was 128.

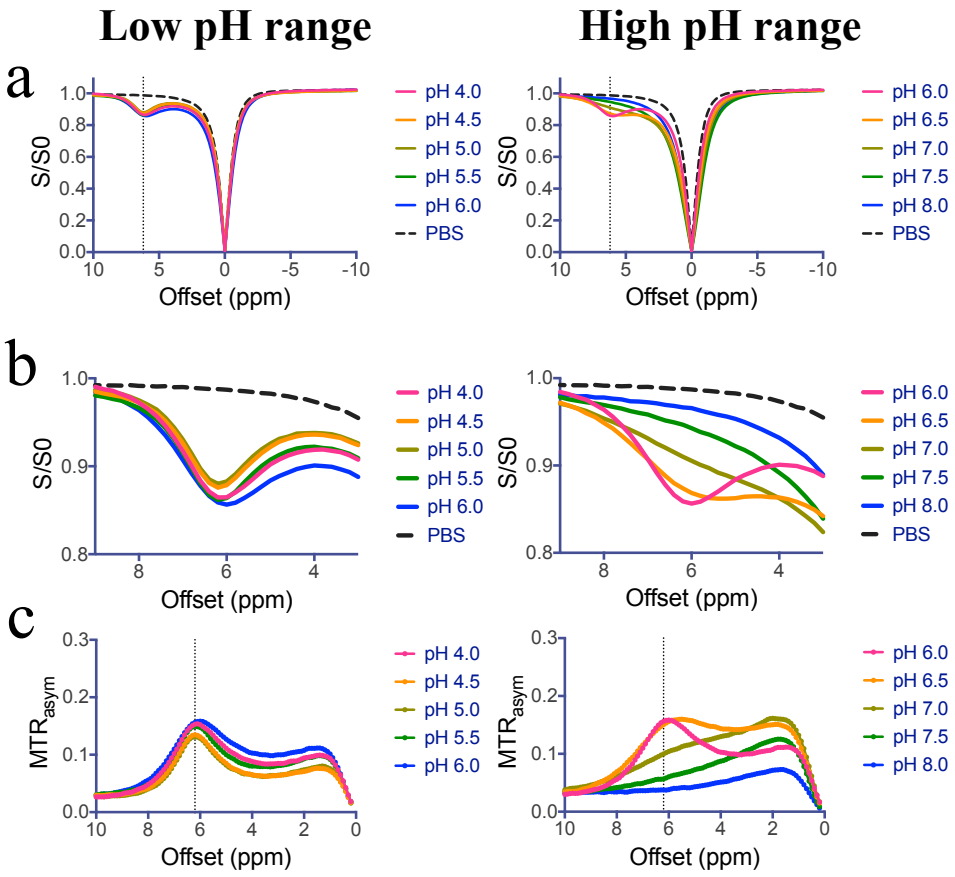

Figure S-5. CEST MRI contrast of H_2O_2 solutions at different pHs. a) Z-spectra (-10 to 10 ppm) of H_2O_2 in the low and high pH range; **b**) The zoomed view of the corresponding Z-spectra in (**a**) around 6.2 ppm; and (**c**) MTR_{asym} plots in the low and high pH range. It is interesting that the peak at ~1 ppm has a much less sensitivity to the change in pH. For example, at high pH, the 6.2 ppm peak completely disappears. While the 1 ppm peak also dramatically decreased, it still has detectable signal, for example 0.054 ± 0.018 at pH 8.0. Thus, the pH-dependence study indicates the exchange rate of 1 ppm is much slower than that of 6.2 ppm.

S5. Repeatability of CEST MR measurement

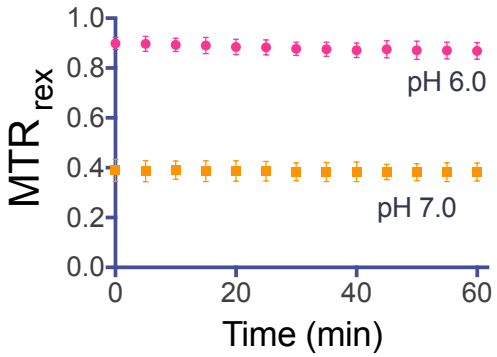

Figure S-6. Repeatability of CEST UFZ NMR measurement. For each pH, triplicate samples were prepared. Each sample was measured using the UFZ method intermittently every five minutes for one hour. The temperature was maintained at 37 ^{o}C throughout the study.

S6. Detectability of CEST MR measurement

Table S-1. The minimal concentration H_2O_2 using CEST NMR method and the corresponding P values (n=3)

		pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0
Minimal conc.	**mM**	1.5	1.5	1.5	0.7	0.7	1.5	1.5	1.5	14.7
	%	0.005	0.005	0.005	0.0025	0.0025	0.005	0.005	0.005	0.05
P value		0.0159	0.0147	0.0806	0.0028	0.0369	0.0123	0.0113	0.0192	0.0114

References

(1) Yadav, N. N.; Xu, J.; Bar-Shir, A.; Qin, Q.; Chan, K. W.; Grgac, K.; Li, W.; McMahon, M. T.; van Zijl, P. C. *Magn Reson Med* **2014**, *72*, 823-828.
(2) Liu, G.; Gilad, A. A.; Bulte, J. W.; van Zijl, P. C.; McMahon, M. T. *Contrast Media Mol. Imaging* **2010**, *5*, 162-170.
(3) Kim, M.; Gillen, J.; Landman, B.; Zhou, J.; van Zijl, P. *Magnetic Resonance in Medicine* **2009**, *61*, 1441-1450.
(4) McMahon, M. T.; Gilad, A. A.; Zhou, J.; Sun, P. Z.; Bulte, J. W.; van Zijl, P. C. *Magnetic Resonance in Medicine* **2006**, *55*, 836-847.