Electronic Supplementary Information for:

Ratiometric Quantitation of Redox Status with a Molecular Fe₂ Magnetic Resonance Probe

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Experimental Section

General considerations. Unless otherwise specified, chemicals and solvents were purchased from commercial vendors and used without further purification. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Bovine blood plasma was obtained from commercial sources (Sigma Aldrich lot P4639). When necessary for moisture sensitive experiments, glassware was flame dried or stored in an oven at 150 °C for at least 4 hours, followed by cooling in a desiccator. Air- and water-free manipulations were carried out using standard Schlenk techniques. Acetonitrile was dried using a commercial solvent purification system from Pure Process Technology and stored over 4 Å molecular sieves prior to use. Water was obtained from a purification system from EMD Millipore. Elemental analysis was conducted by Midwest Microlab Inc. Preparative reverse-phase HPLC was performed on a Waters 19×250 mm² Xbridge C18 Column, using the Varian Prostar 500 system equipped with a Varian 363 fluorescence detector and a Varian 335 UV/Visible Detector. During HPLC, water was used as solvent A and acetonitrile as solvent B. The absorbances at 220 and 285 nm were monitored. Anhydrous hydrogen chloride gas was generated by adding concentrated hydrochloric acid to a stirring solution of concentrated sulfuric acid. The gas was passed through a bubbler filled with sulfuric acid. Synthesis of N,N'-[(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)]bis[N-(carboxymethyl)glycinamide] (HL) was based off a procedure reported previously.¹

Synthesis of (NMe4)[LFe2L']·2.7H2O·THF (1). To a stirred suspension of HL (20 mg, 0.051 mmol) in MeOH (2 mL) was added dropwise a solution of anhydrous FeCl₂ (13 mg, 0.10 mmol) in MeOH (2 mL) to give a light purple solution. A solution of etidronic acid monohydrate (11.4 mg, 0.051 mmol) in MeOH (2 mL) was then added dropwise to give a light orange solution, followed by addition of NMe4OH·5H₂O (46 mg, 0.26 mmol) in MeOH (2 mL) to give a light yellow slurry. After stirring for 12 h, the yellow solid was collected by filtration and washed with MeOH (10 mL). The solid was then stirred in THF (15 mL) for 20 min, collected by filtration, and dried under reduced pressure for 12 h to give 1 (20 mg, 50%). Solution magnetic moment $\chi_M T = 7.3(3) \text{ cm}^3 \text{K/mol}$ (310 K). Anal. Calcd. for C₂₇H_{54.4}Fe₂N₇O_{15.7}P₂: C, 35.99; H, 6.08; N, 10.87. Found: C, 36.00; H, 6.15; N, 10.91. ICP-OES: Fe:P = 0.96:1. UV-Vis absorption spectrum: 453 nm (ε = 48 M⁻¹ cm⁻¹). FT-IR (ATR, cm⁻¹): 3289 (m, broad); 3158 (m, broad); 1668 (s); 1615 (s); 1475 (s); 1447 (w); 1311 (s); 1262 (w); 1097 (s); 1055 (s); 991 (m); 877 (m); 802 (w); 660 (m); 560 (s) (see Figure S43).

Synthesis of LFe₂L'•0.7H₂O•0.2THF (2). To a stirred suspension of HL (20 mg, 0.051 mmol) in MeOH (2 mL) was added dropwise a solution of anhydrous FeCl₃ (8.2 mg, 0.051 mmol) in MeOH (2 mL) to give a dark purple solution. Anhydrous FeCl₂ (6.4 mg, 0.051 mmol) in MeOH (2 mL) was then added dropwise to this solution, with no significant color change observed. A solution of etidronic acid monohydrate (11 mg, 0.051 mmol) in MeOH (2 mL) was then added dropwise, resulting in a dark red solution. A solution of NMe₄OH•5H₂O (46 mg, 0.26 mmol) in MeOH (2 mL) was then added dropwise to give a red slurry. After stirring for 12 h, the a redbrown solid was collected by filtration and was washed with MeOH (10 mL). The solid was then stirred in THF (15 mL) for 20 min, collected by filtration, and dried under reduced pressure for 12 h to give **2** (34 mg, 95%). Solution magnetic moment $\chi_M T = 7.0(6) \text{ cm}^3\text{K/mol}$ (310 K). Anal. Calcd. for C_{19.8}H₃₂Fe₂N₆O_{12.9}P₂: C, 32.40; H, 4.39; N, 11.45. Found: C, 32.40; H, 4.27; N, 11.38.

ICP-OES: Fe:P = 0.97:1. UV-Vis absorption spectrum: 470 nm (ε = 861 M⁻¹ cm⁻¹), 801 nm (ε = 168 M⁻¹ cm⁻¹), 1383 nm (ε = 83 M⁻¹ cm⁻¹). FT-IR (ATR, cm⁻¹): 3278 (m, broad); 2969 (m, broad); 1655 (s); 1613 (s); 1479 (m); 1453 (w); 1311 (m); 1263 (m); 1120 (s); 1049 (s); 988 (s); 877 (m); 805 (w); 662 (m); 562 (s) (see Figure S43). Slow diffusion of THF vapor into a concentrated solution of **2** in H₂O over the course of 3 d gave dark red plate-shaped crystals of LFe₂(etidronate)·7H₂O suitable for single-crystal X-ray diffraction analysis.

Synthesis of [LFe₂L'](NO₃)·0.9H₂O·1.5THF (3). To a stirred suspension of HL (20 mg, 0.051 mmol) in MeOH (2 mL) was added dropwise a solution of Fe(NO₃)₃·9H₂O (41 mg, 0.10 mmol) in MeOH (2 mL) to give a dark purple solution. A solution of etidronic acid monohydrate (11 mg, 0.051 mmol) in MeOH (2 mL) was then added dropwise to give a red solution. A solution of NMe₄OH·5H₂O (46 mg, 0.26 mmol) in MeOH (2 mL) was then added dropwise give a red slurry. After stirring for 12 h, the ensuing red solution was dried under reduced pressure to give a red solid. The solid was stirred in DMF (10 mL) for 30 min, and was then collected by filtration and washed with THF (10 mL) and Et₂O (10 mL). The residue was dried under reduced pressure for 2 h to give **3** (20 mg, 51%) Solution magnetic moment $\chi_M T = 8.9(3) \text{ cm}^3 \text{K/mol}$ (310 K). Anal. Calcd. for C_{20.5}H_{36.7}Fe₂N₇O_{17.4}P₂: C, 29.52; H, 4.44; N, 11.78. Found: C, 29.52; H, 4.53; N, 11.84. ICP-OES: Fe:P = 1.1:1. FT-IR (ATR, cm⁻¹): 3110 (m, broad); 1652 (s); 1591 (s); 1477 (m); 1417 (w); 1386 (m); 1315 (m); 1095 (m); 1001 (s); 880 (m); 800 (m); 664 (w); 573 (m); 468 (s); 448 (m); 414 (m) (see Figure S43).

X-ray structure determination. A single crystal of $LFe_2(etidronate) \cdot 7H_2O$ was directly coated with Paratone-N oil and mounted on a MicroMountsTM rod. The crystallographic data were collected at 100 K on a Bruker APEX II diffractometer equipped with MoK α sealed tube source. Raw data were integrated and corrected for Lorentz and polarization effects using Bruker APEX2 v. 2009.1.² The program SADABS was used to apply absorption correction.³ Space group assignments were determined by examining systematic absences, E-statistics and successive refinement of the structure. Structures were solved by SHELXT⁴ using direct methods and refined by SHELXL within the OLEX interface.⁽³⁾ Partially occupied solvent H₂O molecules that were potentially hydrogen bonded were modeled isotropically. Thermal parameters for all other non-hydrogen were refined anisotropically. Crystallographic data and the details of data collection are listed in Table S1.

¹**H** NMR experiments. Variable temperature ¹H NMR spectra were collected on an Agilent DD MR-400 system (9.4 T) system. The T_1 of H₂O was obtained by fitting H₂O intensities from experiments with an array of relaxation times implemented in the program *vnmr*. Linewidth analyses were obtained in the program MNOVA.

CEST experiments. Variable temperature CEST experiments were performed on an Agilent DD MR-400 system (9.4 T) system. In a typical experiment, samples containing 100% **1**, 100% **2** or mixture of the two at a desired ratio in buffer containing 100 mM NaCl and 100 mM of HEPES at pH desired were used for CEST experiments. Z-spectra (CEST spectra) were obtained according to the following protocol. NMR spectra were acquired using the presaturation pulse applied for 7 s at a power level of 24 μ T. The saturation frequency offsets were screened with a step increase of 1 ppm. The obtained NMR spectra were plotted as normalized water intensity

against frequency offset to produce a Z-spectrum. Direct saturation of the water signal was set to 0 ppm. D₂O was placed in an inner capillary to lock the sample. Exchange rate constants were calculated based off a reported method.⁵ The B_1 values are calculated based on the calibrated 90-degree pulse on a linear amplifier. The NMR spectra were acquired at various presaturation powers ranging from 14 to 24 μ T applied for 7 s. To correct for baseline, reported values of %CEST are the difference in percent H₂O signal reduction between applied on-resonance and off-resonance pre-saturations.

Solid state magnetic measurements. Magnetic measurements were carried out using a Quantum Design MPMS-XL SQUID magnetometer. Powder samples were sealed in 2 mL polyethylene bags. Dc susceptibility data were collected from 1.8 to 300 K at applied dc fields of 1, 1.5 and 2 T. Dc susceptibility data were corrected for diamagnetic contribution from the sample holders and from the sample (estimated using Pascal's constants⁶). The temperature dependent magnetic susceptibility data for **1** (10-300 K) and **2** (1.8-300 K) and were model using spin Hamiltonian $\hat{H} = -2J(\hat{S}_{Fe1}\cdot\hat{S}_{Fe2})$,⁷ where *J* is the magnetic superexchange coupling constant; and \hat{S}_{Fe1} and \hat{S}_{Fe2} are the spin operators for the Fe ions. The best fits of the data give g = 2.20(3) and 2.00(4) for **1** and **2**, respectively.

Solution magnetic measurements. Magnetic moments of metal complexes were carried out using Evan's method⁸ at 310 K. In a typical experiment, compounds (about 4 mM) were dissolved in a mixture of 0.5 w/w % of DMSO in D₂O. A capillary containing same solvent mixture (without the to-be-characterized compound) was inserted into each NMR sample as reference. Diamagnetic correction was carried out based on the empirical formula of each compound (as determined by elemental analysis) using Pascal's constants.⁶

Electrochemical measurements. Cyclic voltammetry was carried out in a standard onecompartment cell inside a nitrogen glove box at room temperature, equipped with a platinum working electrode, a platinum wire as counter electrode and a SCE reference electrode using a CHI 760c potentiostat. The analyte solution was with 100 mM NaCl and 100 mM HEPES buffered at pH 7.4. The voltammogram was converted and shown as values referred to the normal hydrogen electrode (NHE), using a literature conversion factor.⁹ Open circuit potentials were measured by the built-in technique "open circuit potential – time" within the CHI660E electrochemical workstation software. The open circuit potential readings were recorded 10 minutes after the experiment started, at which time the reading was stabilized.

Mössbauer spectroscopy. Zero-field ⁵⁷Fe Mössbauer spectra were obtained at various temperatures with a constant acceleration spectrometer and a ⁵⁷Co/rhodium source. Prior to measurements, the spectrometer was calibrated at 295 K with α -iron foil. Samples were prepared in an MBraun nitrogen glove box. A typical sample contained approximately 60 mg of compounds (~10 mg of Fe) suspended in a plastic cap in heated eicosane, which solidified upon cooling to ambient temperature, in order to immobilize the sample. Another cap with a slightly smaller diameter was squeezed into the previous sample cap to completely encapsulate the solid sample mixture. All spectra were analyzed using the WMOSS Mössbauer Spectral Analysis Software (www.wmoss.org).

Other physical measurements. Infrared spectra were recorded on a Bruker Alpha FTIR spectrometer equipped with an attenuated total reflectance accessory. Solution and solid-state UV-vis-NIR spectra were obtained using an Agilent Cary 5000 spectrophotometer.

Estimation of electron transfer rate by IVCT analysis.¹⁰ The calculation of ambient temperature electron-transfer rate in 2 is based on a method described in a similar mixed-valence Fe₂ analog. Location of the IVCT v_{max} , extinction coefficient (ε) and Fe····Fe distance (d) were obtained experimentally as described in the main text. The full width at half maximum ($\Delta v_{1/2}$) was determined by fitting IVCT to a Gaussian model in the software OriginPro. The electron-transfer rate (k_{et}) in 2 can be calculated using the following equation:

$$k_{\rm et} = v_{\rm et} \exp(-\Delta G^*/RT)$$

where *R* is the ideal gas constant and *T* is temperature. The frequency factor for electron transfer, v_{et} , and the thermal free energy, ΔG^* , are given by:

 $v_{\rm et} = 2\pi^{3/2} v_{\rm ab} h^{-1} (kT v_{\rm max})^{-1/2}$ and $\Delta G^* = v_{\rm max} (4 - v_{\rm ab})^{-1}$

where *h* is the Planck constant, *k* is the Boltzmann constant and v_{max} is the wavenumber of the IVCT peak maximum. The resonance matrix element, v_{ab} , is given by:

$$v_{ab} = \alpha v_{max},$$

where the extent of electron delocalization $\alpha^2 = 4.2 \times 10^{-4} \varepsilon \Delta v_{1/2} (v_{\text{max}} d^2)^{-1}$. Here, ε is the extinction coefficient of IVCT, and *d* is the Fe···Fe distance determined by X-ray structural analysis.

Viability experiment. Melanoma B16F10 cells (ATCC) were cultured in Dulbecco's Modified Eagle's Media (Life Technologies) supplemented with 10% fetal bovine serum (Fisher), 1 mM each of sodium pyruvate, non-essential amino acids and L-glycine at 37 °C and 5% CO₂. Cells for the experiment were subcultivated for 3 to 4 times after thawing the cell stocks. Cells were incubated with media containing the desired concentration of **3** for 24 h before viability measurements. Cell viability was measured by a Guava EasyCyte Mini Personal Cell Analyzer (EMD Millipore). Each sample subjected for analysis contained 50 μ L of well-mixed cell suspension and 150 μ L of Guava ViaCount reagent. Stained samples were vortexed for 20 s and immediately subjected to counting using the ViaCount software module. Viability was measured using the EasyFit software module. Cells not treated by **3** were used as a control to account for normal cell death. Reported % viability was normalized with respect to the control samples.

MRI phantom experiment. Samples contained 100 mM of NaCl, 100 mM of HEPES buffered at pH 7.4 and overall 10 mM Fe₂ concentration with **1**:2 ratio ranging from 9:1 to 1:9. ~0.5 mL of each sample was stored in a 0.5 mL Eppendorf tube, which was placed within another scintillation vial filled with H₂O solution containing 1 mg/mL CuSO₄ and 100 mM NaCl for T_1 matching. CEST experiments were carried out on a Bruker Biospec 9.4 T MRI scanner running ParaVision 6.0.1 (Bruker Biospin, Billerica, MA, USA). Temperature was maintained at 37 °C using a warm water circulating system with feedback control from a temperature probe (SA Instruments, Stonybrook, NY, USA). CEST images were acquired using an accelerated spin echo based sequence with a pre-saturation pulse (14 μ T, 2 s duration) applied at offsets of 83 and 40 ppm (M_z). Other imaging parameters: TR/TE = 2034/14.9 ms, RARE factor 16, matrix = 64 × 64,

FOV = 3.2×3.2 cm, 2 mm slice thickness, and 2 averages). Matched unsaturated images were acquired using identical parameters except that the pulse amplitude was set to 0 μ T (M_0). %CEST = $(1 - M_z/M_0) \times 100\%$. Only regions of the inner Eppendorf tube, where the sample containing the Fe₂ probe is shown in Figure 5. Averaged intensities of the same regions were used to calculate CEST_{83 ppm}/CEST_{40 ppm} for fitting. For the fitting, sample A was a significant outlier likely due to weak CEST signal, and therefore was not taken into account for fitting. Trace amounts of precipitation occurred for sample D, E, F during the phantom experiment, likely due to the affected solubility of **1** and/or **2** in the presence of high buffer concentration. Such precipitate did not alter either the phantom experiment or OCP measurement.

	LFe2(etidronate)·7H2O
Empirical formula	$C_{19}H_{38}Fe_2N_6O_{19}P_2$
Formula weight, g mol ⁻¹	828.19
Crystal system	Orthorhombic
Space group	P na 2_1
Wavelength, Å	0.71073
Temperature, K	100
<i>a</i> , Å	21.3916(7)
b, Å	9.4099(3)
<i>c</i> , Å	16.1292(5)
α, ⁰	90
β, °	90
γ, ^o	90
<i>V</i> , Å ³	3246.7(2)
Ζ	4
$ ho_{ m calcd},{ m Mg}~{ m m}^{-3}$	1.694
μ , mm ⁻¹	1.081
Reflections coll./unique	47460/45560
R _{int}	0.0293
${}^{\mathrm{a}}R_{1} (I > 2\sigma(I))$	0.0497
$^{b}wR_{2}$ (all)	0.1170
GooF	1.2370
${}^{a}R_{1} = \Sigma F_{0} - F_{C} / \Sigma F_{0} $	
^b $wR_2 = [\Sigma w (F_0^2 - F_C^2)^2 / \Sigma w (F_o^2)^2]^{1/2}$	

Table S1 | Crystallographic data for LFe₂(etidronate) \cdot 7H₂O



Figure S1. CV of **1** in solution containing 100 mM NaCl, 100 mM HEPES, buffered at pH 7.4. 50 mV/s scan rate.



Figure S2. Variable temperature Mössbauer spectra of **2**. Crosses, black line, red line and blue line represent the experimental data, global fit, fit of high spin Fe^{II} and fit of high spin Fe^{III}, respectively.



Figure S3. Mössbauer spectrum of **1** at 80 K. Crosses and red line represent the experimental data and fit, respectively.



Figure S4. UV-vis-NIR spectra of 1 (black) and 2 (red) in D₂O.



Figure S5. Diffuse reflectance spectrum of **2**. The flat feature at ca. 12000 cm^{-1} is an instrument artifact.



Figure S6. Variable temperature dc magnetic susceptibility data for 1 (blue circles) and 2 (red circles) collected under an applied field of 1 T. The black lines correspond to fits of the data.



Figure S7. Stacked NMR spectra of 1 in neutral H₂O (red) and D₂O (black) at 37 °C.



Figure S8. Stacked NMR spectra of 2 in neutral H₂O (red) and D₂O (black) at 37 °C.



Figure S9. CEST spectra collected at 37 °C for solutions containing 4.9 mM of **1** (top) and **2** (bottom) with 100 mM HEPES and 100 mM NaCl buffered at pH 7.4.



Figure S10. Omega plot of the CEST effect at 29 ppm (red) and 40 ppm (blue) of 4.0 mM **1** in pH 7.4 H₂O with 100 mM HEPES and 100 mM NaCl. Circles represent experimental data and the lines represent the linear fit.



Figure S11. Omega plot of the CEST effect at 74 ppm (red) and 83 ppm (blue) of 4.0 mM **2** in pH 7.4 H₂O with 100 mM HEPES and 100 mM NaCl. Circles represent experimental data and lines represent linear fits.



Figure S12. OCPs of solutions containing overall 4.9 mM Fe₂, with the ratio of **1**:2 ranging from 9:1 (bottom most) to 1:9 (top most), are monitored over time upon mixing (at 0 s). Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.4.



Figure S13. OCPs of solutions containing overall 3.8 mM Fe₂, with the ratio of **1**:2 ranging from 9:1 (bottom most) to 1:9 (top most), are monitored over time upon mixing (at 0 s). Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.5.



Figure S14. OCPs of solutions containing overall 2.6 mM Fe₂, with the ratio of **1**:2 ranging from 9:1 (bottom most) to 1:9 (top most), are monitored over time upon mixing (at 0 s). Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.3.



Figure S15. OCPs of solutions containing overall 10 mM Fe₂, with the ratio of **1**:2 ranging from 9:1 (bottom most) to 1:9 (top most), are monitored over time upon mixing (at 0 s). Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.4



Figure S16. CEST spectra collected at 37 °C for a sample containing 4 mM of **1** mixed with 1 mM of KO_2 in a solution containing 100 mM HEPES and 100 mM NaCl buffered at pH 7.4. Refer to the CEST experimental section for details on baseline correction.



Figure S17. CEST spectra collected at 37 °C for a sample containing 4 mM of **2** mixed with 200 mM of cysteine in a solution containing 100 mM HEPES and 100 mM NaCl buffered at pH 7.4. Refer to the CEST experimental section for details on baseline correction.



Figure S18. OCPs of two solutions containing 4 mM **1** with 1 mM KO₂ (black) and 4mM **2** with 200 mM cysteine (red) are monitored over time upon mixing (at 0 s). Measurements were stopped when OCP varied less than 1 mV within 5 minutes. Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.4.



Figure S19. CEST spectra for 2.6 mM aqueous solutions of **1** and **2**, with ratios of **1**:**2** from 9:1 (blue) to 1:9 (red) at 37 °C. Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.3. The legend gives the independently obtained OCP of each sample (mV vs NHE). Inset: Expanded view of the relevant CEST peaks.



Figure S20. Open circuit potentials for solutions, containing 100 mM HEPES, 100 mM NaCl and 2.6 mM Fe₂ buffered at pH 7.3 at 37 °C, is plotted against both the ratio of CEST effects from application of presaturation at 83 and 40 ppm and the natural log of the ratio (inset). Black circles and the red line represent the experimental data and the fit, respectively.



Figure S21. CEST spectra for 3.8 mM aqueous solutions of **1** and **2**, with ratios of **1**:2 from 9:1 (blue) to 1:9 (red) at 37 °C. Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.5. The legend gives the independently obtained OCP of each sample (mV vs NHE). Inset: Expanded view of the relevant CEST peaks.



Figure S22. Open circuit potentials for solutions, containing 100 mM HEPES, 100 mM NaCl and 3.8 mM Fe₂ buffered at pH 7.5 at 37 °C, is plotted against both the ratio of CEST effects from application of presaturation at 83 and 40 ppm and the natural log of the ratio (inset). Black circles and the red line represent the experimental data and the fit, respectively.



Figure S23. Comparison of Nernstian fits (from Figures 4, S20, and S22) obtained from data at various pH values.



Figure S24. CEST spectra for 3.8 mM aqueous solutions of **1** and **2**, with ratios of **1**:**2** from 9:1 (blue) to 1:9 (red) at 35 °C. Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.5. The legend gives the independently obtained OCP of each sample (mV vs NHE). Inset: Expanded view of the relevant CEST peaks.



Figure S25. Open circuit potentials for solutions, containing 100 mM HEPES, 100 mM NaCl and 3.8 mM Fe₂ buffered at pH 7.5 at 35 °C, is plotted against both the ratio of CEST effects from application of presaturation at 83 and 40 ppm and the natural log of the ratio (inset). Black circles and the red line represent the experimental data and the fit, respectively.



Figure S26. CEST spectra for 3.8 mM aqueous solutions of **1** and **2**, with ratios of **1**:**2** from 9:1 (blue) to 1:9 (red) at 39 °C. Each solution contains 100 mM NaCl and 100 mM HEPES buffered at pH 7.5. The legend gives the independently obtained OCP of each sample (mV vs NHE). Inset: Expanded view of the relevant CEST peaks.



Figure S27. Open circuit potentials for solutions, containing 100 mM HEPES, 100 mM NaCl and 3.8 mM Fe₂ buffered at pH 7.5 at 39 °C, is plotted against both the ratio of CEST effects from application of presaturation at 83 and 40 ppm and the natural log of the ratio (inset). Black circles and the red line represent the experimental data and the fit, respectively.



Figure S28. Comparison of Nernstian fits (from Figures S22, S25 and S27) obtained from data at various temperatures.



Figure S29. NMR spectra of 4 mM of **1** in pH 7.4 buffer with (top) and without (bottom) presences of 4 mM of each NaOAc, Na₂CO₃, NaH₂PO₄ and Na₂SO₄.



Figure S30. NMR spectra of 4 mM of **1** in D₂O with (top) and without (bottom) presences of 4 mM of each NaOAc, Na₂CO₃, NaH₂PO₄ and Na₂SO₄.



Figure S31. NMR spectra of 4 mM of **2** in pH 7.4 buffer with (top) and without (bottom) presences of 4 mM of each NaOAc, Na₂CO₃, NaH₂PO₄ and Na₂SO₄.



Figure S32. NMR spectra of 4 mM of **2** in D₂O with (top) and without (bottom) presences of 4 mM of each NaOAc, Na₂CO₃, NaH₂PO₄ and Na₂SO₄.



Figure S33. NMR spectra of 4 mM of **1** in pH 7.4 buffer with (top) and without (bottom) presences of 4 mM of $Ca(NO_3)_2$.



Figure S34. NMR spectra of 4 mM of **1** in D_2O with (top) and without (bottom) presences of 4 mM of $Ca(NO_3)_2$.



Figure S35. NMR spectra of 4 mM of **2** in pH 7.4 buffer with (top) and without (bottom) presences of 4 mM of $Ca(NO_3)_2$.



Figure S36. NMR spectra of 4 mM of **2** in D_2O with (top) and without (bottom) presences of 4 mM of $Ca(NO_3)_2$.



Figure S37. CEST spectra collected at 37 °C for solutions containing 4.0 mM of **1** (top) and **2** (bottom) in bovine blood plasma at pH 7.4.



Figure S38. UV-Vis-NIR spectra of 0.4 mM of **3** (top) and 0.4 mM of **2**, buffered at pH 7.4 with different hours of air exposure (bottom, legend indicates hours of exposure).



Figure S39. Stacked NMR spectra for 4 mM of **3** in solutions with 100 mM NaCl and 100 mM HEPES buffered at pH 7.4 in the presence of 0 (black), 4 (red), 8 (blue) and 16 (green) mM of glutathione.



Figure S40. Stacked NMR spectra for 4 mM of **3** in D_2O in the presence of 0 (black), 4 (red), 8 (blue) and 16 (green) mM of glutathione monosodium salt, which is used to avoid acidity build-up caused by glutathione in an unbuffered solution.



Figure S41. Cells survival (in %) after incubation with different concentrations of 3.



Figure S42. Open circuit potentials for solutions for phantom experiments, containing 100 mM of HEPES, 100 mM of NaCl and 10 mM of Fe₂ buffered at pH 7.4, is plotted against both the ratio of CEST effects at 37 °C from the averaged phantom image intensity with presaturation at 83 and 40 ppm and the natural log of the ratio (inset). Black circles and the red line represent the experimental data and the fit (equation displayed), respectively. Refer to Experimental Section for fitting details.



Figure S43. Stacked IR spectra of 1 (blue), 2 (red) and 3 (black).

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