Hyperpolarized Multi-metal ¹³C-Sensors for MRI

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SUPPLEMENTAL RESULTS

Table S1. T ₁ relaxation times of metal-coordinating compounds as well as chemical shifts in the presence of c	al-
cium. T_1 times (uncertainties of the measurements were ~10 ⁻² seconds).	

Name	Formula	MW (g/mol)	ppm (δ): <i>Τ₁ (sec)</i> @5.9 Tesla	ppm (δ): <i>Τ₁ (sec)</i> @11.7 Tesla	Chemical Shift δ (ppm) to Ca ²⁺
¹³ C-EDTA	${}^{12}C_{6}{}^{13}C_{4}H_{16}N_{2}O_{8}$	296.11	170.70: <i>13.1</i>	174.79: 6.8 179.98: 5.6 w/CaCl ₂	5.2
¹³ C-EGTA	${}^{12}C_{10}{}^{13}C_4H_{24}N_2O_{10}$	384.16	170.24 : <i>15.0</i>	170.34: <i>7.6</i> 180.13: <i>5.5 w/CaCl₂</i>	9.8
¹³ C-EGTA-d ₈	${}^{12}C_{10}{}^{13}C_4H_{16}{}^2H_8N_2O_{10}$	392.31	-	170.34 : <i>10.0</i>	9.8
¹³ C-Br-Pyro-EGTA	${}^{12}C_{14}{}^{13}C_4H_{23}N_2O_{10}$	511.29	179.40: <i>7.8</i>	179.97 : <i>4.5</i>	0.8
APTRA	¹² C ₁₂ H ₁₃ NO ₇	283.23	-	179.60: <i>3.9</i> 177.11: <i>5.6</i> 150.74: <i>1.3</i>	~0
ВАРТА	$^{12}C_{22}H_{24}N_2O_{10}$	476.43	-	178.86: <i>2.9</i> 149.83: <i>2.2</i> 140.06: <i>5.1</i> 178.56: <i>2.0 w/CaCl</i> ₂	0.3
HIDA	¹² C ₆ H ₁₁ NO ₅	177.15	-	170.31: <i>10.0</i> 170.72: <i>3.5 w/CaCl</i> ₂	0.3
Carboxy Glutamate (CGlu)	¹² C ₇ H ₁₀ O ₆	191.14	-	178.38: <i>9.7</i> 177.94: <i>10.1</i> 174.59: <i>10.8</i>	-
¹³ C-Glycine	$^{12}C_1^{13}C_1H_5NO_2$	76.07	172.39: <i>24.8</i>	-	-
Glycine-1- ¹³ C,2,2-d ₂	$^{12}C_{1}^{13}C_{1}H_{3}^{2}H_{2}NO_{2}$	78.08	172.39: <i>37.0</i>	-	-



Figure S1. T_1 relaxation (11.7 T) times as a function of molecular weight.

Table S2. T_1 relaxation times obtained from hyperpolarized ¹³C-sensors at 1 Tesla tabulated for each NMR signal peak shown in Figure 3.

	sans Ca ²⁺	subsat. Ca ²⁺	sat. Ca ²⁺
¹³ C-EGTA	15.1 / -	12.6 / 11.6	- / 8.7
T ₁ (sec) @ 171 / 181 ppm			
¹³ C-EDTA	13.5 / -	11.9 / 11.5	- / 10.4
T ₁ (sec) @ 174 / 181 ppm			
¹³ C-EGTA-d ₈	-	25.6 / 26.2	-
T ₁ (sec) @ 171 / 181 ppm			



Scheme S1. Synthetic scheme. A) $^{13}\text{C}\text{-}\text{EGTA}$ and $^{13}\text{C}\text{-}\text{EGTA-d}_8;$ B) $^{13}\text{C}\text{-}\text{EDTA};$ C) $^{13}\text{C}\text{-}\text{Br}\text{-}\text{Pyro}\text{-}\text{EGTA}.$



Figure S2. Response of ¹³C-sensors to pH. Chemical shifts are plotted for the calciumbound and free chelators (1 mM ¹³C-EGTA and ¹³C-EDTA) dissolved in 15 mM MOPS buffer adjusted to 5-8 pH without (open symbols) or with (filled symbols) equimolar concentrations of calcium. 0.5 μ L ¹³C-methanol was used as an internal reference. Measurements were taken at 310 K, 500 MHz (¹³C: 125.83 MHz)



Figure S3. Binding curves and multiplexed metal detection. (a) The Area under the Curve (AUC) was plotted for ^{13}C -EDTA (4mM) with increasing amounts of Ca²⁺ in the absence of Mg²⁺ (filled red circles, slope 0.257) or in the presence of 1.4 mM Mg²⁺ (open yellow circles, slope 0.261) or unbound chelator (gray circles, slope -0.257). (b) Exemplary spectrum showing multiplexed detection of Ca²⁺ and Mg²⁺.



Figure S4. Calcium detection in human serum by hyperpolarized ¹³C-EGTA. The sensor was subjected to DNP for one hour (1.3 K and 3.35 Tesla), dissolved, mixed with human serum, (1:1 in 0.1 M MOPS, 7.5 mM final concentration of ¹³C-EGTA) and measured at 1 Tesla. The two peaks correspond to the unbound carboxylic moieties (171 ppm, T₁ = 12.9 s) and the Ca²⁺-bound species (181 ppm, T₁ = 11.6 s).



Figure S5. Chemical Shift Imaging of metal distributions with non-hyperpolarized sensors. Two NMR tubes filled with ¹³C-EDTA or ¹³C-EGTA at thermal equilibrium (200 mM) were concentrically positioned inside the bore of a MR microimaging system with saturating Ca²⁺ concentrations (300 mM) added to the inner tube. NMR spectra obtained from chemical shift images are overlaid on a proton MSME (Multi Slice Multi Echo) image and areas under the curve (AUC) of the Ca²⁺-bound (left column) or free chelator (right column) are overlaid on a hot color scale. The corresponding spectra are shown on the right.

EXPERIMENTAL SECTION

General

The chemicals and anhydrous solvents were purchased from commercial suppliers (Aldrich, Fluka, and VWR) and were used without further purification unless otherwise stated. All reactions were carried out under an inert (nitrogen) atmosphere. All glassware was washed with a mixed acid solution, thoroughly rinsed with deionized, distilled water and predried with a heat gun under vacuum. Ultra-pure deionized water (18 M Ω cm⁻¹) was used throughout.

Chromatography

Flash column chromatography was performed by using flash silica gel 60 (70–230 mesh) from Merck. Thin layer chromatography (TLC) was performed on aluminum-backed silica gel plates with 0.2 mm thick silica gel 60 F_{254} (E. Merck) using different mobile phases. The compounds were visualized by UV irradiation (254 nm), iodine and Dragondorff staining reagent.

Spectroscopy

Each synthetic step was characterized by NMR and Mass spectroscopy. ¹H and ¹³C NMR spectra were recorded on a Bruker AV250 and Bruker AV500 spectrometer equipped with a cryoprobe (¹H; internal reference CDCl₃ at 7.27 ppm or D₂O at 4.75 ppm; ¹³C; internal reference CDCl₃ at 77.0 ppm). All experiments were performed at 23°C. Electrospray mass spectra (ESI-MS) were recorded on SL 1100 system (Agilent, Germany) with ion-trap detection in positive and negative ion mode. HRMS were measured on a Thermo Finnigan LQT.

Synthesis of chemical shift metal sensors. (1) ¹³C-labeled EGTA:

[3,12-bis[carboxy(¹³C)methyl](1,14-¹³C,)-6,9dioxa-3,12-diazatetradecanedioic acid].

¹³C-Labeled EGTA was synthesized in two steps as described in the literature on non-¹³C labelled EGTA. A solution of 2,2'-(ethane-1,2diylbis(oxy))diethanamine (0.1 g, 0.68 mmol) and K_2CO_3 (0.56 g, 4.06 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. Ethyl bromoacetate-1¹³C (0.34 mL, 3.04 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatog-raphy (100 % DCM to 95:5 DCM/MeOH; $R_f=0.4$) to give *dimethyl* 3,12-bis(2-methoxy(¹³C)-2-oxoethyl)-6,9-dioxa-3,12-diazatetradecane-1,14-dioate(¹³C) (0.295 g, 88%) as a yellow gum.

¹H NMR (CDCl₃, 250 MHz) δ ppm: 1.24 (t, *J*=6 Hz, 12H), 2.94 (t, *J*=6 Hz, 4H), 3.42 - 3.70 (m, 16H), 4.13 (q, *J*=7 Hz, 8H).¹³C NMR (CDCl₃, 62.9 MHz) δ ppm: 14.1, 53.6, 55.3, 56.2, 60.4, 70.1, 171.4. HR-MS (ES⁺) m/z ¹²C₁₈⁻¹³C₄H₄₁N₂O₁₀ requires 497.2895 [M+H]⁺; found 497.2880 [M+H]⁺.

The tetra-acid $(3,12\text{-bis}[\text{carboxy}(^{13}\text{C})\text{methyl}](1,14\text{-}^{13}\text{C}_2)\text{-}6,9\text{-dioxa-}3,12\text{-diazatetradecanedioic acid}) was obtained by deprotection of the ethyl group on$ *dimethyl* $3,12-bis(2-methoxy(^{13}\text{C})\text{-}2\text{-}oxoethyl)\text{-}6,9\text{-}dioxa-3,12\text{-}diazatetradecane-1,14\text{-}dioate(^{13}\text{C})$ (0.25 g, 0.65 mmol) with NaOH (0.25 g, 3 mmol) in 10 mL MeOH: H₂O (9:1) for 2 h at room temperature. After completion of the reaction, the pH was adjusted to 7.4 by 3N HCI. The solvent was evaporated under reduced pressure to afford ¹³C-labeled EGTA (0.19 g, 100% w/w) as an off-white solid.

¹H NMR (D₂O, 250 MHz) δ ppm: 3.23 (t, *J*=7 Hz, 4H), 3.54 - 3.76 (m, 12H), 3.80 (t, *J*=7 Hz, 4H). ¹³C NMR (D₂O, 62.9 MHz) δ ppm: 54.5, 58.7, 65.9, 69.6, 170.4. HR-MS (ES⁺) m/z ¹²C₁₀¹³C₄H₂₅N₂O₁₀ requires 385.1643 [M+H]⁺; found 385.1634 [M+H]⁺.

¹³C-d₈-Labeled EGTA was synthesized in similar way as compound 1 (13C-Labeled EGTA). A solution of 2,2'-(ethane-1,2-diylbis(oxy))diethanamine (0.1 g, 0.68 mmol) and K₂CO₃ (0.56 g, 4.06 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. Ethyl 2-bromo($^{2}H_{2}$)acetate-1 ^{13}C (0.52, 3.06 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f=0.4) to give 1,14-diethyl 3,12-bis[2ethoxy-2-oxo(²H₂)ethyl](2,2,13,13-²H₄(¹³C))-6.9dioxa-3, 12-diazatetradecanedioate (0.10 g, 29%) as a vellow gum.

¹H NMR (CDCl₃, 500 MHz) δ ppm: 1.30 (t, *J*=7 Hz, 12H), 3.21 (t, *J*=4.5 Hz, 4H), 3.66 (t, *J*=5 Hz, 4H), 3.79 (s, 4 H), 4.22 (qd, *J*=7, 3 Hz, 8H). ¹³C NMR (CDCl₃, 126 MHz) δ ppm: 14.0, 29.7, 53.4, 61.8, 68.1, 69.6, 173.5. LR-MS (ES⁺) m/z ¹²C₁₈¹³C₄H₃₆²H₈N₂O₁₀ requires 505.3 [M+H]⁺; found 505.5 [M+H]⁺.

The tetra-acid $(3,12-bis[carboxy({}^{13}C, {}^{2}H_2)methyl](2,2,13,13- {}^{2}H_4({}^{13}C_4))-6,9-dioxa-3,12-diazatetradecanedioic acid) was obtained by deprotection of the ethyl group on 1,14-diethyl 3,12-bis[2-ethoxy-2-oxo({}^{2}H_2)ethyl](2,2,13,13- {}^{2}H_4)-6,9-dioxa-3, 12-diazatetradecanedioate({}^{13}C_4) (0.1 g, 0.65 mmol) with NaOH (0.25 g, 3 mmol) in 10 mL MeOH: H_2O (9:1) for 2 h at room temperature. After completion of the reaction, the pH was adjusted to 7.4 by 3N HCI. The solvent was evaporated under reduced pressure to afford <math display="inline">{}^{13}C-{}^{2}H_8$ -Labeled EGTA (0.078 g, 100% w/w) as an off-white solid.

¹H NMR (D₂O, 500 MHz) δ ppm: 3.40 (t, *J*=5 Hz, 4H), 3.62 (s, 4 H), 3.80 (t, *J*=5 Hz, 4H).¹³C NMR (D₂O, 126 MHz) δ ppm: 38.8, 54.7, 64.7, 69.9, 170.4. LR-MS (ES⁻) m/z ¹²C₁₀⁻¹³C₄H₁₆⁻²H₈N₂O₁₀ requires 391.2 [M-H]⁻; found 391.6 [M+-H]⁻.

(3) ¹³C-labeled EDTA:

[2-[(2-

{bis[carboxy(¹³C)methyl]amino}ethyl)[carboxy(¹³ C)methyl]amino](1-¹³C)acetic acid].

A solution of ethane-1,2-diamine (0.1 g, 1.67 mmol) and K_2CO_3 (1.38 g, 10 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. ¹³C labeled ethyl bromoacetate-1¹³C (0.84 mL, 7.5 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f =0.5) to give *tetraethyl-2-[(2-{bis[carboxy(^{13}C)methyl]amino}ethyl)[carboxy(^{13}C)m ethyl]amino](1-^{13}C) acetate (0.625 g, 92%) as a yellow gum.*

¹H NMR (CDCl₃, 250 MHz) δ ppm: 1.28 (t, *J*=7 Hz, 12H), 2.92 (t, *J*=6 Hz, 4H), 3.61 (s, 8H), 4.17 (q, *J*=7 Hz, 8H).¹³C NMR (CDCl₃, 62.9 MHz) δ ppm: 14.1, 51.4, 55.3, 60.4, 171.3. HR-MS (ES⁺) *m/z* ${}^{12}C_{14}{}^{13}C_{4}H_{33}N_2O_8$ requires 409.2371 [M+H]⁺; found 409.2365 [M+H]⁺.

The tetra-acid (2-[(2-{bis[carboxy(¹³C)methyl]amino}ethyl)[carboxy(¹³C)m ethyl]amino](1-¹³C)acetic acid) was obtained by deprotection of the ethyl group on *tetraethyl-2-[(2-{bis[carboxy(^{13}C)methyl]amino}ethyl)[carboxy(^{13}C)methyl]amino](1-^{13}C) acetate* (0.6 g, 2.05 mmol) with NaOH (0.36 g, 9 mmol) in 10 mL MeOH: H₂O (9:1) for 2 h at room temperature. After the reaction was completed, the pH was adjusted to 7.4 by 3N HCl. The solvent was evaporated under reduced pressure to afford ¹³C-labeled EDTA (0.435 g, 100% w/w) as an off-white solid.

¹H NMR (D₂O, 250 MHz)) δ ppm: 3.71 (s, 4H), 3.94 (d, *J*=4.5, 8H). ¹³C NMR (D₂O, 62.9 MHz) δ ppm: 51.7, 58.5, 170.8. HR-MS (ES⁺) m/z ${}^{12}C_{6}^{-13}C_{4}H_{17}N_{2}O_{8}$ requires 297.1119 [M+H]⁺; found 297.1118 [M+H]⁺.

(4) Bromo derivative of ¹³C-labeled Pyro-EGTA

[2-({2-[2-(2-

{bis[carboxy(¹³C)methyl]amino}ethoxy)-5bromophe-

noxy]ethyl}[carboxy(¹³C)methyl]amino)(1-¹³C)acetic acid].

The bromo-derivative of ¹³C-labeled Pyro-EGTA was synthesized in 5 steps following a similar synthesis from the literature (Mishra *et al.*, 2011). The methyl groups were removed by using successive addition of borontribromide in the solution of *4-bromo-1,2dimethoxybenzene* in anhydrous CH_2Cl_2 at -4°C to room temperature for 2 h. The reaction was quenched by MeOH and solvent was evaporated under reduced pressure to afford *4-bromobenzene-1,2-diol*. Stepwise alkylation of *4-bromobenzene-1,2-diol*. Stepwise alkylation of *4-bromobenzene-1,2-diol* with *tert*-butyl 2-bromoethylcarbamate in anhydrous MeCN (6 h at 80°C) afforded compound di*tert*-butyl 2,2'-(4-bromo-1,2-phenylene)bis(oxy)bis(ethane-2,1-

diyl)dicarbamate, which was de-Boc by using 4N HCl dioxane solution (30 min at room temperature) to give 2,2'-(4-bromo-1,2phenylene)bis(oxy)diethanamine in good yield.

2,2'-(4-bromo-1,2-А solution of phenylene)bis(oxy)diethanamine (0.1 a. 0.37 mmol) and K₂CO₃ (0.40 g, 2.91 mmol) in anhydrous MeCN was stirred at room temperature for 30 min. Ethyl 2-bromoacetate-1¹³C (0.185 mL, 1.64 mmol) was added dropwise and the reaction mixture was heated at 80°C for 4 h. The reaction progress was monitored by TLC. Upon consumption of starting materials, the solvent was filtered through G-4 sintered funnel and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (100 % DCM to 95:5 DCM/MeOH; R_f=0.55) to give tetramethyl 2-({2-[2-(2-{bis[carboxy(13C)methyl]amino}ethoxy)-5-

bromophenoxy]ethyl}[carboxy(¹³C)methyl]amino)(1-¹³C)acetate (0.06 g, 38%) as a yellow gum. ¹H NMR (CDCl₃, 250 MHz) δ ppm: 1.25 (t, J=7.0, 12H), 3.05 - 3.33 (m, 4 H), 3.68 (d, J=5.0 Hz, 8H), 3.99 - 4.29 (m, 12H), 6.73 (d, J=9.0 Hz, 1H), 6.92 - 7.13 (m, 2H). ¹³C NMR (CDCl₃, 62.9 MHz) δ ppm: 14.2, 53.4, 55.5, 56.4, 60.5, 112.9, 116.5, 119.3, 123.7, 149.2. 151.4, 171.4. HR-MS (ES⁺) m/z $^{12}C_{22}^{-13}C_4H_{40}BrN_2O_{10}$ requires 623.2001 [M+H]⁺; found 623.1996 [M+H]⁺.

[2-({2-[2-(2-The tetra-acid {bis[carboxy(13C)methyl]amino}ethoxy)-5bromophenoxy]ethyl}[carboxy(13C)methyl]amino)(1-¹³C)acetic acid] was obtained by deprotection of the group ethyl on tetramethyl 2-({2-[2-(2-{bis[carboxy(13C)methyl]amino}ethoxy)-5bromophenoxy]ethyl}[carboxy(13C)methyl]amino)(1-¹³C)acetate (0.06 g, 0.096 mmol) with NaOH (0.03 g, 0.75 mmol) in 10 mL MeOH: H₂O (9:1) for 2 h at room temperature. After reaction completion, the pH was adjusted to 7.4 by 3N HCl. The solvent was evaporated under reduced pressure to afford ¹³Clabeled m-Br-PyroEGTA (0.05g, 100% w/w) as an off-white solid.

¹H NMR (D₂O, 250 MHz)) δ ppm: 3.02 (t, *J*=5 Hz, 4H), 3.18 - 3.45 (m, 8H), 4.08 (t, *J*=5 Hz, 4H), 6.92 (d, *J*=8.5 Hz, 1H), 7.07 - 7.26 (m, 2H).¹³C NMR (D₂O, 62.9 MHz) δ ppm: 53.5, 58.3, 65.0, 112.4, 113.3, 115.2, 123.7, 146.6, 147.9, 179.4. HR-MS (ES⁺) m/z ¹²C₁₄¹³C₄H₂₄BrN₂O₁₀ requires 511.0749 [M+H]⁺; found 511.0746 [M+H]⁺.

NMR-based metal detection of thermally polarized compounds

NMR experiments were performed at 23°C at 500 MHz (¹³C 125 MHz) or 250 MHz (¹³C 62.5 MHz) and as indicated. pH values were measured with a standard pH electrode. All solutions were prepared in (3-(N-morpholino)propanesulfonic acid) MOPS buffer (concentrations as indicated in the respective experiment) and deuterated water in a ratio of 1:1, at pH 7.4 and were measured at 300K. ¹³C-methanol was used as reference. A thermal spectrum was acquired (number of scans, ns, 1000, number of points, np, 16) using a 30° pulse and 90 degree pulse for decoupling. ¹³C T₁ values were calculated from a fit to a series of spectra acquired with varying repetition times (from 0.01 s to 50 s). Compounds were dissolved in 15 mM MOPS with H₂0/D₂0 (1:1), 1 µL ¹³C-methanol was used as an internal reference. For NMR-based determination of calcium concentrations, human serum was diluted to 50% v/v in 0.1 M MOPS buffer. The reference binding curve for calcium and ¹³C-EGTA was obtained from 0.1 M MOPS buffer containing 0.45 mM Mg²⁺ and 4 g/dL Bovine Serum Albumin (BSA). In parallel, metal concentrations in the serum sample were also obtained from the routine colorimetric test conducted at the clinical chemistry department of the Hospital of the Technical University of Munich.

Dynamic Nuclear Polarization of ¹³C-EGTA and ¹³C-EDTA and NMR detection

Samples were prepared dissolving 4.2 mg of trityl radical (OX063, GE-Healthcare, Amersham UK) in 100 µL of 0.5 M of ¹³C-EGTA (or ¹³C-EDTA) solution (DMSO/D2O) and a trace amount of Dotarem (Guerbet, Birmingham UK). The samples were polarized via dynamic nuclear polarization on a Hyper-Sense DNP polarizer system (Oxford Instruments Molecular Biotools, Oxford, UK) using 60-100 min of 94.1 GHz microwave irradiation at 1.3 K and 3.35 T. The polarized samples were dissolved in a heated and pressurized solution of 100 mM MOPS buffer prepared in D₂O/DMSO-d6 (9:1) with or without saturating concentrations of CaCl₂, leading to a ~13 mM solution of the hyperpolarized substrate with a pH of 7.4 +/- 0.2. Polarization levels were on average 1-2% for ¹³C-EDTA and ¹³C-EGTA and 4-5% for ¹³C-EGTA-d₈. Build-up time constant was ~800 s. Hyperpolarized samples were measured at 1T (42.5 MHz proton and 10.8 Carbon) with NMR benchtop spectrometer (Spinsolve, Magritek) and NMR spectra were analyzed with Mestrenova 10 (Mestrelab Research). Final calcium concentrations were confirmed by ICP-MS. The average time interval between the end of polarization and start of the NMR measurement was 12.0 +/- 0.6 seconds.

Chemical Shift Imaging of ¹³C-EGTA and ¹³C-EDTA at equilibrium polarization

200 mM of either $^{13}\mbox{C-EDTA}$ or $^{13}\mbox{C-EGTA}$ (in 200 mM MOPS in D₂O buffer pH 7.4) were filled in an NMR tube with 5 mm diameter. The same solution with addition of 300 mM calcium was filled into a 3 mm diameter NMR tube that was positioned concentrically inside the 5 mm NMR tube. Microimaging was performed on a Bruker WideBore UltraShield 500WB PLUS with Avance3HD Console Great60 -60A Gradient Amplifiers. The instrument was equipped with Micro5 gradient system (5G/cm/A -> 300G/cm = 3T/M), BLAX500 - 500W rf power amplifier, and 5 mm double tuned ¹H/¹³C coil. The anatomical proton images were acquired with Multi-Slice Multi-Echo (MSME) pulse sequence (TR=5000ms, TE=2.8ms, FA=90°, FOV=8mm, and MTX=128). The CSI sequence was run with TR=8000ms, TE=0.865ms. FOV=8mm, and MTX=128. The data were pre-analyzed by CSI tool software ¹ and analyzed with ParaVision 6.0 and TopSpin 3.2.

Chemical Shift Imaging of hyperpolarized ¹³C-EGTA and ¹³C-EDTA

CSI experiments with hyperpolarized chelators (10 mM) and saturating metal concentrations (15 mM) were performed on a 7T small animal scanner (GE/Agilent MRI 901 7T). A fast Spin Echo se-

quence was used to acquire the proton images with a dual-tuned ¹H-¹³C-volume coil for radiofrequency transmission and reception. The Fast Spin Echo was acquired using 256 steps of frequency and phase encoding, TE=20 ms, TR=3000 ms, FA=90°, echo train length=4, bandwidth=15.63, FOV=6 cm and slice thickness=1 mm. The ¹³C scans were acquired using a Flex Surface Coil ¹³C/¹H (RAPID Biomedical

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GmbH). A spectro-spatial (SPSP) IDEAL spiral² sequence was used for fast Chemical Shift Imaging with TR=125 ms, FA=8°, FOV=6 cm, slice thickness=8 mm. Chemical shifts were referenced against ¹³C-Urea measured before each experiment. Data processing was performed using custom-written routines³ in MATLAB (R2015a, Mathworks).