Supporting Information for

Ratiometric Method for Rapid Monitoring of Biological Processes Using Bioresponsive MRI Contrast Agents

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General remarks

Commercially available reagents and solvents were used without further purification. **MSCA** and isothiocyanate **1** for synthesis of dendrimeric **DSCA** were synthesized according to a published procedure.[1] G4 Starburst® PAMAM dendrimer with cystamine core was purchased from Dendritic Nanotechnologies, Inc., USA. Dendrimer **2** was purified using lipophilic Sephadex®LH-20 (bead size: 25-100 μm) from Sigma-Aldrich (Germany). Brain extracellular model (BEM) solution was prepared from Ca-free Dulbecco's Modified Eagle's Medium (DMEM, without L-glutamine, sodium pyruvate and calcium chloride), Ham's F-12 Nutrient Mixture (F-12) and N-2 supplement (N-2) from Life Technologies GmbH, Germany. MALDI-TOF-MS analysis was performed by The Scripps Center for Mass Spectrometry, La Jolla, CA. ^{1}H and ^{13}C- NMR spectra, relaxometric experiments and NMR diffusion measurements were performed on a Bruker Avance III 300 MHz spectrometer at 25 ^{o}C using 5 mm NMR tubes. Processing was performed using TopSpin 2.1 (Bruker GmbH) and ACD/SpecManager 9.0 (Advanced Chemistry Development, Inc.). The NMR spectra were obtained either in $CDCl_3$ or D_2O, using the deuterium lock frequency. The concentration of Gd^{3+} in analyzed solutions was determined using the bulk magnetic susceptibility shift (BMS).[2] Diffusion experiments were carried out on samples filtered through 0.20 μm PTFE filters from Carl Roth GmbH + Co. KG, Germany. DLS measurements were done on a Malvern-Nano-ZS (Zetasizer, software ver. 6.2) instrument. MRI measurements were performed on a Bruker BioSpec 70/30 USR magnet (software ver. Paravision 5.1) using Bruker dual frequency volume coil (RF RES 300 1H/19F 075/040 LIN/LIN TR).

Synthesis of DSCA

Dendrimer 2. G4 PAMAM dendrimer (80 mg, 5.6 μmol) and isothiocyanate 1 (650 mg, 539 μmol) were dissolved in dimethylformamide (5 mL) and triethylamine (200 μL, 1.4 mmol) was added to the solution. The reaction mixture was stirred at 45 °C for 24 h. The solvent was evaporated and the unreacted ligand was removed using a lipophilic Sephadex column with methanol as eluent to obtain protected dendrimeric chelator **2** (270 mg, 53%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.2 (br, Ar*H*), 7.04 (br, Ar*H*), 4.10–1.70 (overlapping m), 1.55–1.25 (overlapping m, C(C*H*$_3$)$_3$). MALDI-TOF/MS (m/z): $[M+44Na]^{2+}$ calcd. for $C_{3024}H_{5372}N_{610}Na_{44}O_{684}S_{42}^{2+}$, 31790, found 31792.

Dendrimer 3. The protected dendrimeric chelator **2** (150 mg, 2.9 μmol) was dissolved in formic acid (5 mL) and the mixture was stirred at 60 ^oC for 48 h. The residue was purified by centrifugation using 3 KDa molecular weight cut-off filters and freeze-dried to give dendrimeric chelator **3** as a light brown solid (135 mg, 90%).

^{1}H NMR (300 MHz, D_2O): δ 7.24 (br, Ar*H*), 4.25−2.45 (overlapping m), 1.84 (br. s). MALDI-TOF/MS (m/z): $[M+46Na+12H_2O]^{2+}$ calcd. for $C_{2224}H_{3772}N_{610}Na_{46}O_{684}S_{42}(H_2O)_{12}^{2+}$, 26311, found 26308.

Dendrimer DSCA. Dendrimeric chelator **3** (138 mg, 2.4 μmol) was dissolved in water and the pH was adjusted to 7.0 with aqueous sodium hydroxide (0.1 M). A solution of $GdCl_3·6H_2O$ (61 mg, 164 μmol) in water was added and pH was maintained at 7.0. The mixture was stirred at room temperature for 24 h. EDTA (123 mg, 329 μmol) was added into the solution to remove excess Gd^{3+} while maintaining pH at 7.0. Excess GdEDTA and EDTA were removed by centrifugation using 3 KDa molecular weight cut-off filters and the resulting solution was lyophilized to give **DSCA** as a brown solid (151 mg, 85%).

MALDI-TOF/MS (m/z): $[M+53Na+78H_2O]^{2+}$ calcd. for $C_{2224}H_{3652}Gd_{40}N_{610}Na_{53}O_{684}S_{42}(H_2O)_{78}^{2+}$, 30070, found 30066; $[M+36Na+43H_2O]^{2+}$ calcd. for $C_{2145}H_{3534}Eu_{38}N_{592}Na_{36}O_{656}S_{40}(H_2O)_{43}^{2+}$, 28386, found 28385.

NMR relaxometric experiments

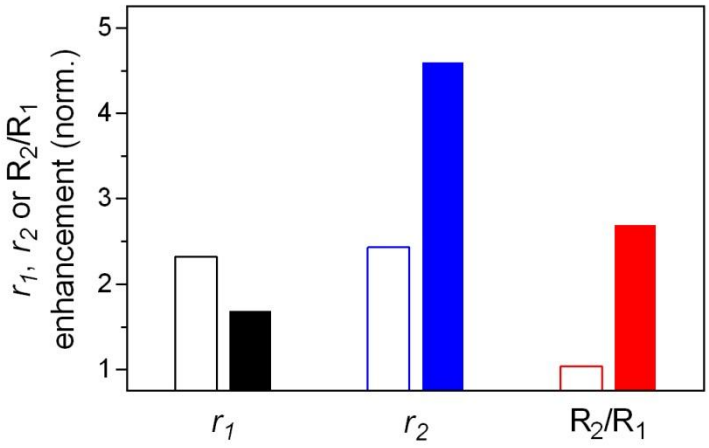

Figure S1. Increase of the r_1, r_2 and the R_2/R_1 ratio for **MSCA** (clear bars) and **DSCA** (color-filled bars) upon saturation with Ca^{2+} (HEPES, pH 7.4).The plots show normalized values where the final r_1, r_2 or R_2/R_1 ratio value (at Ca^{2+} saturation) is divided by the initial value (in absence of Ca^{2+}).

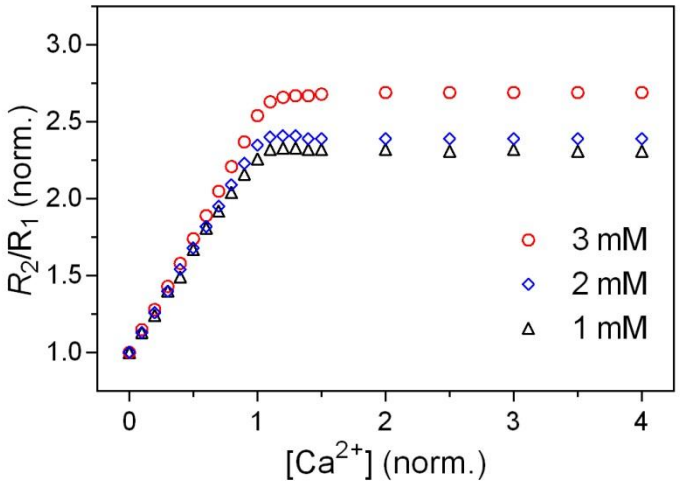

Figure S2. Longitudinal and transverse relaxometric titrations of **DSCA** with Ca^{2+} at 7 T and different Gd^{3+} concentrations (pH 7.4, HEPES). The plots show normalized values where the final r_1, r_2 or R_2/R_1 ratio value (at Ca^{2+} saturation) is divided by the initial value (in absence of Ca^{2+}).

DLS experiments

Measurements were carried out with Eu^{3+} complex of **DSCA** (0.75 mM Eu^{3+}) with and without addition of 2 equiv. of Ca^{2+}. They included 5 repetitions of 15 scans (1 scan = 12 sec, refractive index 1.345, absorption 1 %), without delays in between the scans, and with equilibration of 30 sec prior to recording. For comparison with NMR diffusion experiments, the diffusion coefficient was calculated from the obtained diameter using the Stokes-Einstein equation (Eq. 1) and assuming spherical sample approximation, where k is the Boltzmann constant, T – absolute temperature (298.15 K), r – hydrodynamic radius of a sample, η – viscosity of the water (0.8872 mPa s). The reversed procedure was performed for the diffusion coefficient obtained with NMR measurements of **MSCA** (see below).

$$D = \frac{k\,T}{6\,\pi\,r\,\eta} \qquad \text{[Eq. 1]}$$

NMR diffusion experiments

Determination of diffusion coefficient was performed using 2D – Diffusion Ordered NMR Spectroscopy (DOSY).[3] Experiments included 3 repetitions on Eu^{3+} complex of **MSCA** (15 mM) with and without 2 equiv. of Ca^{2+} (δt= 2 ms, ΔT= 330 ms). Data analysis was done with TopSpin 2.1 using 16 linear points between 5–95 % gradient strength.

MRI phantom experiments

MRI experiments were performed on tubes containing solutions of **DSCA** or **MSCA** (1 mM Gd^{3+}) to which 0, 0.4, 0.8 and 1.2 equiv. of Ca^{2+} were added (DSCA1=MSCA1=0 mM Ca^{2+}, DSCA2=MSCA2=0.4 mM Ca^{2+}, DSCA3=MSCA3=0.8 mM Ca^{2+}, DSCA4=MSCA4=1.2 mM Ca^{2+}). Each set (DSCA1–4 or MSCA1–4) was placed in 4×200 µl plastic tubes and inserted into a 20 mL syringe filled with solution of Dotarem® in water (162 mM) in order to avoid susceptibility artifacts.

Firstly, T_1 and T_2 times were determined from respective T_1 and T_2 maps using rapid acquisition with relaxation enhancement (RARE) with inversion recovery (IR-RARE) and multi-slice multi-

echo (MSME) sequences, respectively. IR-RARE was performed with the following parameters: field-of-view (FOV)=40x40 mm^2, matrix size (MTX)=256x256, 1 slice, slice thickness 1 mm, echo time (TE)=9.725 ms, repetition time (TR)=1500 ms, Rare factor=8, inversion times (TI): 90, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1150, 1300 ms, number of averages (NA)=1, total acquisition time (TA)=36 s, while MSME with FOV=40x40 mm^2, MTX=128x128, 1 slice, slice thickness 1 mm, TR=1500 ms, TE: 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 135, 170, 200 ms; NA=1,TA=3 min 12 s. Obtained T_1 and T_2 times were further employed for numerical CNR optimization of acquisition parameters for T_1-, T_2- and T_2/T_1-weighted imaging. Numerical CNR optimization for FLASH, bSSFP and RARE was based on Bloch simulations that optimize the signal difference for different contrast agents. The resulting optimized TR and flip angle were then used for measurements and the signal difference was normalized with the square root of the total measuring time.[4]

MRI was accomplished using T_1-, T_2- and T_2/T_1-weighted sequences: fast low angle shot (FLASH), RARE and balanced steady state free precession (bSSFP) pulse sequence, respectively, with FOV=25x25 mm^2, MTX=256x256, 1 slice and slice thickness 1 mm.

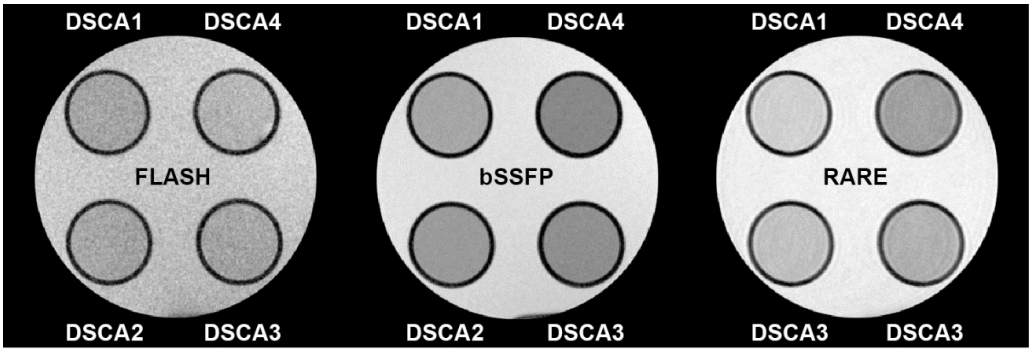

Figure S3. Comparison of MRI sequences with **DSCA** at different Ca^{2+} concentrations ([Gd^{3+}]= 1 mM, 7T, pH 7.4, HEPES). Parameters for FLASH: TR/TE= 3.797/1.494 ms, FA= 19, NA=16, TA= 15.552 s, for bSSFP: TR/TE=3/1.5 ms, FA=81, NA=16, TA=12.288 s, and for RARE: TR/TE= 620/9.284 ms, Rare factor=16, NA=16, TA= 2 m 38 s 720 ms.

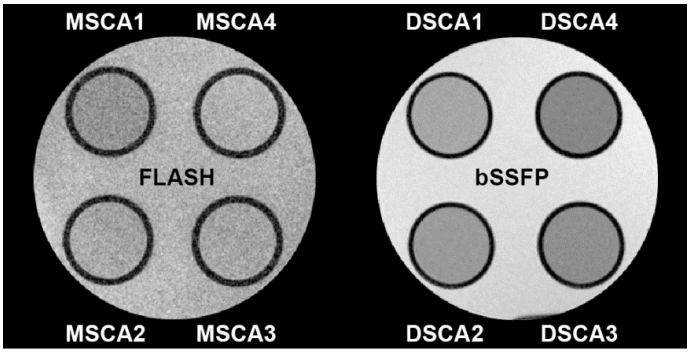

Figure S4. Comparison of FLASH sequence with **MSCA** and bSSFP sequence with **DSCA** at different Ca^{2+} concentrations ([Gd^{3+}]= 1mM, 7T, pH 7.4, HEPES). Parameters for FLASH: TR/TE= 3.4/1.494 ms, FA= 21, NA=16, TA= 13.926 s, and for bSSFP: TR/TE=3/1.5 ms, FA=81, NA=16, TA=12.288 s.

Table S1. SNR values obtained for **DSCA** and **MSCA** in the presence or absence of 1.2 equiv. of Ca^{2+}, using T_1-, T_2- and T_2/T_1-weighted sequences.

Contrast agent	**T_1-weighted (FLASH) [a)]**		**T_2-weighted (RARE) [b)]**		**T_2/T_1-weighted (bSSFP) [c)]**	
	No Ca^{2+}	+ Ca^{2+} (1.2 equiv.)	No Ca^{2+}	+ Ca^{2+} (1.2 equiv.)	No Ca^{2+}	+ Ca^{2+} (1.2 equiv.)
DSCA	5.41	6.27	11.83	8.50	9.75	7.39
MSCA	4.81	6.12	13.44	12.60	10.41	10.95

a) **DSCA**: TA=3888 ms, **MSCA**: TA=3481 ms; b) **DSCA**: TA=9920 ms, **MSCA**: TA=14400 ms; c) TA=767.5 ms.

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