Supporting Information

Multifunctional Mesoporous Silica Nanospheres with Cleavable Gd(III) Chelates as Magnetic Resonance Imaging Contrast Agents: Synthesis, Characterization, Target-Specificity, and Renal Clearance**

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Materials and Methods. All reagents were purchased from Aldrich and used without further purification, except for 3-aminopropyltriethoxysilane (AP-TES) and 3-mercaptopropyltriethoxysilane (MP-TES), which were purchased from Gelest. Thermogravimetric analysis (TGA) was carried out with a Shimadzu TGA-50 equipped with a platinum pan and using a heating rate of 3 °C/min under air. A Hitachi 4700 Field Emission Scanning Electron Microscope (SEM) was used to determine particle size and morphology. A Cressington 108 Auto Sputter Coater equipped with an Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat the sample with a thin conductive layer (~3 nm) before SEM imaging. Each SEM sample was prepared by suspending the nanoparticles in ethanol. A drop of the suspension was placed on a glass slide and the solvent was allowed to evaporate. Gd^{3+} ion

concentration was measured using an Applied Research Laboratories (ARL) SpectraSpan 7 DCP spectrometer or a Varian 820-MS Inductively Coupled-Plasma Mass Spectrometer. MR images were acquired on a Siemens 3T Allegra scanner (Siemens Medical Systems, Erlagen, Germany) with a CP coil. For images acquired on the 3 T scanner, a 3D FLASH sequence was utilized to compute T1 maps with seven different flip angles (2, 5, 10, 20, 30, 40, and 60). Imaging parameters were: FOV=190 x 190 x 64 mm^3, matrix size= 128 x 128 x 32, TR/TE= 40/1.64 ms; total data acquisition time was 30 minutes. A 2-D multiple echo spin echo sequence was used to estimate T2 maps. In total, 32 echoes with an echo spacing of 6.2 ms were obtained. The first echo time was 6.2 ms. TR was 3000 ms. FOV and matrix size were set to 190 x 190 mm^2 and 128 x 128. The slice thickness was 2 mm, and the total data acquisition time was about 6 minutes and 29 seconds. All images were acquired on a Siemens Allegra 3T system.

Synthesis of 2-(pyridin-2-yldisulfanyl)ethanamine hydrochloride (PDSEA·HCl) (1). The synthesis of PDSEA was carried out by a procedure reported in the literature.[1] A solution of cystamine hydrochloride (1.132g, 10.0 mmol) in methanol (10 mL) was added dropwise to a magnetically stirred mixture of 2,2'-dipyridyl disulfide (4.4062 g, 20.0 mmol) and acetic acid (800 μL) in methanol (20 mL) over a period of 30 min. After magnetically stirring the solution for an additional 24 hours, the solvent was removed by rotary evaporation. The product was isolated by dissolution of the bright yellow oil in a minimal volume of methanol, precipitated with diethyl ether, and isolated by centrifugation. This washing procedure was repeated up to 5 times before drying the white crystalline product under high vacuum. Yield: 1.935 g (87 %). NMR: ^{1}H (400 MHz, MeOD-d_4, ppm): 3.13 (t, 2H), 3.29 (t, 2H), 7.31 (t, 1H), 7.67 (d, 1H) 7.80 (t, 1H), 8.53 (d, 1H)

Synthesis of 2-triethoxypropyldisulfanyl ethylamido diethylenetriaminepentaacetic acid gadolinium complex (Si-DS-DTPA-Gd) (4; Scheme S1). DTPA-bisanhydride (0.2 g, 0.56 mmol) was dissolved in 4 mL of anhydrous DMSO in a 2-neck round bottom flask. PDSEA-HCl (**1**) (0.1 g, 0.45 mmol) was dissolved in 1.0 mL of anhydrous DMSO. This solution was added dropwise to the DTPA-bisanhydride solution, and the reaction was stirred at room temperature, under N_2, overnight. The product precipitated upon the addition of acetone and diethyl ether. The product (PDS-DTPA, **2**) was isolated by centrifugation as a brownish viscous liquid, which was stirred with additional acetone and diethyl ether for 6 h until an off-white precipitate formed. The final product was isolated by centrifugation and dried under high vacuum. Yield: 0.227 g (0.405 mmol, 90%). NMR: ^{1}H (400 MHz, MeOD-d_4, ppm): 2.82 (t, 2H), 3.34 (s, 8H), 3.40 (t, 2H), 3.45-3.50 (10H), 7.07 (t, 1H), 7.66 (m, 2H), 8.26 (t, 1H). PDS-DTPA (**2**) (0.20 g, 0.3561 mmol) was dissolved in 2.0 mL of nanopure water. To this solution, 660 μL of NaOH (2 M) were added until a pH of approximately 9.4 was achieved. At the same time, $GdCl_3 \cdot 6H_2O$ (0.1324 g, 0.356 mmol) was dissolved in 0.5 mL of water. This solution was slowly added to the previously prepared ligand solution. The final mixture was stirred at room temperature for an additional 2 h. The product (**3**) precipitated upon the addition of acetone, collected by centrifugation, and dried under high vacuum. Yield: 0.205 g (0.28 mmol, 78.5%). The synthesis of Si-DS-DTPA-Gd (**4**) was carried out by a disulfide-thiol exchange reaction with 3-mercaptopropyltriethoxysilane. Briefly, PDS-DTPA-Gd (0.175 g, 0.238 mmol) was dissolved in 5 mL of dry methanol. The solution was filtered to remove any insoluble impurities. Then, 3-mercaptopropyltriethoxysilane (115.3 μL, 0.476 mmol) was added, and the reaction was stirred overnight at room temperature under N_2 atmosphere. The reaction solution was concentrated by rotary evaporation and the product was precipitated upon the addition of diethyl ether. Yield: 0.152 g (0.177 mmol, 74.6%).

Synthesis of large pore mesoporous silica nanoparticles (MSNs).[2] Cetyltrimethylammonium bromide (CTAB) (0.1 g, 0.274 mmol) was dissolved in 48 mL of distilled water, along with 0.35 mL of 2 M NaOH and mesitylene (0.7 mL, 4.88 mmol). This solution was heated to 80 °C for 30 min, and tetraethylorthosilicate (TEOS) (0.5 mL, 2.24 mmol) was rapidly injected. The reaction was stirred for an additional 2 h at 80 ºC, andhe nanoparticles were isolated by centrifugation at 10,000 rpm for 10 min. The as-made MSNs were washed with H_2O and ethanol, and then redispersed in 40 mL of ethanol.

Synthesis of MeO-PEG(5K) carboxylic acid. Methyl ether PEG(5K) (2.0 g, 0.4 mmol; dried under high vacuum at 90 ºC overnight) was dissolved in 50 mL of dry THF. To this solution, sodium hydride (60% suspension in mineral oil, 85 mg, 2.125 mmol) was added, and the dispersion was vigorously stirred for 1 h. After stirring, t-butyl bromoacetate (236.3 µL, 1.62 mmol) was slowly added, and the dispersion was stirred overnight. The final dispersion was filtered to remove the formed NaBr salts. This reaction solution was concentrated by rotary evaporation, and the product precipitated upon the addition of diethyl ether. The final product was dried under high vacuum. To deprotect the carboxylic acid group, a mixture of dichloromethane/trifluoroacetic acid (70/30, 50 mL) was used. The reaction solution was concentrated by rotary evaporation, and the product precipitated upon the addition of diethyl ether. Yield: 1.5 g (0.296 mmol, 74.1%). NMR: ^{1}H (400 MHz, $CDCl_3$, ppm): 3.33 (s, 3H), 3.40 (t, 2H), 3.45-3.65 (m, 444H), 3.68 (t, 2H), 3.75 (t, 2H), 4.25 (s, 2H)

Release Profile. From a suspension of PEG-Gd-MSNs (18.08 mg/mL), 1.5 mL was centrifuged to isolate the particles, which were then redispersed in 2 mL of PBS (1 mM, pH 7.4). This suspension was placed inside a section of dialysis tubing (MWCO 3500). A volume of 400 mL of PBS (1.0 mM, pH 7.4) was placed in a vessel and heated to 37 °C in an oil bath. The aqueous

solution was then degassed by bubbling with N_2 for 20 min. The dialysis bag containing the particles was then submerged; aliquots were removed at various time points as a control. After 12 h, a 50 mL aliquot of PBS was removed from the vessel and replaced with 50 mL PBS solution containing 0.6304 g (4.0 mmol) of L-cysteine·HCl, for a final cysteine concentration of 10 mM. Aliquots were then removed at various time points. Each removed aliquot was diluted in 3.0 mL of 0.6 M nitric acid; the Gd^{3+} concentration of each sample was then measured using ICP-MS.

Synthesis of anisamide(AA)-Gd-MSNs. First, a AA-PEG(5K) triethoxysilane derivative was synthesized as follow: firstly, diamino PEG(5K) was synthesized according to a literature procedure.[3] Diamino PEG(5K) (1 g, 0.2 mmol) was dissolved in 100 mL of dichloromethane. Subsequently, 4-methoxybenzoic acid (36.5 mg, 0.22 mmol), dicyclohexylcarbodiimide (61.6 mg, 0.3 mmol) and 4-dimethyl-aminopyridine (28 mg, 0.22 mmol) were added to the diamino PEG solution. The solution was stirred at room temperature for 24 h, and the dicyclohexylurea byproduct was removed by filtration. Finally, the desired anisamide-PEG(5K)-NH_2 was obtained by removing the dichloromethane by rotary evaporation. Yield: 0.35 g (0.0679 mmol, 34%). NMR: ^{1}H (400 MHz, $CDCl_3$, ppm): 2.94 (t, 4H), 3.35 (s, 3H), 3.40 (t, 4H), 3.45-3.65 (m, 428H), 3.75 (t, 4H), 4.37 (t, 2H), 6.86 (d, 2H), 7.93 (d, 2H). This AA-PEG-NH_2 derivative (120 mg, 23.3 μmol) was further reacted with isocyanatopropyltriethoxysilane (12 μL, 48 μmol) in 4 mL of dry DMSO for 6 h at room temperature. A portion of this solution (1 mL) was added to a dispersion of 50 mg of PEG-Gd-MSNs in 10 mL of ethanol. The final mixture was reacted for 12 h at 60 ºC. The resulting material was washed several times with ethanol to afford the desired AA-Gd-MSN material.

Synthesis of FITC- and RITC-labeled MSN materials. A similar procedure was used to prepare both FITC- and RITC-labeled MSN samples. Briefly, a solution of FITC-AP-TES was prepared by adding 6.8 mg (0.0175 mmol) of FITC in 1.1 mL of dry DMSO; to this solution 4.95 μL (4.65 mg, 0.0211 mmol) of 3-aminopropyltriethoxysilane was added, and the reaction was stirred at room temperature, in the dark, for 6 h. The final solution was diluted to 3 mL with additional dry DMSO to make a 6 mM FITC-AP-TES solution. A dispersion of 25 mg of Gd-MSNs in 10 mL of ethanol was prepared. To this mixture, 50 μL of the FITC-AP-TES solution was added. The final dispersion was heated at 60 ºC overnight. The sample was centrifuged and washed several times with water and ethanol. PEG-Gd-MSN and AA-Gd-MSN materials were FITC-labeled following the same protocol.

Competitive binding (Confocal Microscopy): AsPC-1 cells were cultured in a 6-well plate containing silanized coverslips at 1 x 10^5 cells/well. Each well contained a total of 3.0 mL of cell media and the plate was incubated (37 °C, 5 % CO_2) overnight to promote cell attachment. For the competitive binding assay, the cells were incubated in the presence of anisamide ligand (1mM) for 45 min. After that, the cell media was removed and the wells were washed once with PBS (1.5 mL). The cells were inoculated with 2.0 mL of media containing the FITC-labeled materials of PEG-Gd-MSNs, and AA-Gd-MSNs in a concentration of 50 μg/mL and incubated for either 1h or 4 h. Each well was washed twice with PBS (1.5 mL), refilled with 1 mL of cell media, and the coverslips were mounted onto slides for confocal imaging. The micrographs were obtained with an Olympus FV500 inverted confocal laser scanning microscope. The FITC dye was imaged using 490 nm excitation and a 525 nm long pass emission filter.

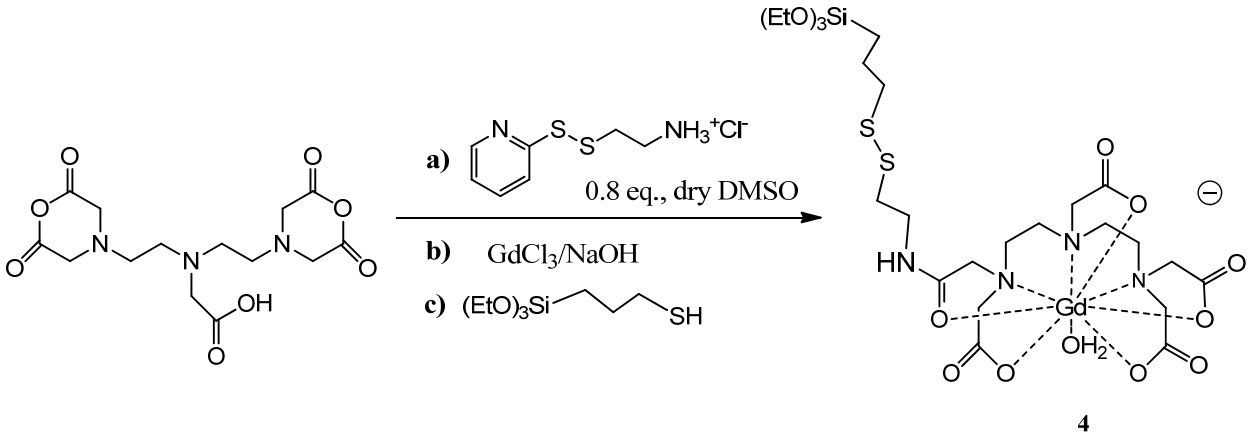
Competitive binding assay (ICP-MS). Confluent AsPC-1 cells were trypsinized, and an aliquot of cell suspension was added to 10 mL RPMI-1640 media (Cellgro) in each 25 mL culture flask

to obtain a cell density of 5.0 x 10^4 cells/flask. The flasks (four total) were incubated (37 °C, 5 % CO_2) for 24 hours. To test the specific binding ability of the AA-Gd-MSN material, two of the cell culture flasks were then incubated in 10 mL fresh media containing the anisamide ligand (1 mM) for 45 min. After that, the media was then removed and replaced with 10 mL fresh media containing either AA-Gd-MSN or PEG-Gd-MSN in a concentration of 50 μg/mL. The same procedure was followed for the cell culture flasks that were not incubated in presence of anisamide ligand. The flasks were incubated (37 °C, 5 % CO_2) for 1 hour; cells were trypsinized, and centrifuged at 3,000 rpm for 15 minutes to obtain cell pellets. The amount of Gd^{3+} uptaken by AsPC-1 cells was determined by ICP-MS as it is described in the Experimental Section.

Gd-chelate stability and physisorption experiments: To further demonstrate the stability of the Gd-chelate grafted on the MSN material, a series of assays were carried out. A sample of 1.5 mg each of PEG-Gd-MSN and AA-Gd-MSN were redispersed in 1.0 mL of acidic PBS (10 mM; pH 3.1) to wash out any Gd^{3+} weakly bound to DTPA chelate. The dispersion was sonicated for 5 min and centrifuged down at 10,000 rpm for 10 min. This procedure was repeated twice more and the washing solutions were stored for Gd^{3+} analysis. The final product was also digested for ICP-MS analysis following the procedure described in the Experimental section. In addition, to test the amount of Gd^{3+} and Gd-chelate (compound **3**) that could be physisorbed on MSN, 1.0 mg of MSN was stirred for 4h in an aqueous solution (1.0 mL) containing 90 μg and 382 μg of Gd^{3+} and Gd-chelate (compound **3**), respectively. The samples were centrifuged down and washed with PBS (10 mM, pH 3.1) as was described above. The amount of Gd^{3+} in the supernatant and washing solutions was determined by ICP-MS.

Biodistribution of PEG-Gd-MSN. Female athymic nude mice (6-8 weeks old, 20-25 g, University of North Carolina) were cared for under an approved protocol and the guidelines of

the University of North Carolina Institutional Animal Care and Use Committee. To find the amount of Gd^{3+} in various organs (liver, lung, kidneys, spleen and bladder) a female nude mice was i.v injected (0.08 mmol [Gd]/Kg) and euthanized after 2h post injection. The autopsied organs were stored in formalin solution. For the ICP-MS analysis, the organs were digested in strong acidic conditions (conc. HNO_3) and high temperature (80 ºC) for 18h. The resulting solution was filtered and an aliquot was taken for the ICP-MS analysis.



Scheme S1. 2-triethoxypropyldisulfanyl ethylamido diethylenetriaminepentaacetic acid gadolinium complex (**4)** was synthesized through a multistep procedure. **(a)** 2-(pyridin-2-yldisulfanyl)ethanamine hydrochloride (**1)** was reacted with DTPA bis-anhydride to afford the mono-substituted DTPA ligand (**2**). **(b)** To synthesize the Gd-complex, a metallation reaction was carried out under basic conditions to afford Gd-complex (**3**). **(c)** Finally, by means of a disulfide-thiol exchange reaction with 3-mercaptopropyl triethoxysilane, the desired Gd-complex (**4**) was obtained.

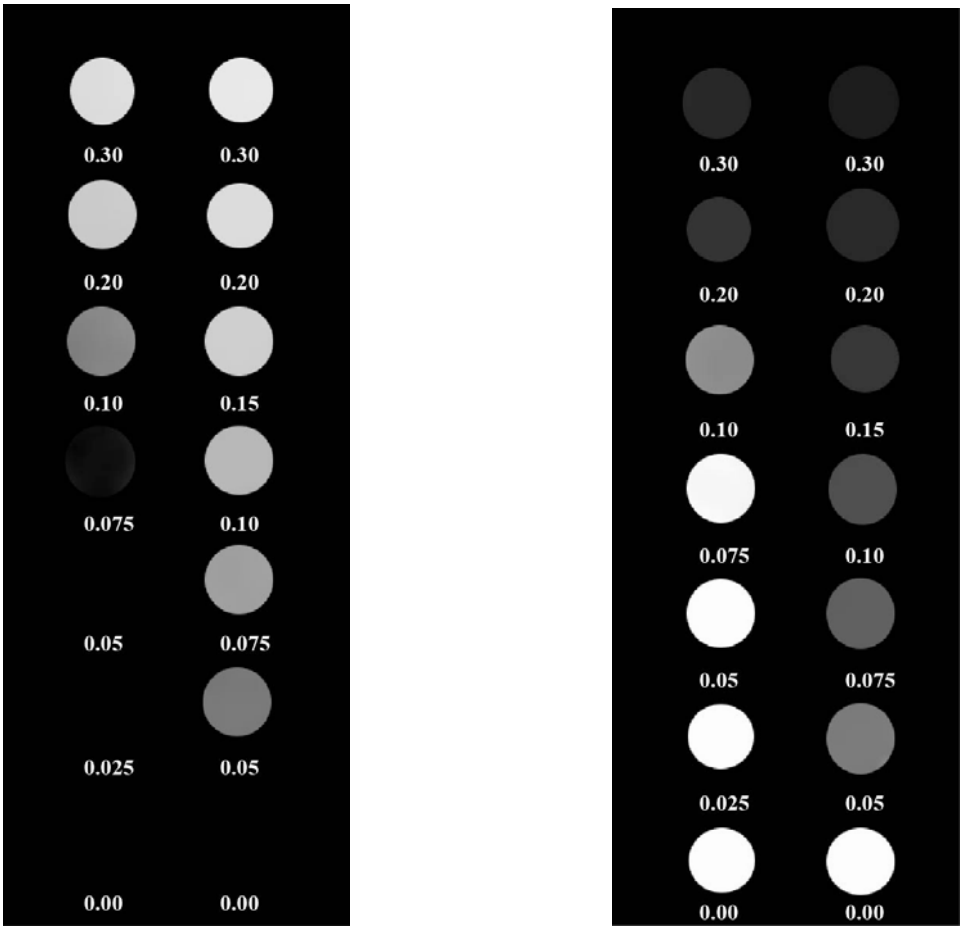


Figure S1. T_1- (left) and T_2-weighted (right) images of water suspensions of Gd-MSN (left) and PEG-Gd-MSN (right) materials. The numbers refer to the corresponding Gd^{3+} concentrations [mM].

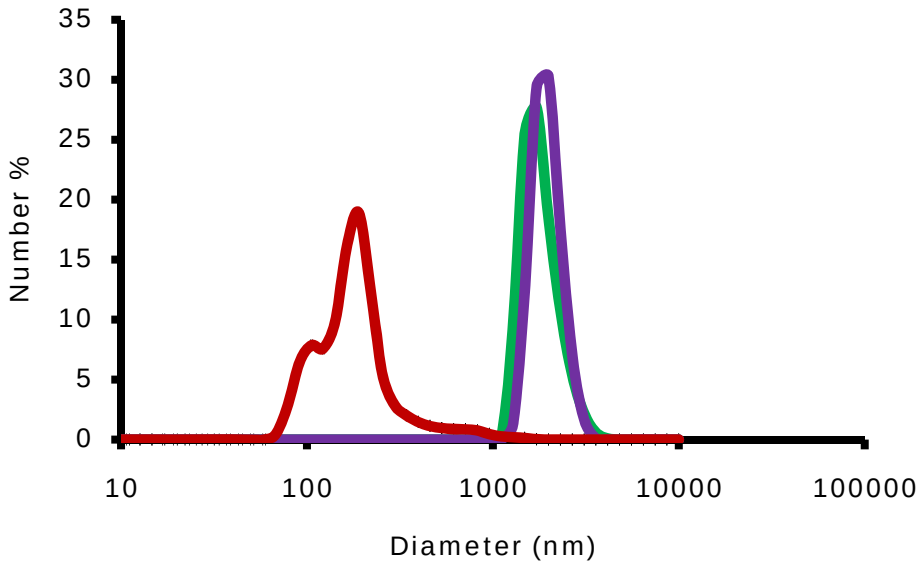


Figure S2. Dynamic light scattering graphs of AP-MSNs (green), Gd-MSNs (purple), and PEG-Gd-MSNs (red) nanomaterials.

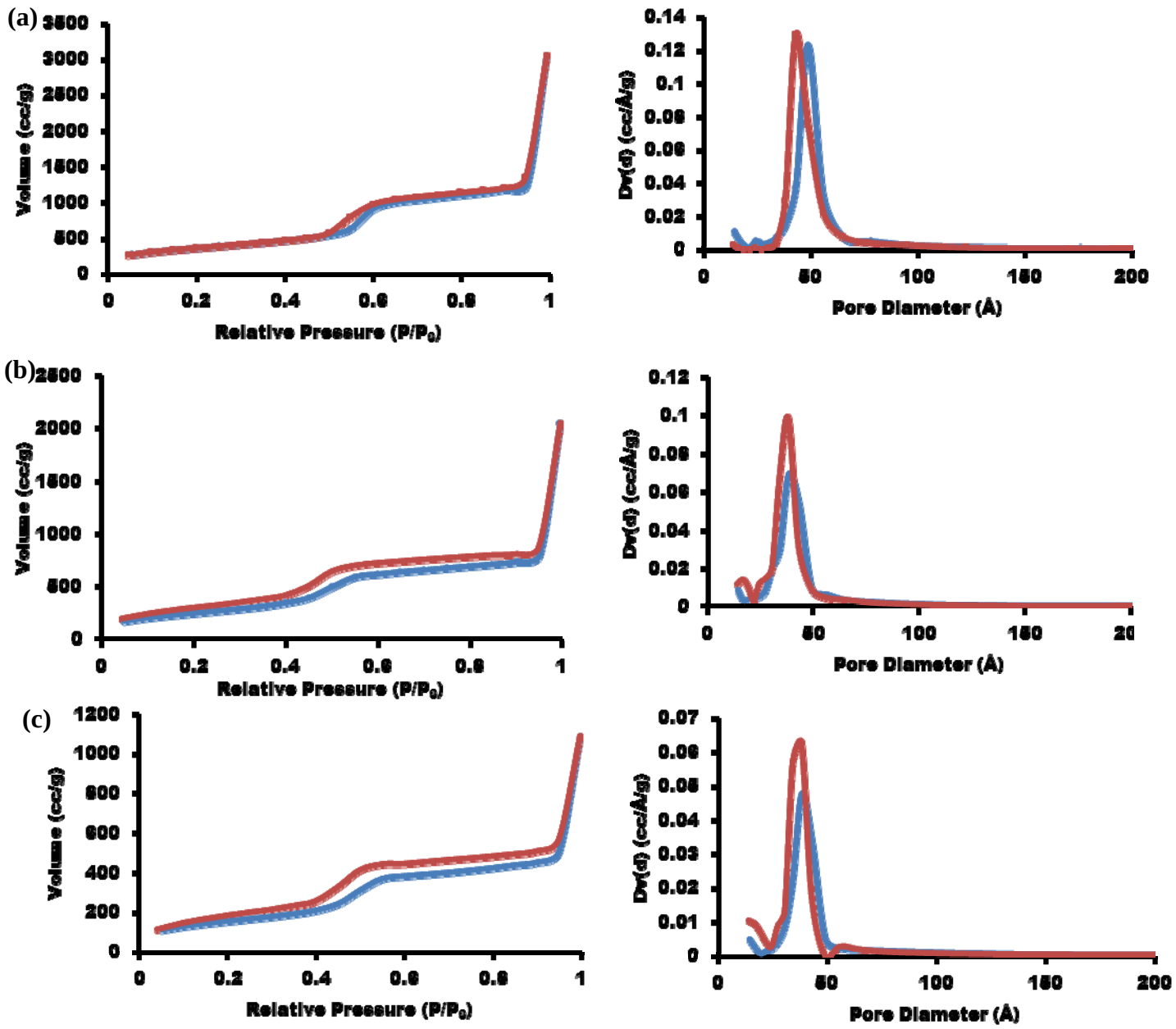


Figure S3. N_2 sorption isotherms: BET surface area (left) and BJH pore size distribution (right) of (a) AP-MSNs, (b) Gd-MSNs, and (c) PEG-Gd-MSNs (blue-adsorption; red-desorption)

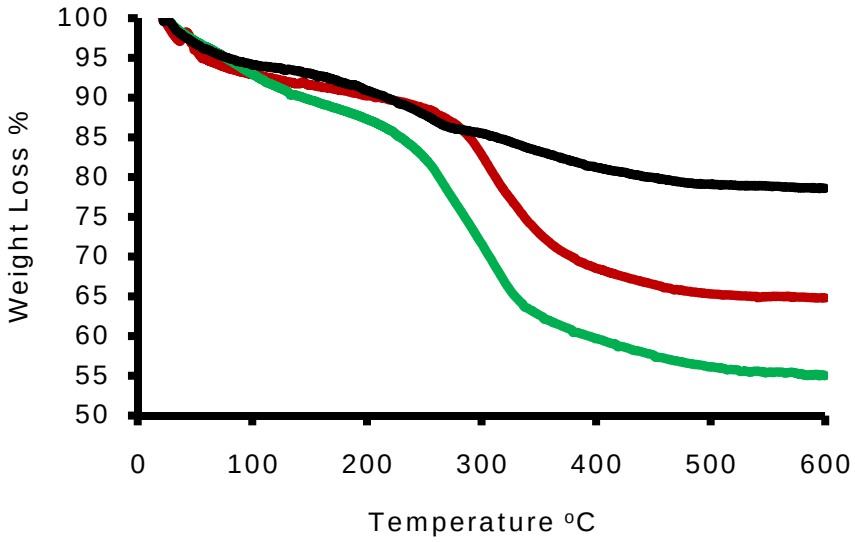


Figure S4. Thermogravimetric analysis of AP-MSNs (black), Gd-MSNs (red), and PEG-Gd-MSNs (green) nanomaterials.

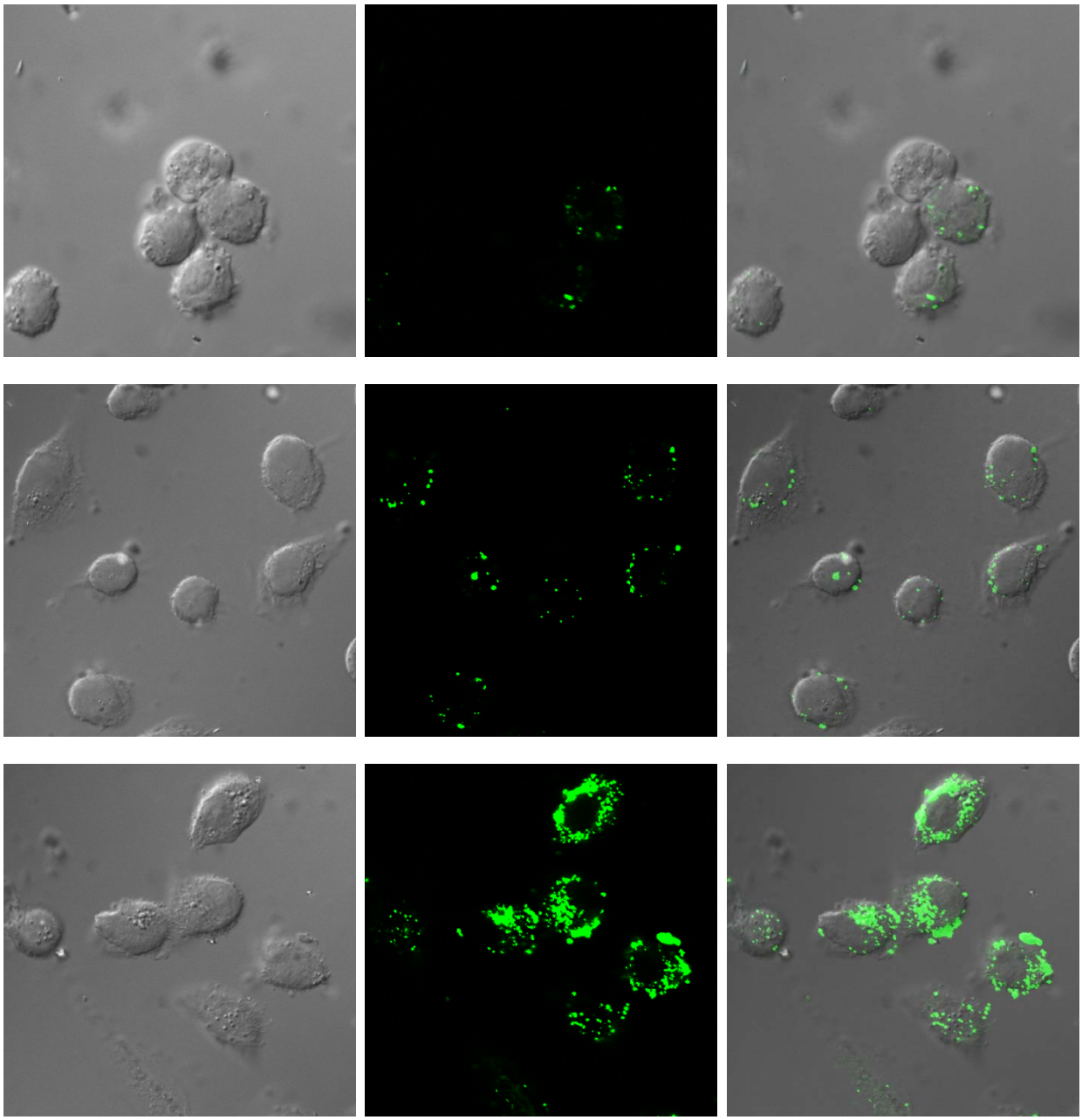


Figure S5. Confocal microscope images of AsPC-1 human pancreatic adenocarcinoma cells incubated with FITC-Gd-MSNs (first row), FITC-PEG-Gd-MSNs (second row), and FITC-AA-Gd-MSNs (third row). The amount of nanomaterial used in each sample was 30 μg/mL. DIC images are on the left, FITC channel in the middle, and the composite images in the right column.

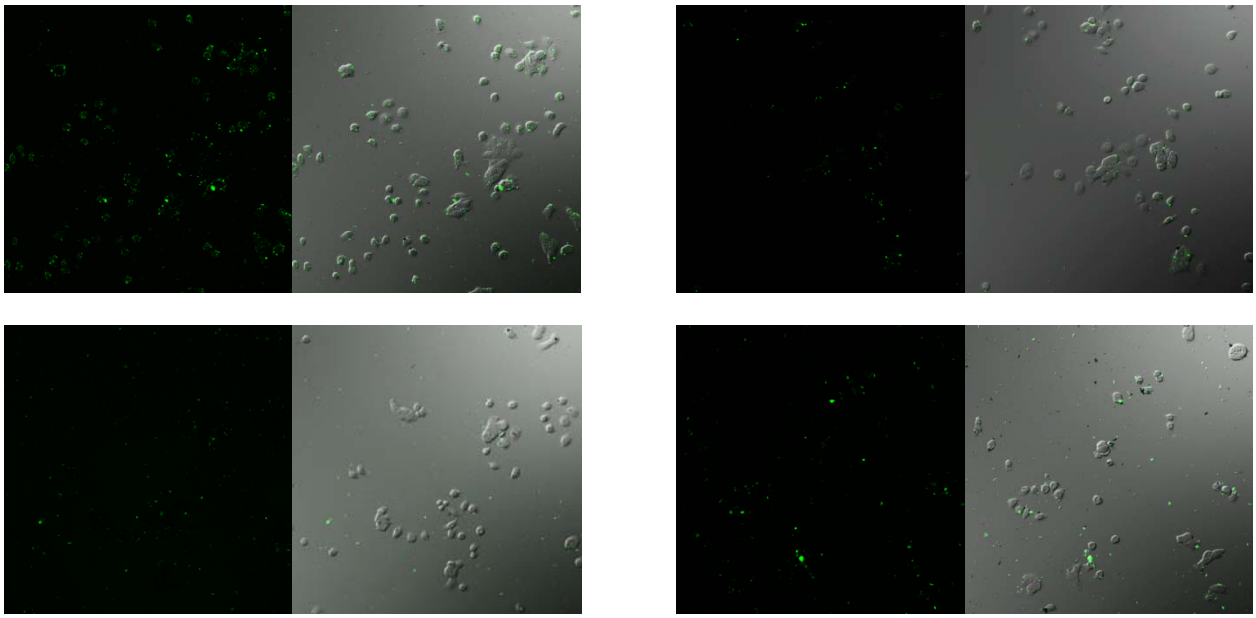


Figure S6. Anisamide competitive binding assay. Confocal microscope images of AsPC-1 human pancreatic adenocarcinoma cells incubated in the absence of (first row) and the presence of (second row) the anisamide ligand (1 mM). The cells were inoculated with AA-Gd-MSNs (left), and PEG-Gd-MSNs (right) materials in a concentration of 50 μg/mL for 1 h. FITC channel images are in the left, and the composite images in the right column.

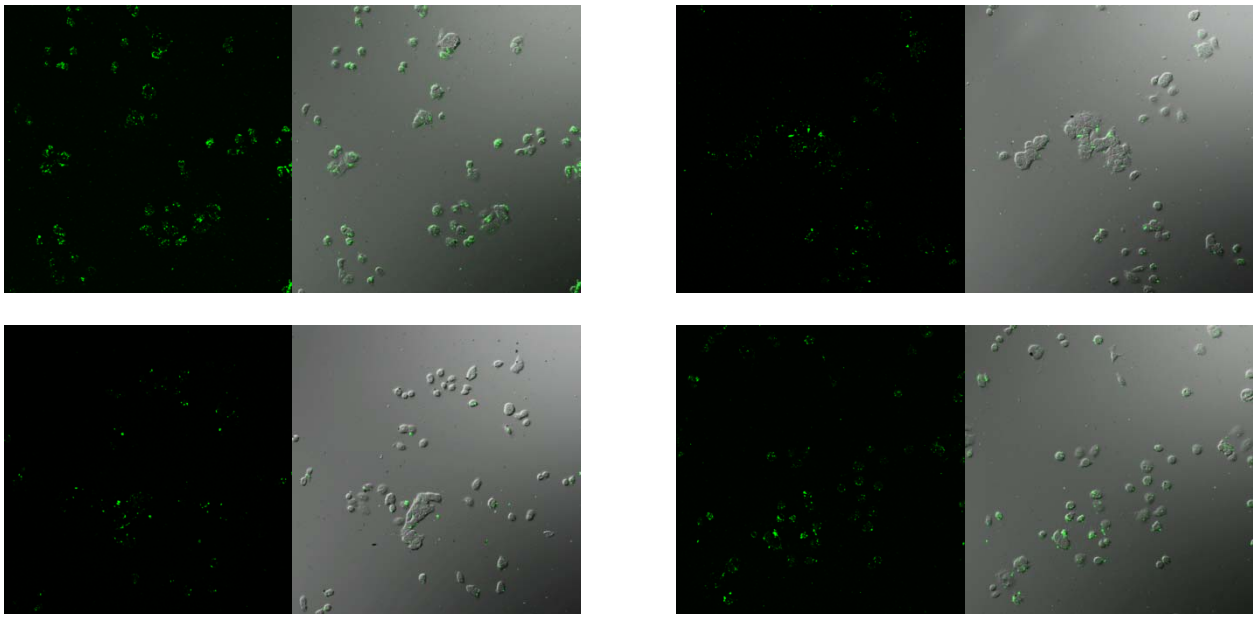


Figure S7. Anisamide competitive binding assay. Confocal microscope images of AsPC-1 human pancreatic adenocarcinoma cells incubated in the absence of (first row) and the presence of (second row) the anisamide ligand (1 mM). The cells were inoculated with AA-Gd-MSNs (left), and PEG-Gd-MSNs (right) materials in a concentration of 50 μg/mL for 4 h. FITC channel images are in the left, and the composite images in the right column.

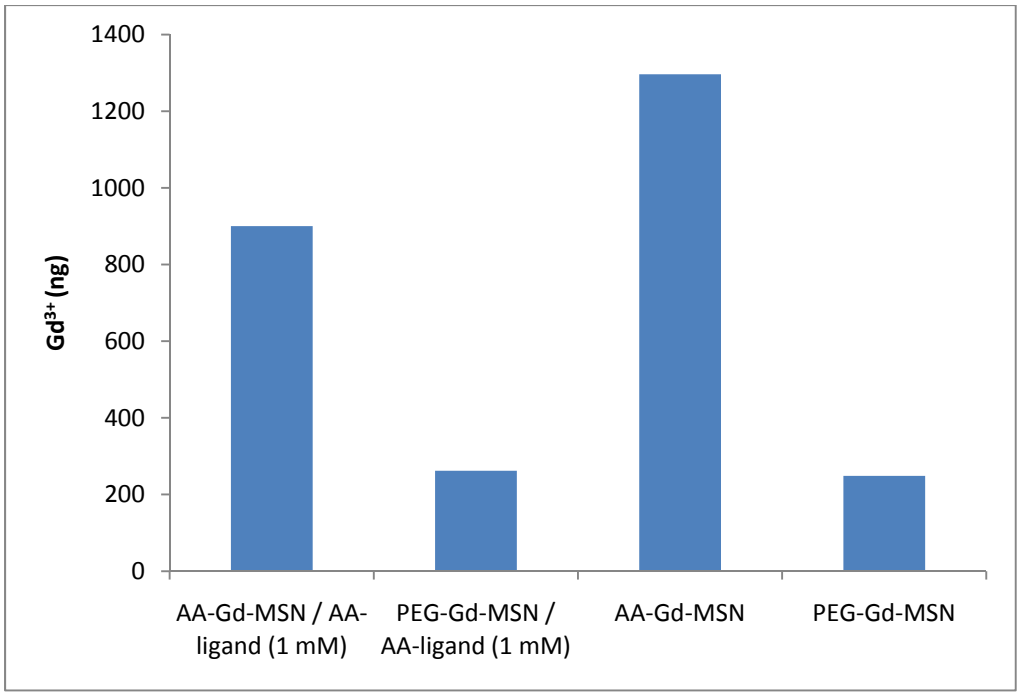
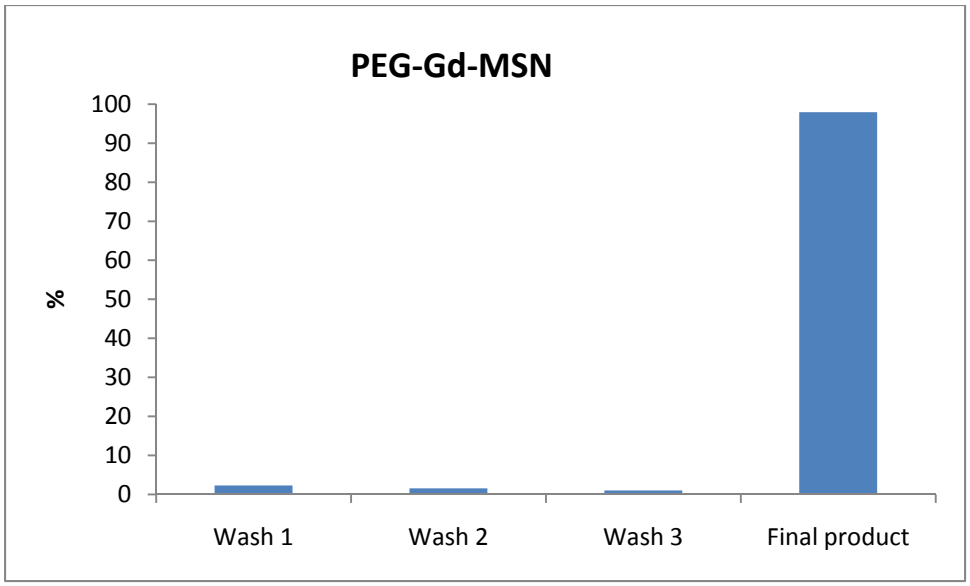


Figure S8. Anisamide competitive binding assay. The amount of Gd^{3+} uptaken by AsPC-1 cells was determined by ICP-MS. The graph shows the effect of the anisamide ligand on the uptake of AA-Gd-MSNs as an indication that the internalization of this material is partially due to a receptor-specific endocytosis pathway.



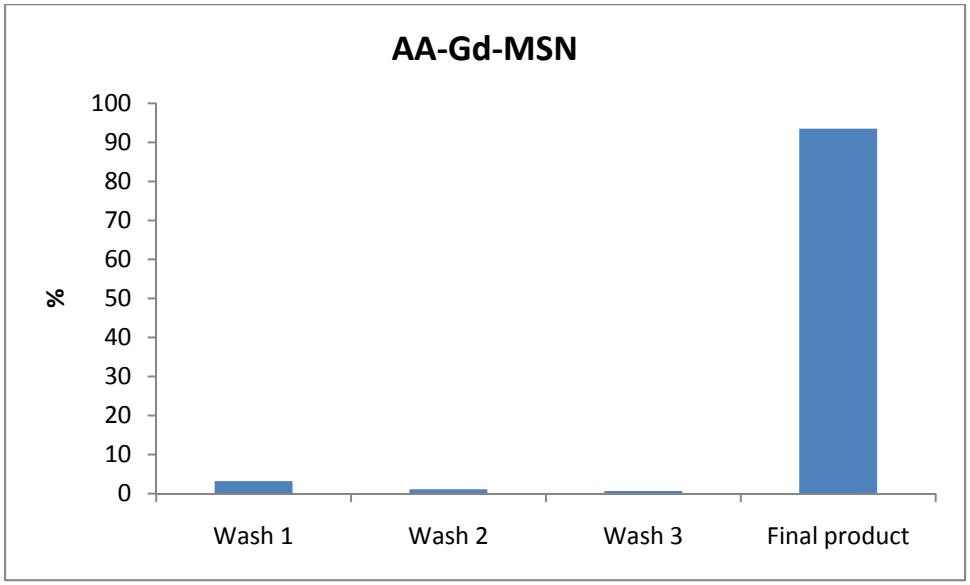
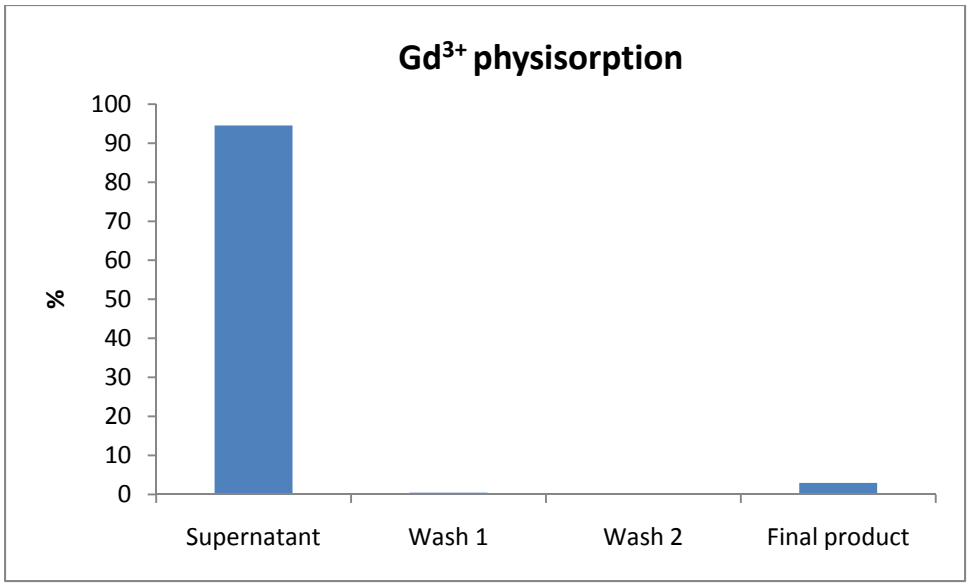


Figure S9. Gd-chelate stability under acidic conditions (PBS 10 mM, pH 3.1). The amount of Gd^{3+} that leached under acidic conditions after several washings was determined by ICP-MS. As it is shown in the graphs; PEG-Gd-MSN (top) retains >97% of the original Gd^{3+}; in a similar way, AA-Gd-MSN (bottom) retains >93% of Gd^{3+}..



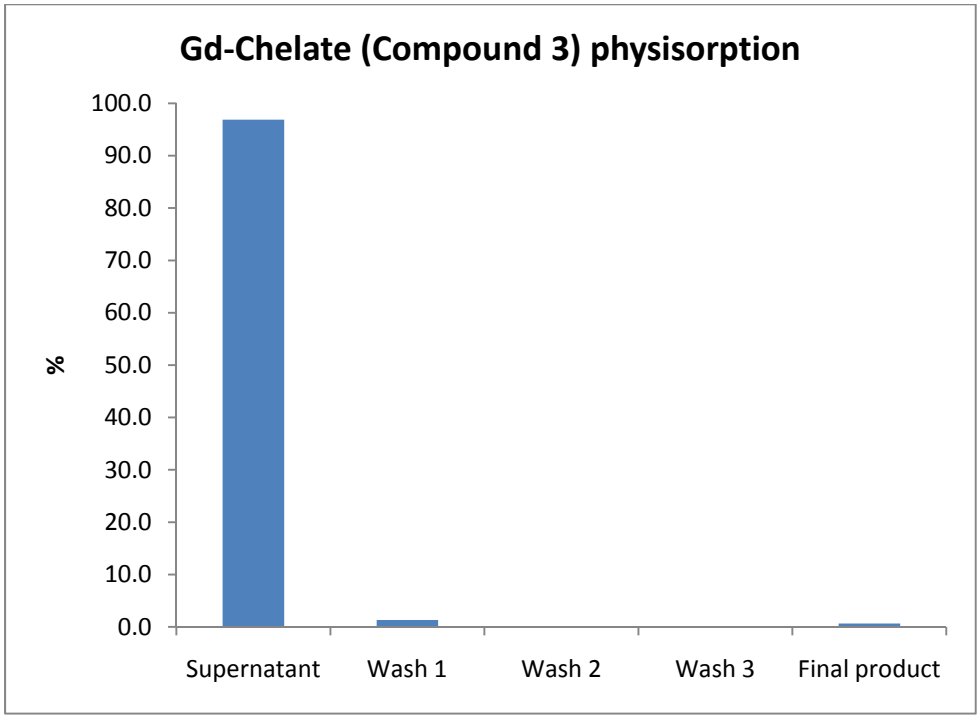


Figure S10. Gd^{3+} and Gd-chelate physisorption assay. The amount of Gd^{3+} that was physisorbed on MSN was determined by ICP-MS. As shown in the graphs, most of the Gd^{3+} (>94%, top) and Gd-chelate (>97%, bottom) was recovered in the supernatant after stirring the MSN sample for 4h. The final materials only contained trace amounts of Gd^{3+} (< 2.5%) and Gd-chelate (< 0.6%), indicating the low physisorption of these species.

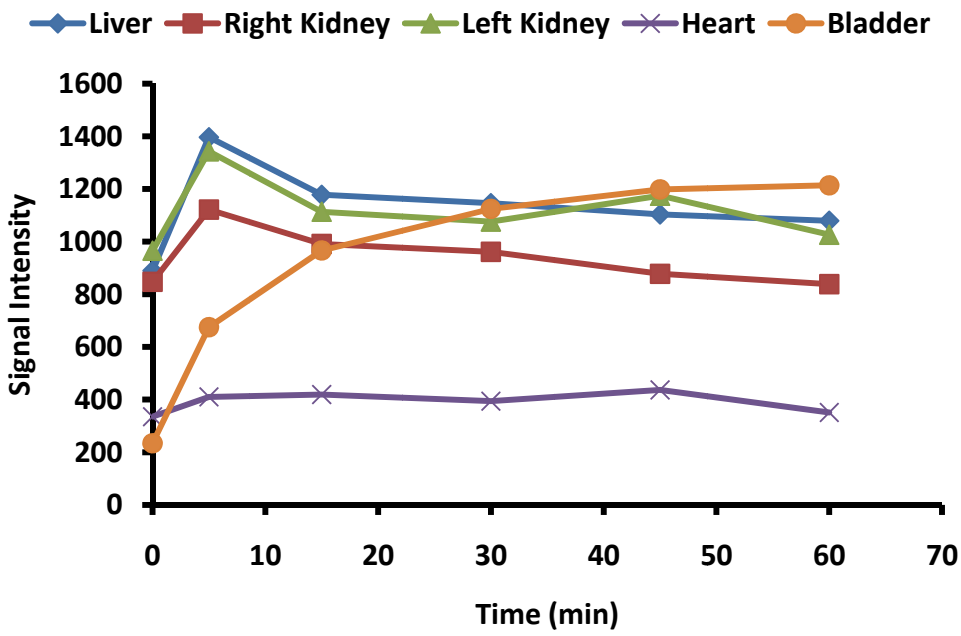


Figure S11. Relative MR signal intensities in the liver (◆), right kidney (■), left kidney (▲), heart (**X**), and urinary bladder (●) at various time points after the i.v. injection of PEG-Gd-MSNs with a dose of 0.080 mmol-Gd/kg.

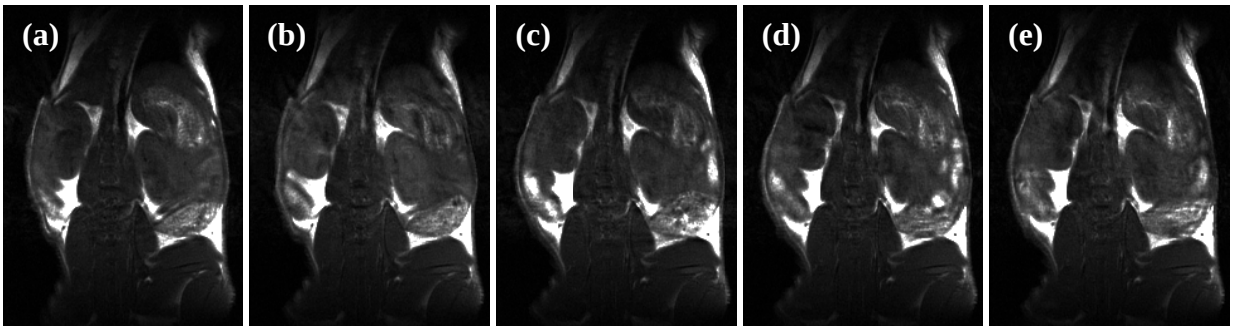


Figure S12. MR images of a mouse injected (i.v.) with PEG-Gd-MSNs (0.080 mmol-Gd/kg dose), focusing on the kidneys. The MR images were taken at different times; **(a)** precontrast, **(b)** 5 min, **(c)** 15 min, **(d)** 30 min, and **(e)** 45 min.

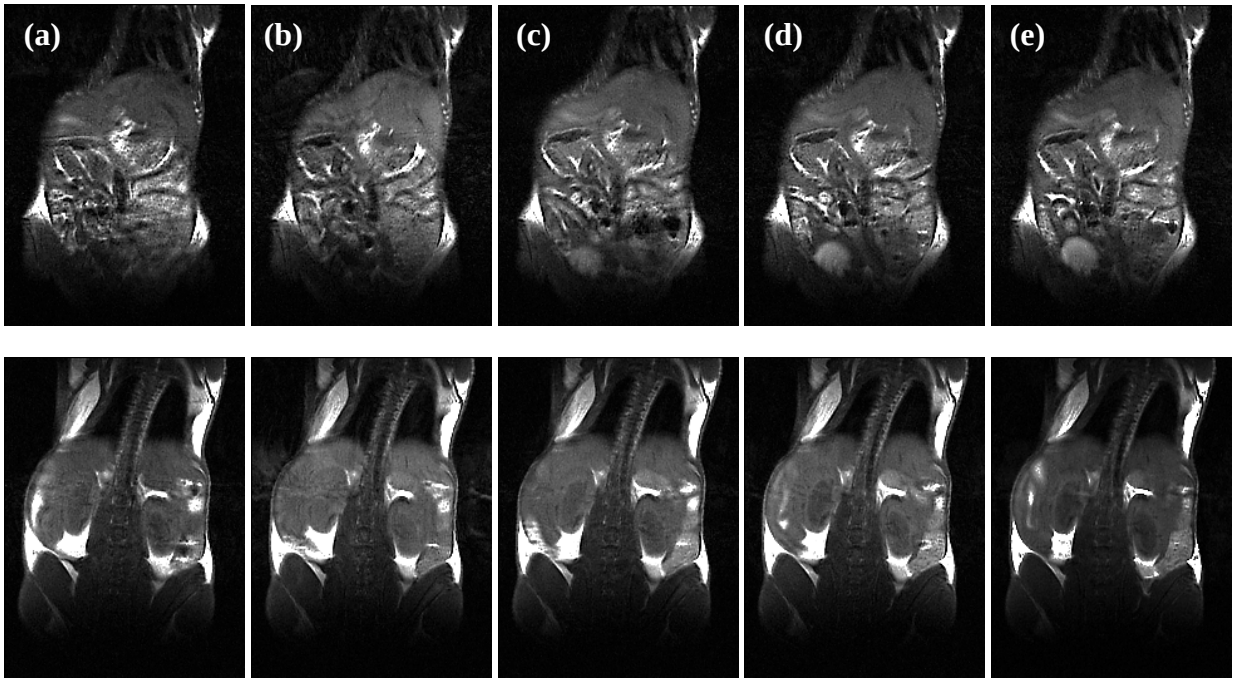


Figure S13. MR images of a mouse injected (i.v.) with PEG-Gd-MSNs (0.096 mmol-Gd/kg dose), focusing on urinary bladder/liver (upper row) and kidneys (lower row). The MR images were taken at different times; **(a)** precontrast, **(b)** 5 min, **(c)** 15 min, **(d)** 30 min, and **(e)** 45 min. The enhanced contrast in the bladder was apparent after 15 min, indicating that the Gd-chelate is biodegraded by the reducing agents in the blood and quickly cleared through the renal pathway.

Table S1. Biodistribution of Gd^{3+} 2h after iv injection.

Organ	**% ID**
Spleen	1.2
Kidney	0.2
Lung	0.2
Bladder	2.9
Liver	3.6

References

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