Supporting Information

Multifunctional Gold Nanoparticles as Smart nanovehicles with Enhanced Tumor Targeting for Intracellular pH mapping and in *vivo* MRI imaging

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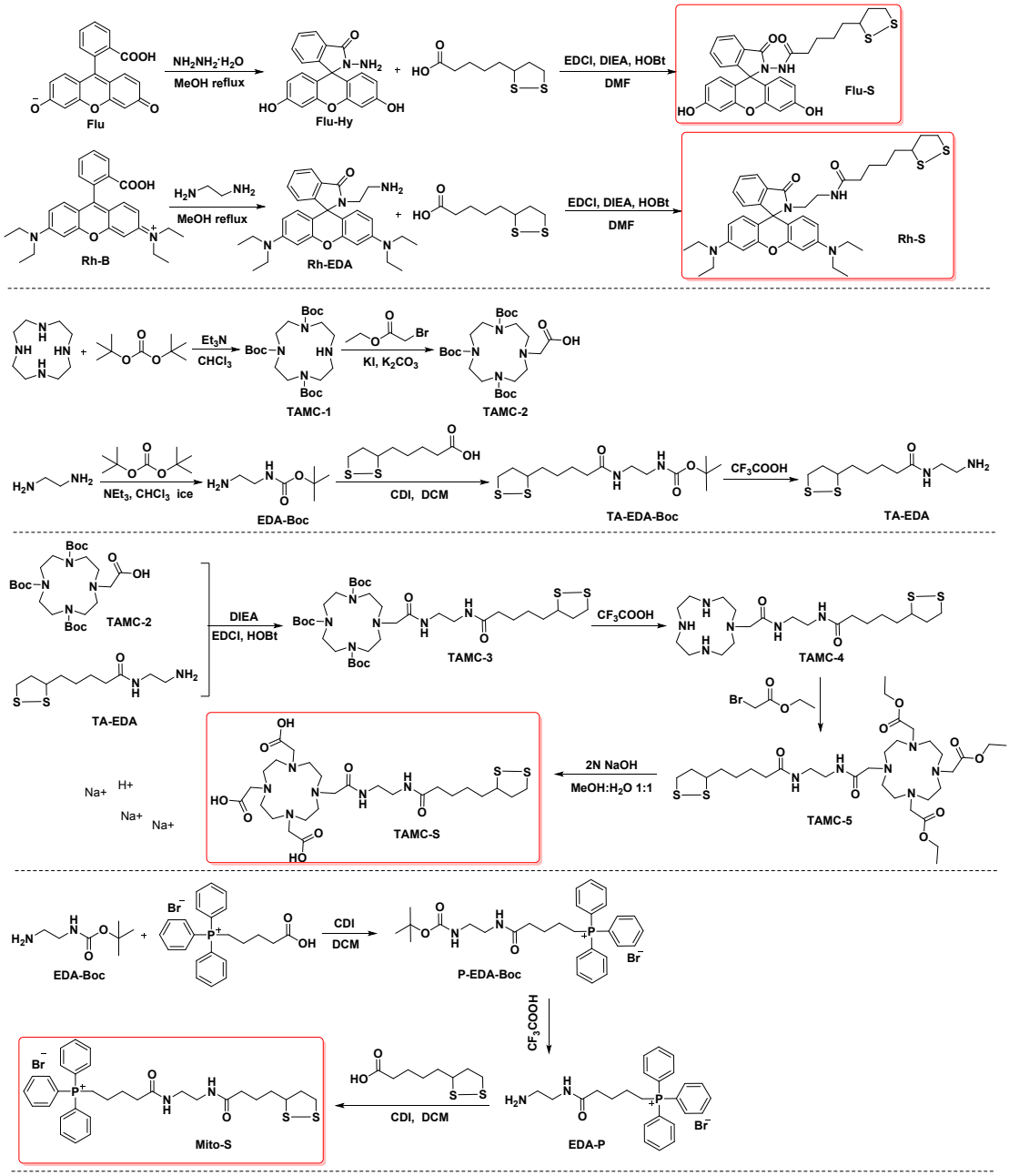
1. Synthesis of various compounds



Scheme S1 Synthesis of target compounds

Flu-Hy, **Flu-S**, **RhEDA**, **Rh-S**, **EDA-Boc**, **TAMC-1**, and **TAMC-2** were synthesized according to our previous literature.[1]

TA-EDA-Boc and **TA-EDS**: To a solution of thioctic acid (3.2 g, 16 mmol) and N,N'-carbonyldiimidazole (CDI, 2.5 g, 16 mmol) in dichloromethane (20 ml), 2.1 g **EDA-Boc** (13 mmol) was added and the resulting mixture was stirred at room temperature overnight. Then the reaction mixture was washed with saturated aqueous NaCl (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography over silica gel to give 3.7 g **TA-EDA-Boc** (10.7 mmol, yield 82.1%).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (s, 1H), 6.78 (s, 1H), 3.66-3.57 (m, 1H), 3.16 (ddd, *J* = 17.8, 8.8, 4.9 Hz, 2H), 3.09-3.00 (m, 2H), 3.00-2.90 (m, 2H), 2.42 (dd, *J* = 12.2, 6.2 Hz, 1H), 2.05 (t, *J* = 7.4 Hz, 2H), 1.92-1.82 (m, 1H), 1.66 (s, 1H),

1.60-1.46 (m, 3H), 1.38 (s, 9H), 1.36-1.28 (m, 2H). After dissolving **TA-EDA-Boc** with anhydrous dichloromethane, excessive trifluoroacetic acid was added to reaction and the mixture reacted at room temperature overnight, then the solvent was removed by vacuum distillation. Compounds **TA-EDA** can be obtained by adding ether washing products and removing ether by vacuum distillation, which needed to be repeated about five times. EMS-MS: m/z 249.10 $[M+H]^+$: (calcd 249.11); m/z 271.10 $[M+Na]^+$:(calcd 271.09).

TAMC-3 and **TAMC-4**: 1.9 g **TAMC-2** (3.6 mmol), 690 mg EDCI (3.6 mmol), 537 mg 1-hydroxybenzotriazole (HOBt, 3.7 mmol) and 2.6 mL N, N-diisopropylethylamine (DIEA, 15 mmol) were dissolved in dichloromethane and stirred in ice bath for 1 hour. Then, 744 mg **TA-EDA** (3 mmol) was dissolved in a small amount of dichloromethane and added to the above reaction solution. The mixture stirred at 0℃ for another 2 hours and the reaction lasted overnight at room temperature. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography over silica gel to give 400 mg white compound **TAMC-3** (0.53 mmol, 17.5%).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.80 (s, 1H), 3.66-3.58 (m, 1H), 3.45 (dd, *J* = 8.6, 6.7 Hz, 4H), 3.24 (d, *J* = 14.1 Hz, 8H), 3.21-3.16 (m, 3H), 3.11 (dt, *J* = 16.7, 7.6 Hz, 5H), 2.73 (s, 4H), 2.42 (dd, *J* = 12.3, 6.2 Hz, 1H), 2.06 (t, *J* = 7.3 Hz, 2H), 1.87 (dd, *J* = 12.8, 6.7 Hz, 1H), 1.71-1.62 (m, 1H), 1.58-1.48 (m, 3H), 1.39 (d, *J* = 10.6 Hz, 27H), 1.33 (d, *J* = 7.5 Hz, 2H)； EMS-MS: m/z 761.43 $[M+H]^+$: (calcd 761.43); m/z 783.40 $[M+Na]^+$:(calcd 783.41). After dissolving **TAMC-3** with anhydrous dichloromethane, excessive trifluoroacetic acid was added to reaction and the mixture reacted at room temperature overnight, then the solvent was removed by vacuum distillation. Compounds **TAMC-4** can be obtained by adding ether washing products and removing ether by vacuum distillation, which needed to be repeated about five times. EMS-MS: m/z 461.27 $[M+H]^+$: (calcd 461.27); m/z 483.24 $[M+Na]^+$:(calcd 483.25).

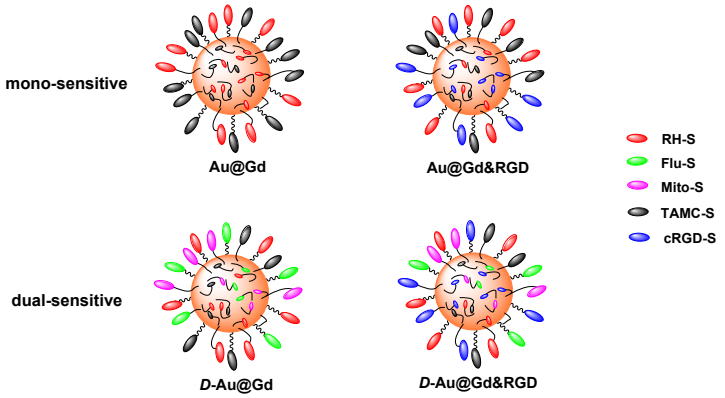
TAMC-5 and **TAMC-S**: 230 mg **TAMC-4** (0.5 mmol) was dissolved in acetonitrile, then pH value of the solution was adjusted to 7.0-8.0 by adding dried potassium carbonate. After that, 210 μL ethyl bromoacetate (2 mmol) and 398 mg dry potassium carbonate (3.6 mmol) were added to the solution. The mixture reacted at 80℃ overnight and then the reaction was cooled to room temperature. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography over silica gel to give 151 mg white compound **TAMC-5** (0.21 mmol, 42%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.85 (d, *J* = 4.7 Hz, 1H), 4.20-4.06 (m, 10H), 3.67-3.56 (m, 3H), 3.22-3.05 (m, 11H), 2.95 (s, 2H), 2.82 (d, *J* = 14.3 Hz, 3H), 2.42 (dd, *J* = 12.1, 6.3 Hz, 3H), 2.07 (t, *J* = 7.3 Hz, 4H), 1.86 (dd, *J* = 12.8, 6.7 Hz, 1H), 1.69-1.63 (m, 1H), 1.58-1.46 (m, 4H), 1.38-1.32 (m, 2H), 1.21 (d, *J* = 7.3 Hz, 12H)； EMS-MS: m/z 741.36 $[M+Na]^+$:(calcd 741.36). After dissolving **TAMC-5** with methanol, excessive 2N NaOH was added to reaction and the mixture reacted at room temperature overnight, then adjusted the pH value of the solvent to 3.0 by using concentrated hydrochloric acid. The solvent then extracted with CH_2Cl_2 (3×30 mL). The combined organic layer was washed with saturated aqueous NaCl (50 mL) successively and dried over anhydrous Na_2SO_4. The solvent was removed under the reduced pressure and **TAMC-S** can be obtained. EMS-MS: m/z 701.20 $[M+H]^+$: (calcd 701.23).

P-EDA-Boc and **EDA-P**: triphenylphosphovalerate (7.4 g, 17.4 mmol) and CDI (2.8 g, 17.3 mmol) were dissolved in dichloromethane, 2.8 g **EDA-Boc** (17.5 mmol) was added and the resulting mixture was stirred at room temperature overnight. Then the reaction mixture was washed with saturated aqueous NaCl (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography over silica gel to give 7.6 g **P-EDA-Boc** (15 mmol, 86.6%)^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92-7.76 (m, 15H), 6.83 (t, *J* = 5.5 Hz, 1H), 3.61 (t, *J* = 14.8 Hz, 2H), 3.03-2.96 (m, 2H), 2.93-2.84 (m,

2H), 2.12 (t, *J* = 7.0 Hz, 2H), 1.76-1.64 (m, 2H), 1.51 (d, *J* = 7.2 Hz, 2H), 1.37 (s, 9H). After dissolving **P-EDA-Boc** with anhydrous dichloromethane, excessive trifluoroacetic acid was added to reaction and the mixture reacted at room temperature overnight, then the solvent was removed by vacuum distillation. Compounds **EDA-P** can be obtained by adding ether washing products and removing ether by vacuum distillation, which needed to be repeated about five times. EMS-MS: m/z 405.20 $[M]^+$: (calcd 405.21).

Mito-S: To a solution of thioctic acid (729 mg, 4.5 mmol) and CDI (656 mg, 4.5 mmol) in dichloromethane (20 ml), 2.0 g **EDA-P** (3.5 mmol) was added and the resulting mixture was stirred at room temperature overnight. Then the reaction mixture was washed with saturated aqueous NaCl (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under the reduced pressure and the residue was further purified by column chromatography over silica gel to give 1.9 g white compound **Mito-S** (2.9 mmol, yield 83%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (s, 1H), 7.95 (s, 1H), 7.93-7.87 (m, 3H), 7.85-7.74 (m, 12H), 4.15 (q, *J* = 5.2 Hz, 1H), 3.67-3.56 (m, 3H), 3.22-3.06 (m, 4H), 2.40 (td, *J* = 12.6, 6.4 Hz, 1H), 2.13 (t, *J* = 7.1 Hz, 2H), 2.04 (t, *J* = 7.3 Hz, 2H), 1.85 (dq, *J* = 13.5, 6.8 Hz, 1H), 1.74-1.61 (m, 3H), 1.57-1.43 (m, 5H), 1.32 (dt, *J* = 13.9, 7.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6 (s), 172.8 (s), 135.2 (m), 133.5 (m), 130.5 (m), 118.4 (s), 117.5 (s), 77.3 (s), 77.0 (s), 76.7 (s), 56.5 (s), 40.12 (s), 39.6 (s), 39.3 (s), 38.4 (s), 36.1 (s), 34.6 (s), 33.7 (s), 28.8 (s), 25.5 (s).

2. Preparation of gold nanoparticles



Scheme S2 Different gold nanoparticles.

Au@Gd: 114 mg **TAMC-S** (0.145 mmol) and 85.6 mg **Rh-S** (0.145 mmol) were dissolved in 3 mL DMF, then 4 mL chloroauric acid aqueous solution ($HAuCl_4$, 0.0485 mmol) was added and the mixture was stirred for 15 minutes at room temperature. Under intense stirring, 10 mL cold fresh sodium borohydride aqueous solution ($NaBH_4$, 0.485 mmol) was added to the mixed solution, which instantly changed the color of the solution from light yellow pink to wine red. Gold nanoparticles can be obtained by high-speed centrifugation and vacuum drying. Then the obtained gold nanoparticles was dissolved in methanol and 30 mg gadolinium chloride hexahydrate (0.08 mmol) was added in the solution. The mixture was stirred overnight at room temperature and **Au@Gd** can be obtained by high-speed centrifugation and vacuum drying.

Au@Gd&RGD: 114 mg **TAMC-S** (0.145 mmol), 85.6 mg **Rh-S** (0.145 mmol), and 12.5 **cRGD-S** (0.01 mmol) were dissolved in 3 mL DMF, then 4 mL chloroauric acid aqueous solution ($HAuCl_4$, 0.0485 mmol) was added and the mixture was stirred for 15 minutes at room temperature. Under intense stirring, 10 mL cold fresh sodium borohydride aqueous solution ($NaBH_4$, 0.485 mmol) was added to the mixed solution, which instantly changed the color of the solution from light yellow pink to wine red. Gold nanoparticles can be obtained by high-speed

centrifugation and vacuum drying. Then the obtained gold nanoparticles was dissolved in methanol and 30 mg gadolinium chloride hexahydrate (0.08 mmol) was added in the solution. The mixture was stirred overnight at room temperature and **Au@Gd&RGD** can be obtained by high-speed centrifugation and vacuum drying.

***D*-Au@Gd**: 190 mg **TAMC-S** (0.2 mmol), 134 mg **Rh-S** (0.2 mmol), 107 mg **Flu-S** (0.2 mmol), and 65.5 **Mito-S** (0.1 mmol) were dissolved in 3 mL DMF, then 4 mL chloroauric acid aqueous solution ($HAuCl_4$, 0.0485 mmol) was added and the mixture was stirred for 15 minutes at room temperature. Under intense stirring, 10 mL cold fresh sodium borohydride aqueous solution ($NaBH_4$, 0.485 mmol) was added to the mixed solution, which instantly changed the color of the solution from light yellow pink to wine red. Gold nanoparticles can be obtained by high-speed centrifugation and vacuum drying. Then the obtained gold nanoparticles was dissolved in methanol and 36 mg gadolinium chloride hexahydrate (0.1 mmol) was added in the solution. The mixture was stirred overnight at room temperature and ***D*-Au@Gd** can be obtained by high-speed centrifugation and vacuum drying.

***D*-Au@Gd&RGD**: 190 mg **TAMC-S** (0.2 mmol), 134 mg **Rh-S** (0.2 mmol), 107 mg **Flu-S** (0.2 mmol), 65.5 **Mito-S** (0.1 mmol), and 25 mg **cRGD-S** (0.02 mmol) were dissolved in 3 mL DMF, then 4 mL chloroauric acid aqueous solution ($HAuCl_4$, 0.0485 mmol) was added and the mixture was stired for 15 minutes at room temperature. Under intense stirring, 10 mL cold fresh sodium borohydride aqueous solution ($NaBH_4$, 0.485 mmol) was added to the mixed solution, which instantly changed the color of the solution from light yellow pink to wine red. Gold nanoparticles can be obtained by high-speed centrifugation and vacuum drying. Then the obtained gold nanoparticles was dissolved in methanol and 36 mg gadolinium chloride hexahydrate (0.1 mmol) was added in the solution. The mixture was stirred overnight at room temperature and ***D*-Au@Gd&RGD** can be obtained by high-speed centrifugation and vacuum drying.

3. UV-Vis Spectroscopy of gold nanovehicles in DMSO

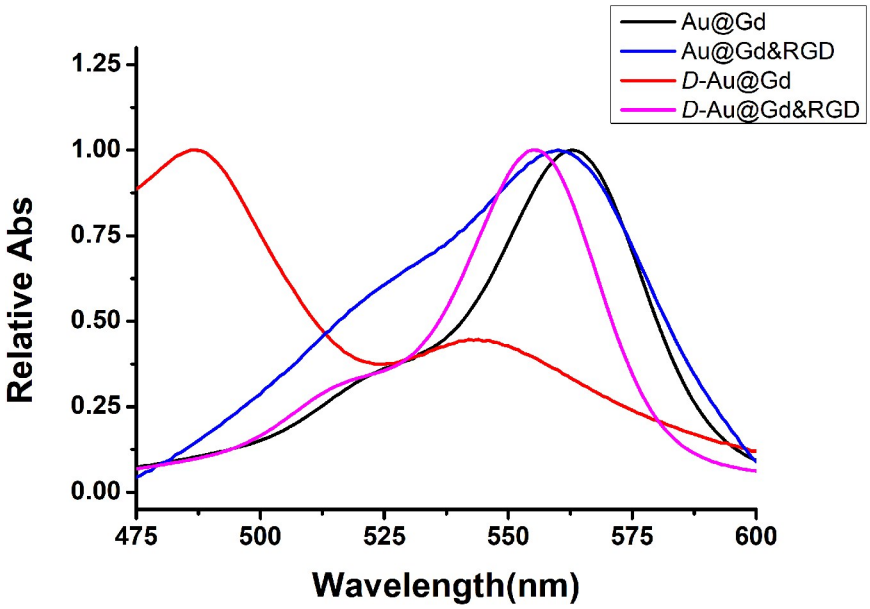


Figure S1 UV-Vis absorption of **Au@Gd**, **Au@Gd&RGD**, **D-Au@Gd**, and **D-Au@Gd&RGD** (200 mg/mL).

4. pH titration of single-sensitive dual-modal agents

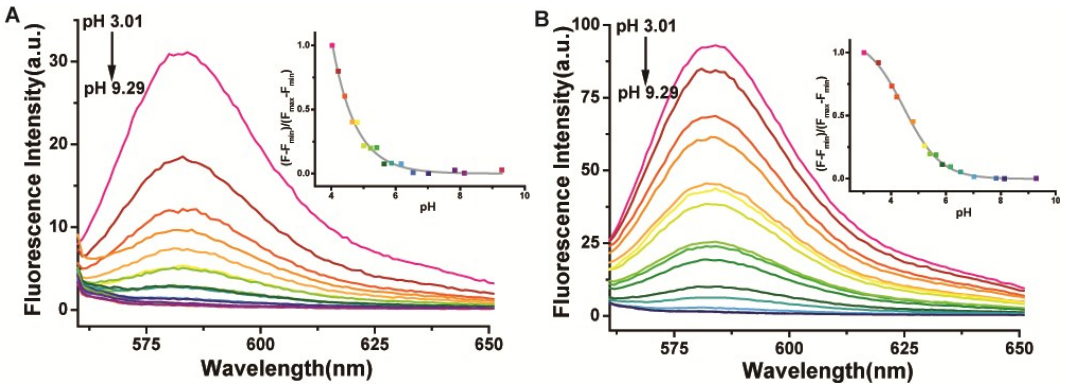


Figure S2 Fluorescence emission spectral changes of A. **Au@Gd** and B. **Au@Gd&RGD** (100 μg/mL) in B–R buffer solution at different pH values. The maximum emission intensity of the two nanovehicles were both at 580 nm (λ_{ex} 550 nm). Embedded graph: Normalized fluorescence intensity as a function of pH for **Au@Gd** and **Au@Gd&RGD** at 580nm. pH 3.01, 3.54, 4.03, 4.22, 4.42, 4.65, 4.80, 5.01, 5.22, 5.41, 5.64, 5.87, 6.17, 6.54, 7.02, 7.83, 8.13, and 9.29.

5. pK_a Calculation

The pKa values of all the dual-modal agents were calculated according to the study of J. W. Aylott (pK_a is generally the pH at which the fluorophore shows half its maximal response) [2].

Table S1. The pK_a of **Au@Gd**, **Au@Gd&RGD**, ***D*-Au@Gd**, and ***D*-Au@Gd&RGD.**

	Sticker Rh-S	Sticker Flu-S-
Au@Gd	4.07	
Au@Gd&RGD	4.62	
***D*-Au@Gd**	4.50	6.17
***D*-Au@Gd&RGD**	4.57	6.02

6. Anti-interference of all the dual-modal agents

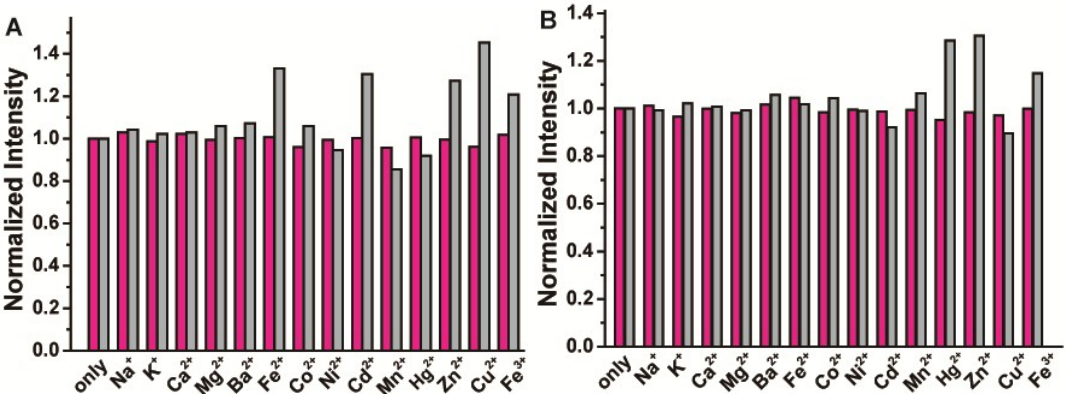


Figure S3. Fluorescent intensity changes at 580 nm of 100 μg/mL A. **Au@Gd** and B. **Au@Gd&RGD** in the presence of different interfering species at pH 4.65 (pink bars) and 7.41 (grey bars). The concentration of interferences: 200 μM.

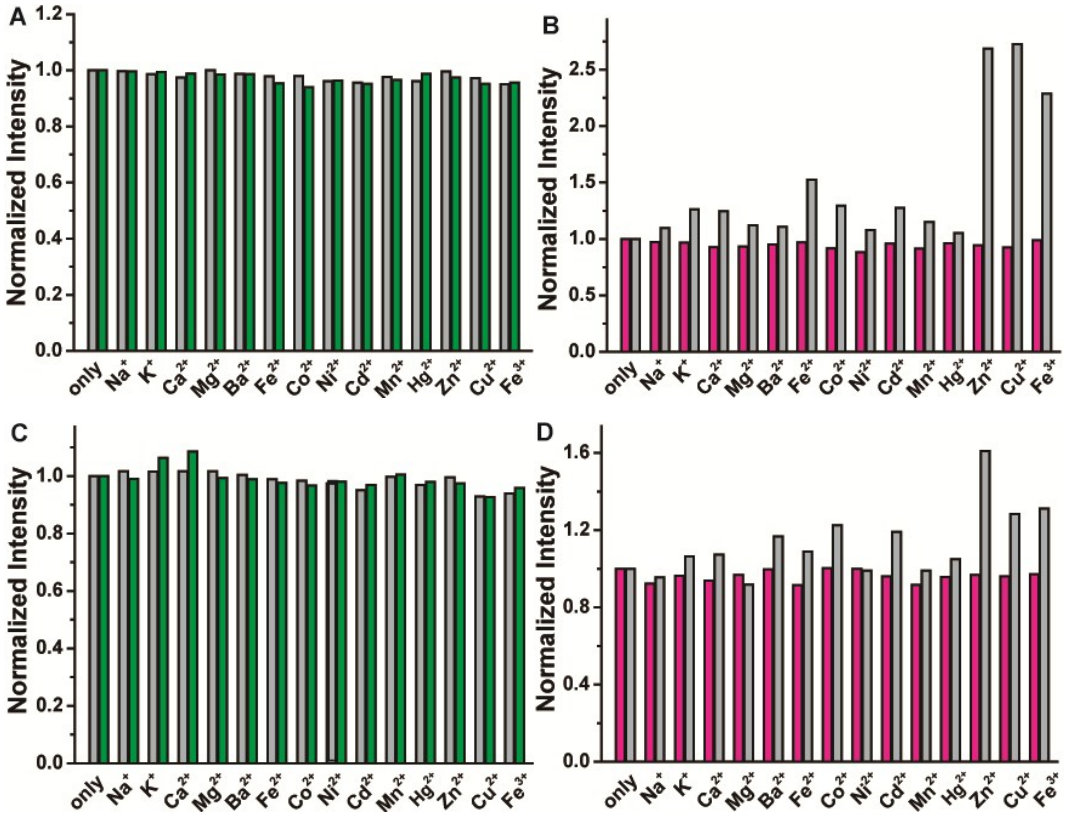


Figure S4. Fluorescent intensity changes of 100 μg/mL ***D*-Au@Gd** (A and B) and ***D*-Au@Gd&RGD** (C and D) in the presence of different interfering species at different pH. A and C: fluorescence was collected at 515 nm, pH values of green bars and grey bars is 7.41 and 4.65; B and D: fluorescence was collected at 580 nm, pH values of pink bars and grey bars is 4.65 and 7.41. The concentration of interferences is 200 μM.

7. T_1-weighted MR imaging of single-sensitive dual-modal agents

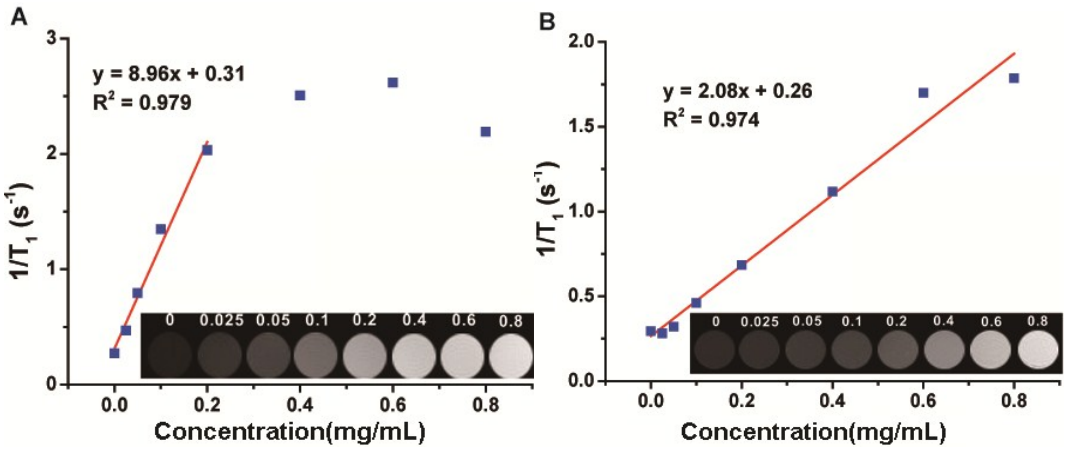


Figure S5. In vitro T_1-weighted MR imaging of A. **Au@Gd** and B. **Au@Gd&RGD** in PBS.

8. Cytotoxicity of all the dual-modal agents

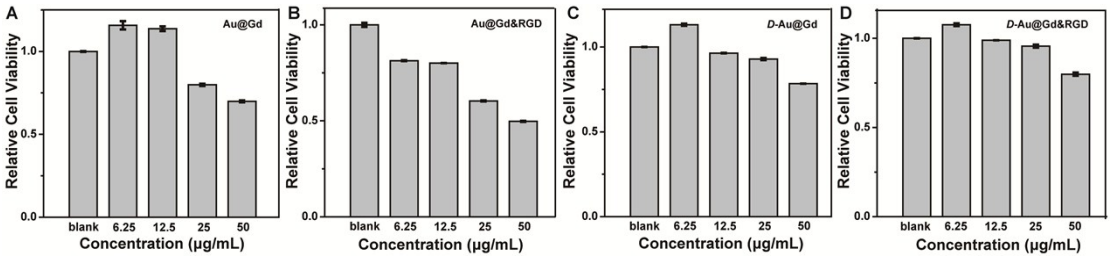


Figure S6. Cell viability by a standard MTS assay, the experiment was repeated three times and the data are shown as mean (±S.D.).

9. Confocal microscopy images of single-sensitive dual-modal nanovehicles

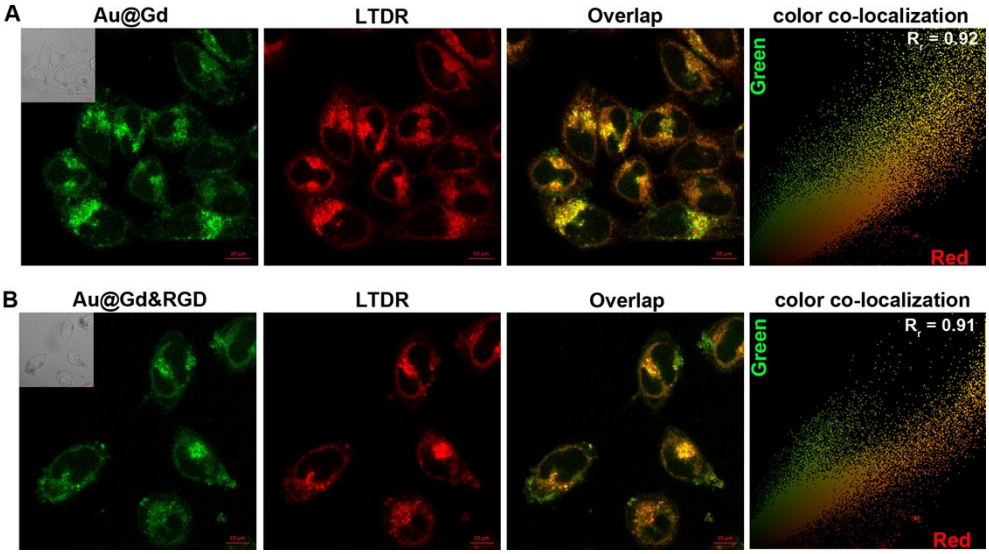


Figure S7. Intracellular distribution of A. **Au@Gd** and B. **Au@Gd&RGD** (50 μg/mL, incubated for 30 min). Confocal microscopy images of green channel: rhodamine unit, 570-620 nm, λ_{ex} 552 nm; red channel (commercial dyes): Lyso Tracker Deep red (LTDR, 1 μM), 650-720 nm, λ_{ex} 638 nm. The color co-localization shown the overlap rate of two different fluorescent dye in cells: the red spots represent signals in red channel and the green spots means signals in green channel. Scale bar: 10 μm.

10. References

[1] a) K. Yu, K. Li, H. Qin, Q. Zhou, C. Qian, Y. Liu and X. Yu, *ACS Appl. Mater. Interf.,* 2016, ***8***, 22839; b) Q. Liu, Q. Jiang, W. Yi, J. Zhang, X. Zhang, M. Wu, Y. Zhang, W. Zhu and X. Yu, *Bioorgan. Med. Chem.,* 2013**,** ***21***, 3105; c) J. Callahan, D. Ashton-Shue, H. G. Bryan, W. M. Bryan, G. D. Heckman, L. B. Kinter, J. E. McDonald, M. L. Moore and D. B. Schmidt, *J. Med. Chem.,* 1989, ***32***, 391;

[2] V. M. Chauhan, G. R. Burnett and J. W. Aylott, *Analyst*, 2011, ***136***, 1799;