Supporting Information

Expanding Nanoparticle Multifunctionality: Size-Selected Cargo Release and Multiple Logic Operations

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Materials and Chemicals

Iron(III) acetylacetonate ($Fe(acac)_3$, 97%), manganese(II) acetylacetonate ($Mn(acac)_2$, 21%−23% Mn), cobalt(II) acetylacetonate ($Co(acac)_2$, 97%), 1,2-dodecanediol (90%), oleic acid (90%), oleylamine (70%), benzyl ether (98%), hexane (98.5+%), chloroform (99.5+%), hexadecyltrimethylammonium bromide (CTAB, 99+%), tetraethyl orthosilicate (TEOS, 98%), (3-aminopropyl)triethoxysilane (APTS, 99%), 4,4'-azobis(4-cyanovaleric acid) (ACVA, 98+%), bisBenzimide H 33342 trihydrochloride (Hoechst 33342, 98+%), *p*-coumaric acid (CA, 98+%), ammonium nitrate (NH_4NO_3, 98+%), 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide (EDC, 98+%), *N*-hydroxysuccinimide (NHS, 98%), and 2-(*N*-morpholino)ethanesulfonic acid (MES, 99+%) were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl, 99+%), and *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS, 99+%) were purchased from CovaChem. Heptakis(6-amino-6-deoxy)-β-cyclodextrin (β-CD-NH_2, 97+%) was purchased from Zhiyuan Biotechnology (Shandong, China). 2,6-Dimethoxyaniline (97%), 3,5-dimethoxyaniline (98%), and sodium nitrite (98%) were purchased from Alfa Aesar. Dimethyl sulfoxide (DMSO, 99.9+%), sodium hydroxide (NaOH, 97+%), methanol (99.8+%), ethyl acetate (EA, 99%), hydrochloric acid (HCl, 36.5%-38%, tracemetal grade), and nitric acid (HNO_3, 67%-70%, tracemetal grade) were purchased from Fisher Scientific. Ethanol (200 proof) was purchased from Decon Laboratories, Inc. All chemicals were used without further purification.

Characterization

The size and morphology of nanoparticles were investigated by transmission electron microscopy (TEM, Tecnai T12) with an operating voltage of 120 kV. $MnFe_2O_4$, $MnFe_2O_4$@$CoFe_2O_4$, or Mag@MSN core@shell nanoparticles were dispersed in hexane or in ethanol at the concentration of 0.1 mg/mL. The suspension (10 μL) of the nanoparticles was dropped onto the carbon-coated copper grid and dried at room temperature. The field-dependent magnetization of $MnFe_2O_4$@$CoFe_2O_4$ was measured using a superconducting quantum interference device (SQUID) Quantum Design MPMS7 magnetometer at 300 K. The iron/manganese/cobalt ratio of the $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles was quantitatively determined by ICP-OES using a Shimadzu ICPE-9000 instrument. Typically, 1 mg of $MnFe_2O_4$@$CoFe_2O_4$ powder was dissolved in 0.5 mL of hydrochloric acid solution. The solution was diluted with 2 % HNO_3 for quantitative measurement. The calibration curves for iron, manganese, and cobalt were created from 0 ppm to 10 ppm. The surface area, pore volume, and pore size of Mag@MSN core@shell nanoparticles were determined by N_2 adsorption-

desorption isotherm measurements at 77 K (Autosorb-iQ, Quantachrome Instruments). Mag@MSNs-ACVA core@shell was degassed at 110 °C for 12 h before the measurement. The surface area and pore size distribution of Mag@MSN core@shell were determined by Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH) methods. The functional groups on the surface of Mag@MSN core@shell were characterized by Fourier transform infrared spectroscopy (FTIR, JASCO FT/IR-420) spectrometer in the range of 4000–400 cm^{-1}. Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer Pyris Diamond TG/DTA machine under air flow. About 5 mg of Mag@MSN core@shell were loaded in aluminum pans and the data were recorded from 50 °C to 600 °C at a scan rate of 10 °C min^{-1}. An empty aluminum pan was used as the reference. The loading capacities and release efficiencies of Hoechst or CA were determined by fluorescence spectroscopy by Acton Spectra Pro 2300i CCD cooled with liquid nitrogen. Hoechst or CA were excited by a CUBE 375-16C laser (Coherent Inc.) at a wavelength of 376 nm and a power of 2 mW. The fluorescence intensities of Hoechst or CA released from the nanoparticles was integrated from 495 to 505 nm or 420 to 430 nm, respectively.

Synthesis of Magnetic $MnFe_2O_4$@$CoFe_2O_4$ Nanoparticles

Synthesis of 6.4 ± 0.8 nm $MnFe_2O_4$ nanoparticles: $MnFe_2O_4$ nanoparticles were synthesized following a previously reported method with a slight modification.[1–4] $Fe(acac)_3$ (2 mmol), $Mn(acac)_2$ (1 mmol), 1,2-dodecanediol (10 mmol), oleic acid (6 mmol), and oleylamine (6 mmol) were dissolved in benzyl ether (20 mL) in a three-neck flask. The reaction was brought to 200 °C with vigorously stirring under the flow of nitrogen and kept at 200 °C for 1 h. The reaction mixture was then heated up to 298 °C and refluxed for 1 h. Then, the resulting solution containing $MnFe_2O_4$ nanoparticles was cooled to room temperature (21 °C). The nanoparticles were precipitated by adding ethanol (80 mL) and further separated by centrifugation (7830 rpm, 7197 g, 20 min). Finally, $MnFe_2O_4$ nanoparticles were dispersed in hexane (10 mL) with oleic acid (50 μL) and oleylamine (50 μL).

Synthesis of 7.9 ± 0.8 nm $MnFe_2O_4$ nanoparticles: The larger $MnFe_2O_4$ nanoparticles were synthesized by growing $MnFe_2O_4$ on the 6.4 ± 0.8 nm $MnFe_2O_4$ nanoparticles prepared previously. In general, $Fe(acac)_3$ (2 mmol), $Mn(acac)_2$ (1 mmol), 1,2-dodecanediol (10 mmol), oleic acid (2 mmol), and oleylamine (2 mmol) were dissolved in benzyl ether (20 mL) in a 100 mL three-neck flask. The $MnFe_2O_4$ nanoparticles (6.4 ± 0.8 nm, 90 mg) obtained previously in hexane (10 mL) were added to the reaction mixture. The reaction mixture was brought to 90 °C and kept at that temperature for 30 min to evaporate hexane. Afterwards, the reaction mixture was heated to 200 °C with vigorously stirring under the flow of nitrogen. After 1 h, the reaction

mixture was heated to 298 °C and refluxed at this temperature for 1 h. Afterwards, the reaction solution was cooled to room temperature (21 °C). The nanoparticles were then precipitated by adding ethanol (80 mL) and further separated by centrifugation (7830 rpm, 7197 g, 20 min). Finally, the 7.9 ± 0.8 nm $MnFe_2O_4$ nanoparticles were re-dispersed in hexane (10 mL) with oleic acid (50 μL) and oleylamine (50 μL).

Synthesis of 9.3 ± 0.8 nm $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles: To coat $CoFe_2O_4$ on the surface of 7.9 ± 0.8 nm $MnFe_2O_4$ nanoparticles, $Fe(acac)_3$ (2 mmol), $Co(acac)_2$ (1 mmol), 1,2-dodecanediol (10 mmol), oleic acid (2 mmol), and oleylamine (2 mmol) were dissolved in benzyl ether (20 mL) in a 100 mL three-neck flask. $MnFe_2O_4$ nanoparticles (7.9 ± 0.8 nm, 180 mg) obtained previously in hexane (10 mL) were added to the reaction mixture. The synthetic procedures and reaction temperature were the same as that of the 7.9 ± 0.8 nm $MnFe_2O_4$ nanoparticles synthesis. Finally, $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles (9.3 ± 0.8 nm) were re-dispersed in hexane (10 mL) with oleic acid (50 μL) and oleylamine (50 μL).

Synthesis of 10.5 ± 0.9 nm $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles: To coat $CoFe_2O_4$ on the surface of $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles (9.3 ± 0.8 nm) to obtain the larger nanoparticles, $Fe(acac)_3$ (2 mmol), $Co(acac)_2$ (1 mmol), 1,2-dodecanediol (10 mmol), oleic acid (2 mmol), and oleylamine (2 mmol) were dissolved in benzyl ether (20 mL) in a 100 mL three-neck flask. $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles (9.3 ± 0.8 nm, 270 mg) obtained previously in hexane (10 mL) were added to the reaction mixture. The synthetic procedures and reaction temperature were the same as that of 9.3 ± 0.8 nm $MnFe_2O_4$@$CoFe_2O_4$ nanoparticle synthesis. Finally, $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles (10.5 ± 0.9 nm) were re-dispersed in hexane (10 mL) with oleic acid (50 μL) and oleylamine (50 μL) for further use.

Synthesis of APTS-functionalized $MnFe_2O_4$@$CoFe_2O_4$@mesoporous Silica (Mag@MSNs-APTS) Core@shell Nanoparticles

Before surface coating of mesoporous silica, the synthesized $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles were phase transferred to be well dispersed in aqueous solution. Generally, the $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles (10.5 ± 0.9 nm, 2.5 mg) were first dispersed in chloroform (0.2 mL). CTAB aqueous solution (40 mg of CTAB dissolved in 2 mL of D.I. water, 54 mM) was then added to the chloroform solution containing $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles. The mixture was sonicated and vigorously stirred for 10 min with a fully sealed cover to generate oil-in-water emulsion. The emulsion was then sonicated for 1 h to evaporate chloroform. The clear and well-dispersed $MnFe_2O_4$@$CoFe_2O_4$ colloidal aqueous solution (2 mL) was obtained and designated as $MnFe_2O_4$@$CoFe_2O_4$@CTAB. Meanwhile, CTAB (40 mg) was dissolved in D.I. water (18 mL) with NaOH solution (120 μL, 2 M) in a 100 mL flask. The previous made

$MnFe_2O_4$@$CoFe_2O_4$@CTAB colloidal aqueous solution (2 mL) was added to the reaction solution with vigorously stirring, and the temperature of the solution was brought to 70 °C. To coat mesoporous silica shell on the surface of $MnFe_2O_4$@$CoFe_2O_4$@CTAB, TEOS (200 μL) and ethyl acetate (1.2 mL) were added dropwise into the solution. After stirring for 2 h, (3-aminopropyl)triethoxysilane (APTS, 40 μL) was added dropwise into the solution and stirred for another 2 h to generate APTS functionalized $MnFe_2O_4$@$CoFe_2O_4$@MSNs (Mag@MSNs-APTS, Mag denotes magnetic). Afterwards, the solution was cooled to room temperature (21 °C). The Mag@MSNs-APTS was centrifuged (14000 rpm, 16873 g, 20 min) and washed 3 times with ethanol (20 mL × 3). Subsequently, the Mag@MSNs-APTS was dispersed in ethanol (20 mL) containing NH_4NO_3 (120 mg) and the reaction was stirred at 60 °C for 1 h to remove the CTAB surfactants. The surfactant removal procedures were repeated twice and the Mag@MSNs-APTS was washed 2 times with D.I. water (20 mL × 2) and ethanol (20 mL × 2), respectively, to obtain the surfactant-free Mag@MSNs-APTS. Finally, the Mag@MSNs-APTS was dispersed in ethanol (5 mL) for further use.

Synthesis of ACVA-functionalized Mag@MSNs-APTS (Mag@MSNs-ACVA)

The conjugation of thermo-responsive 4,4′-azobis(4-cyanovaleric acid) (ACVA) on the surface of Mag@MSNs-APTS was carried out by using an amide bond coupling reaction. At first, the carboxylic acid of ACVA (20 mg) was activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 40 mg) and *N*-hydroxysuccinimide (NHS, 20 mg) in DMSO (4 mL). To crosslink the activated ACVA to the primary amine of APTS, after 30 min activation at room temperature (21 °C), Mag@MSNs-APTS (20 mg) dispersed in DMSO (4 mL) was added dropwise to the activated ACVA in DMSO and stirred for 24 h. The ACVA functionalized Mag@MSNs-APTS (Mag@MSNs-ACVA) was washed, centrifuged (14000 rpm, 16873 g, 20 min), and re-suspended in DMSO 3 times (10 mL × 3) to remove the excess ACVA, EDC, and NHS.

Synthesis of β-CD-NH_2-functionalized Mag@MSNs-ACVA (Mag@MSNs-CD)

The conjugation of heptakis(6-amino-6-deoxy)-β-cyclodextrin (β-CD-NH_2) on the surface of Mag@MSNs-ACVA was carried out through amide bond formation between the carboxylic acid of ACVA and the primary amine of β-CD-NH_2. Typically, the carboxylic acid of Mag@MSNs-ACVA (1.5 mg) were activated by EDC-HCl (3 mg) and sulfo-NHS (2 mg) in MES buffer (0.5 mL, 100 mM, pH = 6.0) for 30 min at room temperature (21 °C). To crosslink ACVA to β-CD-NH_2, β-CD-NH_2 (10 mg) dissolved in MES buffer (1 mL, 100 mM, pH = 6.0) was added dropwise to the activated Mag@MSNs-ACVA in MES buffer and stirred for 24 h at room temperature (21 °C). Finally, β-CD-NH_2-functionalized Mag@MSNs-ACVA

(Mag@MSNs-CD) was washed with 100 mM MES buffer (pH = 6.0) 4 times (1 mL × 4), D.I. water 2 times (1 mL × 2), and ethanol 1 time (1 mL × 1) to remove the excess β-CD-NH_2, EDC-HCl, and sulfo-NHS. The Mag@MSNs-CD was finally dispersed in 1 mL of ethanol for further use.

Loading of Hoechst in Mag@MSNs-ACVA and Capping by β-CD-NH_2 (Mag@MSNs-CD)

The loading of Hoechst was carried out by using MES buffer as the solvent. In general, Mag@MSNs-ACVA (1.5 mg) was dispersed in MES buffer (0.5 mL, 100 mM, pH = 6.0) with 3 mM Hoechst. After stirring for 24 h, EDC-HCl (3 mg) at room temperature (21 °C), sulfo-NHS (2 mg) were added to activate the carboxylic acid of ACVA and the reaction was stirred for 30 min. Then, β-CD-NH_2 (10 mg) dissolved in MES buffer (1 mL, 100 mM, pH = 6.0) was added dropwise to the solution to crosslink β-CD-NH_2 and ACVA to seal the pores and prevent Hoechst from leakage. After mixing for 24 h at room temperature (21 °C), Hoechst-loaded Mag@MSNs-CD was centrifuged (8000 rpm, 5510 g, 5 min) and washed with MES buffer (100 mM, pH = 6.0) 4 times (1 mL × 4), D.I. water 2 times (1 mL × 2), and PBS 1 time (1 mL × 1) to remove the free Hoechst, β-CD-NH_2, EDC-HCl, and sulfo-NHS. The final product was suspended in PBS (1.5 mL) for the water bath heating or magnetic heating triggered release experiments.

Loading Capacity Analysis of Hoechst and CA

For Hoechst, after being loaded with 3 mM Hoechst and capped with β-CD-NH_2, 1.5 mg of Hoechst-loaded Mag@MSNs-CD were washed with MES buffer (100 mM, pH = 6.0) 4 times (1 mL × 4), D.I. water 2 times (1 mL × 2), and PBS 1 time (1 mL × 1). Afterwards, Hoechst-loaded Mag@MSNs-CD in PBS (1 mL) was heated to 80 °C and the solution was kept at this temperature for 30 min to allow Hoechst to release from Mag@MSNs-CD. For CA, after being loaded with 2 mM CA and being plugged with mAzo, 1.5 mg of CA-loaded Mag@MSNs-CD-mAzo were washed with D.I. water 8 times (1 mL × 8) and PBS 1 time (1 mL × 1). Then, CA-loaded Mag@MSNs-CD-mAzo in PBS (1 mL) was heated to 80 °C and the solution was kept at this temperature for 30 min to allow CA to release from Mag@MSNs-CD-mAzo. The amount of released Hoechst or CA in the supernatant was separated from the nanoparticles by centrifugation (14000 rpm, 16873 g, 10 min) and measured by fluorescence spectroscopy. The loading capacity of Hoechst or CA was calculated using the integrated intensities from 495 to 505 nm (for Hoechst) or 420 to 430 nm (for CA), respectively, its calibration curve, and the following definition of loading capacity: (mass of loaded Hoechst or CA / mass of particles) × 100%.

Zeta Potential Value Measurement of Mag@MSNs-ACVA after Bulk or Magnetic Heating

Mag@MSNs-ACVA (0.5 mg) was dispersed in D.I. water (1 mL) in an Eppendorf tube. For bulk heating treatment, the Eppendorf tubes containing the samples were put in a hot water bath at 37, 60, or 80 °C for 10 min. Afterwards, the solution was centrifuged (8000 rpm, 5510 g, 5 min) and the nanoparticles were washed and re-dispersed in D.I. water (1 mL).

For magnetic heating treatment, the Eppendorf tube containing Mag@MSNs-ACVA (0.5 mg) in D.I. water (1 mL) was exposed to an AMF at a power of 5 kW and a frequency of 375 kHz for 10 min. Similarly, the treated solution was centrifuged (8000 rpm, 5510 g, 5 min) and the nanoparticles were washed and re-dispersed in D.I. water (1 mL). The zeta potential values of the samples after bulk heating or magnetic heating were measured by a laser particle analyzer LPA-3100 at the concentration of 50 μg mL^{-1} at room temperature (21 °C).

Triggered Release of Hoechst from Mag@MSNs-CD by AMF

Hoechst-loaded Mag@MSNs-CD (0.5 mg) was dispersed in PBS (1.5 mL) in an Eppendorf tube. Before being triggered by magnetic heating, the Hoechst-loaded nanoparticle solution was centrifuged (8000 rpm, 5510 g, 5 min) and the supernatant (40 μL) was collected every 20 min over a period of 60 min at room temperature (21 °C). The collected supernatants were then measured by fluorescence spectroscopy to evaluate the release amount of Hoechst. After the monitoring process, the sample-containing tube was exposed to an alternating magnetic field (AMF) in the center of a water-cooled five-turn copper coil (5 cm height and diameter, MSI automation) at a power of 5 kW and a frequency of 375 kHz for 5, 10, and 30 min, respectively, followed by the same Hoechst release measurement. The release of Hoechst was recorded every 20 min over a period of 120 min followed by every 30 min over a period of 120 min until the release leveled off. The intensities of the Hoechst released from Mag@MSNs-CD was integrated from 495 nm to 505 nm.

Triggered Release of Hoechst from Mag@MSNs-CD by Bulk Heating

Hoechst-loaded Mag@MSNs-CD (0.5 mg) was dispersed in PBS (1.5 mL) in an Eppendorf tube. Before immersing the Hoechst-loaded nanoparticle-containing tube in a hot water bath, the nanoparticle solution was centrifuged (8000 rpm, 5510 g, 5 min) and supernatant (40 μL) was collected every 20 min over a period of 60 min at room temperature (21 °C). The collected supernatants were then measured by fluorescence spectroscopy to evaluate the release amount of Hoechst. Afterwards, the Hoechst-loaded nanoparticle-containing tubes were immersed in the hot water bath (37 or 50 °C) for 10 min per heating cycle. After each heating cycle, the solution was centrifuged (8000 rpm, 5510 g, 5 min) and the supernatant (40 μL) was

collected and measured by fluorescence spectroscopy. A total of 9 heating cycles were performed. As the control, the release amount of Hoechst from the sample stayed at room temperature (21 °C) was also evaluated.

Synthesis of Red Light-responsive Tetra-*ortho*-methoxy-substituted Azobenzene (mAzo) Plug

The red light-responsive tetra-*ortho*-methoxy-substituted azobenzene (mAo) was synthesized following a previously reported method with slight modification.[5,6] mAzo was synthesized by coupling 2,6-dimethoxyaniline and 3,5-dimethoxyaniline. 2,6-dimethoxyaniline (0.46 g, 3.0 mmol, 1 equiv.) was dissolved in a mixture of D.I. water (0.6 mL) and HCl (0.7 mL, 37 wt.%). The solution was cooled to 0-5 °C in an ice water bath followed by slowly adding D.I. water (2 mL) containing $NaNO_2$ (0.20 g, 3.0 mmol, 1 equiv.). The reaction solution was vigorously stirred for 30 min in an ice water bath. Afterward, the solution containing the diazonium salt was slowly added to a mixture of D.I. H_2O (20 mL) and 3,5-dimethoxyaniline (0.46 g, 3.0 mmol, 1 equiv.) in an ice water bath. The pH value of the solution was subsequently adjusted between 8 to 9 by using saturated sodium carbonate solution. The reaction solution was stirred for another 16 h. The red solid was collected and simply purified by column chromatography (1:1 methanol/ethyl acetate). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (t, J = 8.4 Hz, 1H; Ar-H), 6.71 (d, J = 8.4 Hz, 2H; Ar-H), 3.67 (s; O-CH_3). ESI-MS *m/z* 318.1118 for $[M+H]^+$, calcd for $C_{16}H_{19}N_3O_4$ M 317.14.

UV-Vis Spectra of Mag@MSNs-CD and mAzo-plugged Mag@MSNs-CD (Mag@MSNs-CD-mAzo)

The formation of supramolecular complex by mAzo and CD on the surface of the nanoparticles was validated by UV-Vis spectroscopy. Mag@MSNs-CD (1 mg) was dispersed in D.I. water (1 mL) in an Eppendorf tube, followed by adding 1 mL of water-dissolved mAzo (1 mg) to the solution. The tube was wrapped with aluminum foil to be protected from light. The sample was designated as Mag@MSNs-CD-mAzo. After mixing for 48 h at room temperature (21 °C), the Mag@MSNs-CD-mAzo was centrifuged (8000 rpm, 5510 g, 5 min) and washed with D.I. water 8 times (1 mL × 8) and PBS 1 time (1 mL × 1) to remove unplugged mAzo. Finally, Mag@MSNs-CD or Mag@MSNs-CD-mAzo was dispersed in D.I. water (1 mL) for UV-Vis spectroscopy measurements.

Loading of CA in Mag@MSNs-CD and Capping by mAzo

Mag@MSNs-CD (1 mg) was dispersed in D.I. water (1 mL) with 2 mM CA and stirred for 24 h at room temperature (21 °C). Then, mAzo (1 mg) dissolved in D.I. water (1 mL) was added to the solution and mixed for 48 h at room temperature (21 °C), to form a stable

supramolecular complex with CD to prevent CA from leakage. The resulting sample was designated as CA-loaded Mag@MSNs-CD-mAzo. Afterward, the CA-loaded Mag@MSNs-CD-mAzo was centrifuged (8000 rpm, 5510 g, 5 min) and washed with D.I. water 8 times (1 mL × 8) and PBS 1 time (1 mL × 1), to remove unloaded CA and mAzo. The final product was dispersed in D.I. water (2 mL) for red light triggered release experiments.

Triggered Release of CA from Mag@MSNs-CD-mAzo by a Red-light Illumination

CA-loaded Mag@MSNs-CD-mAzo (0.5 mg) was dispersed in PBS (1.5 mL) in an Eppendorf tube. Before being triggered by a red light, the CA-loaded Mag@MSNs-CD-mAzo solution was centrifuged and the supernatant (40 μL) was collected at room temperature (21 °C) every 30 min over a period of 90 min. The collected supernatants were measured by fluorescence spectroscopy. After the monitoring process, the sample was exposed to red-light LED illumination (625 nm, 785 mW cm^{-2}) for 10, 20, or 30 min, followed by monitoring the release of CA as mentioned above. The release of CA was recorded every 20 min over a period of 160 min. The intensities of CA released from nanoparticles were integrated from 420 nm to 430 nm.

Triggered Release of CA from Mag@MSNs-CD-mAzo by Bulk Heating

CA-loaded Mag@MSNs-CD-mAzo (0.5 mg) was dispersed in PBS (1.5 mL) in an Eppendorf tube. Before immersing the CA-loaded nanoparticle-containing tube in a hot water bath, the nanoparticle solution was centrifuged (8000 rpm, 5510 g, 5 min) and supernatant (120 μL) was collected at room temperature (21 °C) for fluorescence spectroscopy measurements. Afterwards, the samples were heated in the 37, 50, or 80 °C water baths for 90 min. The solution was centrifuged (14000 rpm, 10 min) and the supernatant (120 μL) was collected and measured by fluorescence spectroscopy. As the control, the release amount of CA from the sample stayed at room temperature (21 °C) was also evaluated.

Release Efficiency Analysis of Hoechst or CA

To determine the release efficiency of Hoechst or CA, the Eppendorf tubes containing Hoechst-loaded Mag@MSNs-CD (0.5 mg) or CA-loaded Mag@MSNs-CD-mAzo (0.5 mg) dispersed in D.I. water (1 mL) was immersed in a hot water bath at 80 °C for 1 h. The released Hoechst or CA was separated from the nanoparticles by centrifugation (14000 rpm, 16873 g, 10 min) and recorded by fluorescence spectroscopy. The intensities of the released Hoechst or CA were integrated from 495 nm to 505 nm or 420 nm to 430 nm, respectively, with 100% release set for the amount released by bulk heating (80 °C for 1 h). The release efficiency of Hoechst or CA is defined as (mass of released cargo/mass of loaded cargo) × 100%.

Successive Loading of Size-selected Cargos (Hoechst and CA) in Mag@MSNs-CD-mAzo

The successive loading of size-selected cargos (Hoechst and CA) was carried out as follows: first do Hoechst loading in Mag@MSNs-ACVA followed by CD capping; CA was next loaded in Hoechst-loaded Mag@MSNs-CD followed by mAzo plugging.

The loading of Hoechst in Mag@MSNs-ACVA followed by the capping by CD were followed the same procedures in the "Loading of Hoechst in Mag@MSNs-ACVA and capping by cyclodextrin (Mag@MSNs-CD)" section. Next, the Hoechst-loaded Mag@MSNs-CD (1 mg) was dispersed in D.I. water (1 mL) with 2 mM CA and stirred for 24 h at room temperature (21 °C). mAzo (1 mg) in D.I. water (1 mL) was then added to the solution and mixed for 48 h at room temperature (21 °C) to prevent CA from leakage. Afterward, the Hoechst- and CA-loaded Mag@MSNs-CD-mAzo (designated as Hoechst/CA-Mag@MSNs-CD-mAzo) was centrifuged (8000 rpm, 5510 g, 5 min) and washed with D.I. water 8 times (1 mL × 8) and PBS 1 time (1 mL × 1) to remove unloaded CA and mAzo. The resulting nanoparticles were dispersed in PBS (2 mL) for dual physical stimuli (red light and AMF)-triggered release experiments.

Successive Release of Size-selected Cargos (Hoechst and CA) from Mag@MSNs-CD-mAzo Triggered by Physical Stimuli (Red Light and AMF)

The successive release of size-selected cargos (Hoechst and CA) was achieved by first releasing CA with the trigger of red-light LED illumination, followed by releasing Hoechst with the exposure to an AMF. Hoechst/CA-Mag@MSNs-CD-mAzo (1 mg) was dispersed in PBS (2 mL) in an Eppendorf tube. Before the red-light trigger, the solution containing Hoechst/CA-Mag@MSNs-CD-mAzo was centrifuged and the supernatant (40 μL) was collected every 30 min over a period of 90 min at room temperature (21 °C) and measured by fluorescence spectroscopy. Afterward, the sample was exposed to the red-light LED illumination (625 nm, 785 mW cm^{-2}) for 30 min, followed by monitoring the release of CA. After that, the release of CA was recorded every 20 min over a period of 160 min. The intensities of CA released from nanoparticles were integrated from 420 nm to 430 nm.

Before releasing Hoechst by the AMF trigger, to avoid the interference from the fluorescence of the released CA, the red light-triggered nanoparticles solution was centrifuged (8000 rpm, 5510 g, 5 min) and the supernatant was replaced by a fresh PBS solution (1.5 mL). Then, the red light-triggered Hoechst-loaded nanoparticle solution was centrifuged (8000 rpm, 5510 g, 5 min) and the supernatant (40 μL) was collected every 20 min over a period of 60 min at room temperature (21 °C) and measured by fluorescence spectroscopy. Afterward, the sample was exposed to an AMF at a power of 5 kW and a frequency of 375 kHz for 30 min followed by monitoring the release of Hoechst. The release of Hoechst was recorded every 20

min over a period of 120 min followed by every 30 min over a period of 120 min until the release of Hoechst leveled off. The intensities of the Hoechst released from Mag@MSNs-CD was integrated from 495 nm to 505 nm.

Supporting Figures

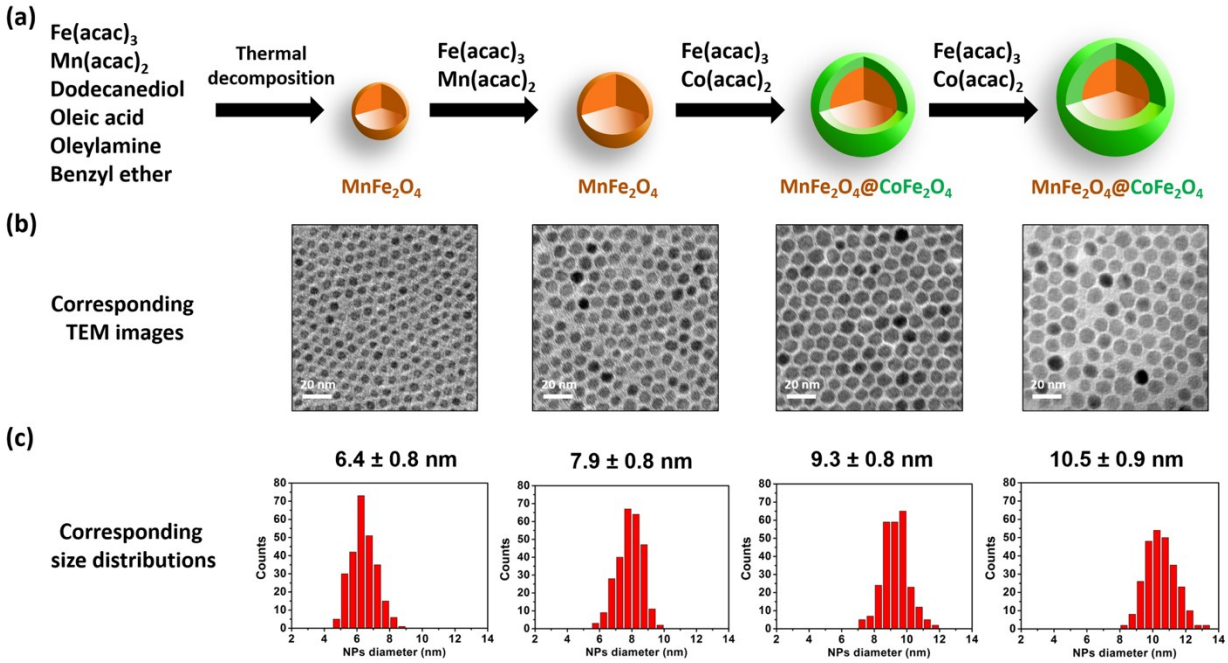

Fig. S1 (a) The synthetic protocol of $MnFe_2O_4$@$CoFe_2O_4$ obtained by a thermal decomposition method. (b) TEM images and (c) nanoparticle size distributions of $MnFe_2O_4$, and $MnFe_2O_4$@$CoFe_2O_4$ nanoparticles.

The calculation of specific loss power of $MnFe_2O_4$@$CoFe_2O_4$ and Fe_3O_4 nanoparticles is by using equations (1), where C is the volumetric heat capacity of toluene, V_s is the sample volume, m is the mass of $MnFe_2O_4$@$CoFe_2O_4$ or Fe_3O_4 nanoparticles, and dT/dt is the initial slope of the time-dependent temperature increase curves.

For $MnFe_2O_4$@$CoFe_2O_4$:

$$\text{Specific loss power} = \frac{CV_s \mathrm{d}T}{m\ \mathrm{d}t} \quad (1)$$

$$= \frac{1.504\ \left(J\,mL^{-1}K^{-1}\right) \times 1\ (mL)}{1 \times 10^{-3}\ (g)} \times \frac{1\ (K)}{1\ (s)}$$

$$= 1504\ W\,g^{-1}_{(Fe + Mn + Co)}$$

For Fe_3O_4:

$$\text{Specific loss power} = \frac{CV_s \mathrm{d}T}{m\ \mathrm{d}t} \quad (1)$$

$$= \frac{1.504\ \left(J\,mL^{-1}K^{-1}\right) \times 1\ (mL)}{1 \times 10^{-3}\ (g)} \times \frac{1\ (K)}{5\ (s)}$$

$$= 301\ W\,g^{-1}_{(Fe)}$$

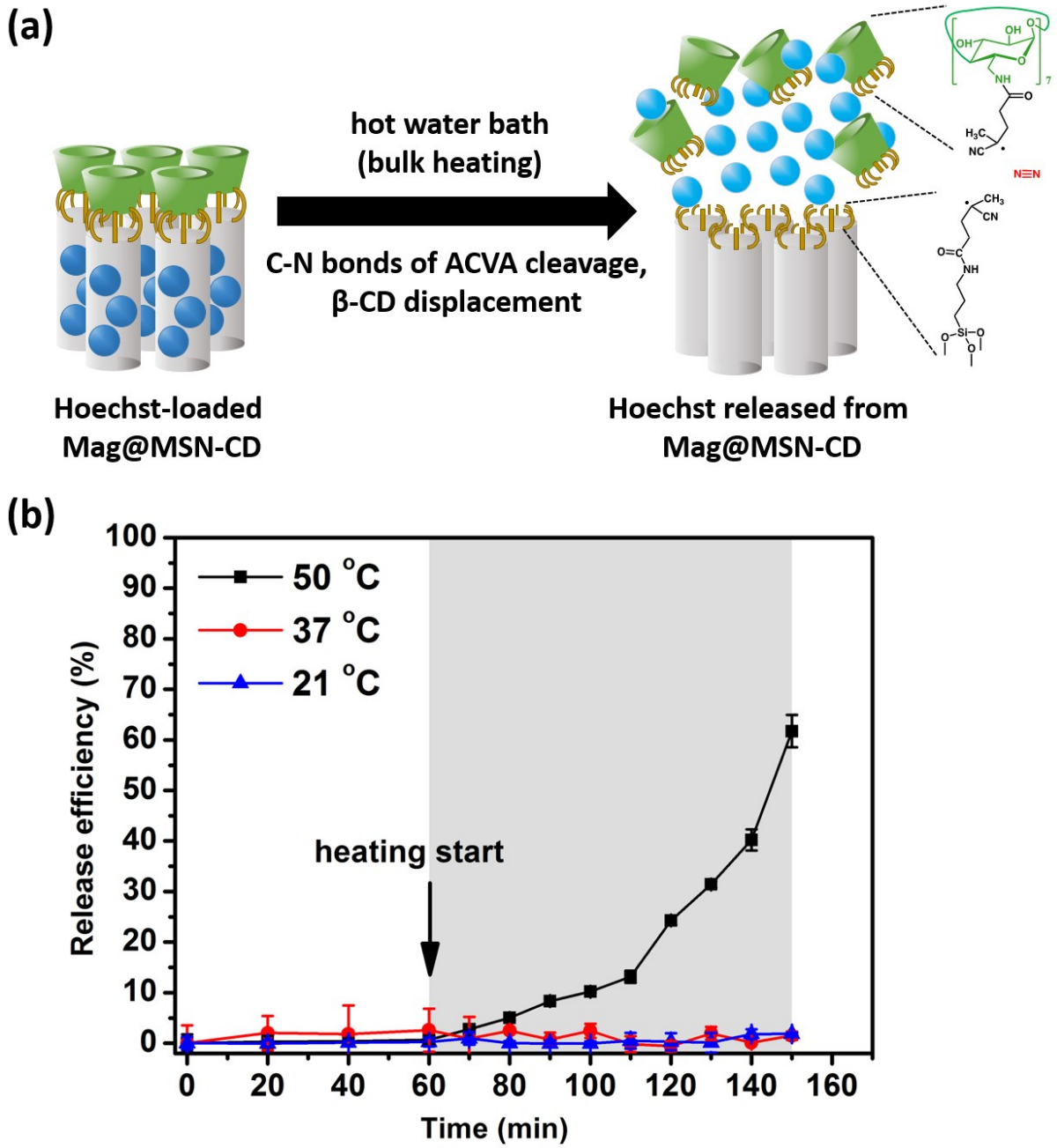

Fig. S2 (a) Release of Hoechst from Mag@MSN-CD caused by bulk heating trigger in a water bath at 37, or 50 °C. The sample stayed at room temperature (21 °C) was also recorded as a control. (b) Time dependent release efficiency of Hoechst from Mag@MSN-CD caused by bulk heating trigger in water bath at 21 °C, 37 °C, and 50 °C.

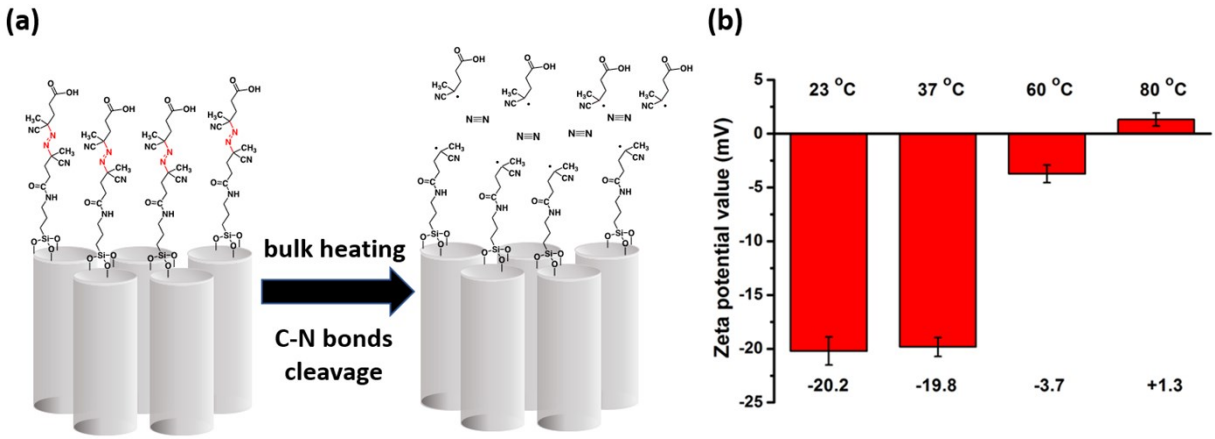

Fig. S3 (a) Cleavage of C-N bonds of Mag@MSNs-ACVA triggered by 10 min of water bath heating. (b) Zeta potential values of Mag@MSNs-ACVA before and after 10 min of water bath heating at 37, 60, and 80 °C in deionized water. The sample stayed at 23 °C served as a control. The concentration of Mag@MSNs-ACVA in D.I. water is 0.5 mg mL^{-1}.

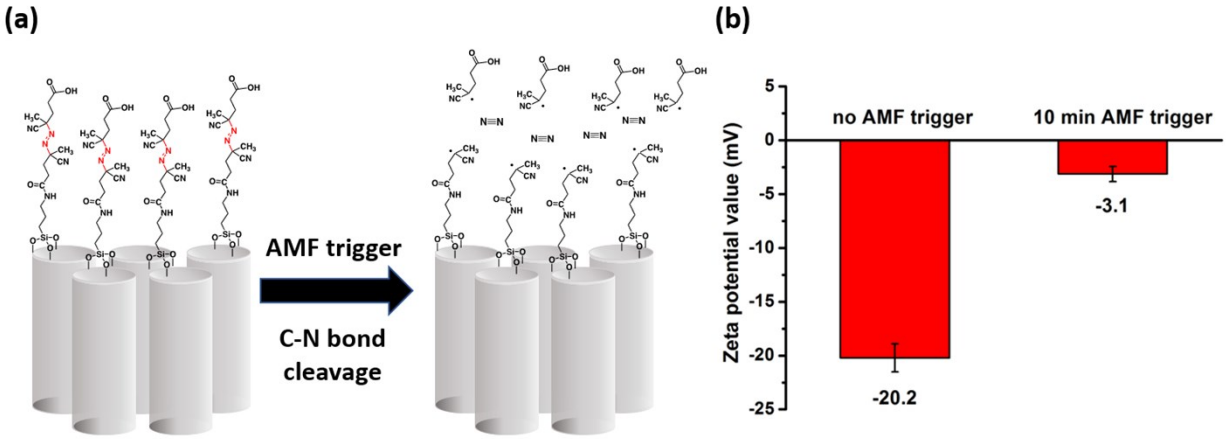

Fig. S4 (a) Cleavage of C-N bonds of Mag@MSNs-ACVA triggered by 10 min of AMF exposure. (b) Zeta potential values of Mag@MSNs-ACVA before and after 10 min of AMF trigger in D.I. water. The concentration of Mag@MSNs-ACVA in deionized water is 0.5 mg mL^{-1}.

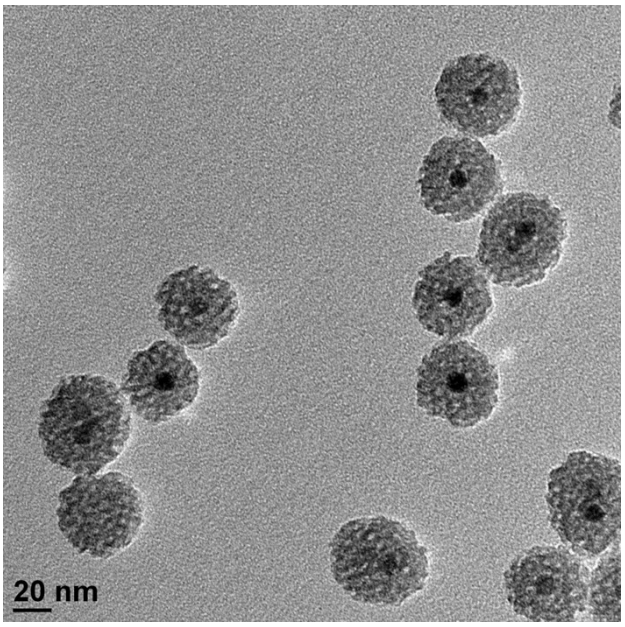

Fig. S5 TEM image of Hoechst-loaded Mag@MSN-CD after 10 min of AMF exposure.

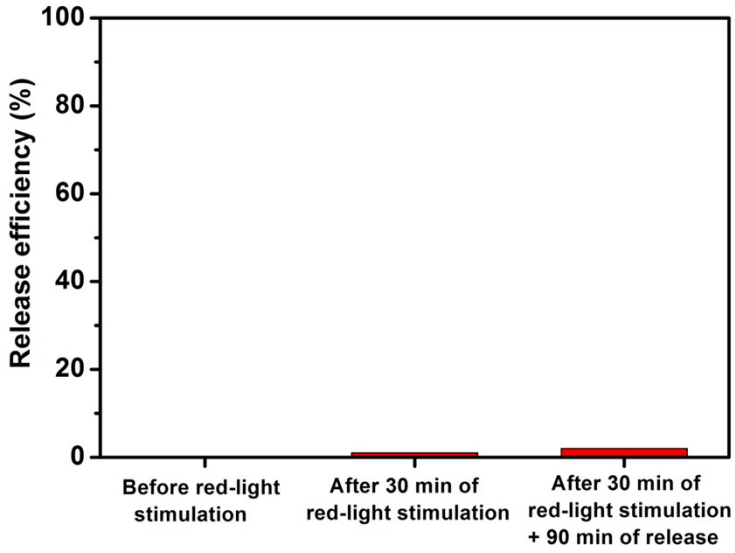

Fig. S6 Release efficiency of Hoechst from Mag@MSNs-CD stimulated by a red light (625 nm, 785 mW cm^{-2}) for 30 min.

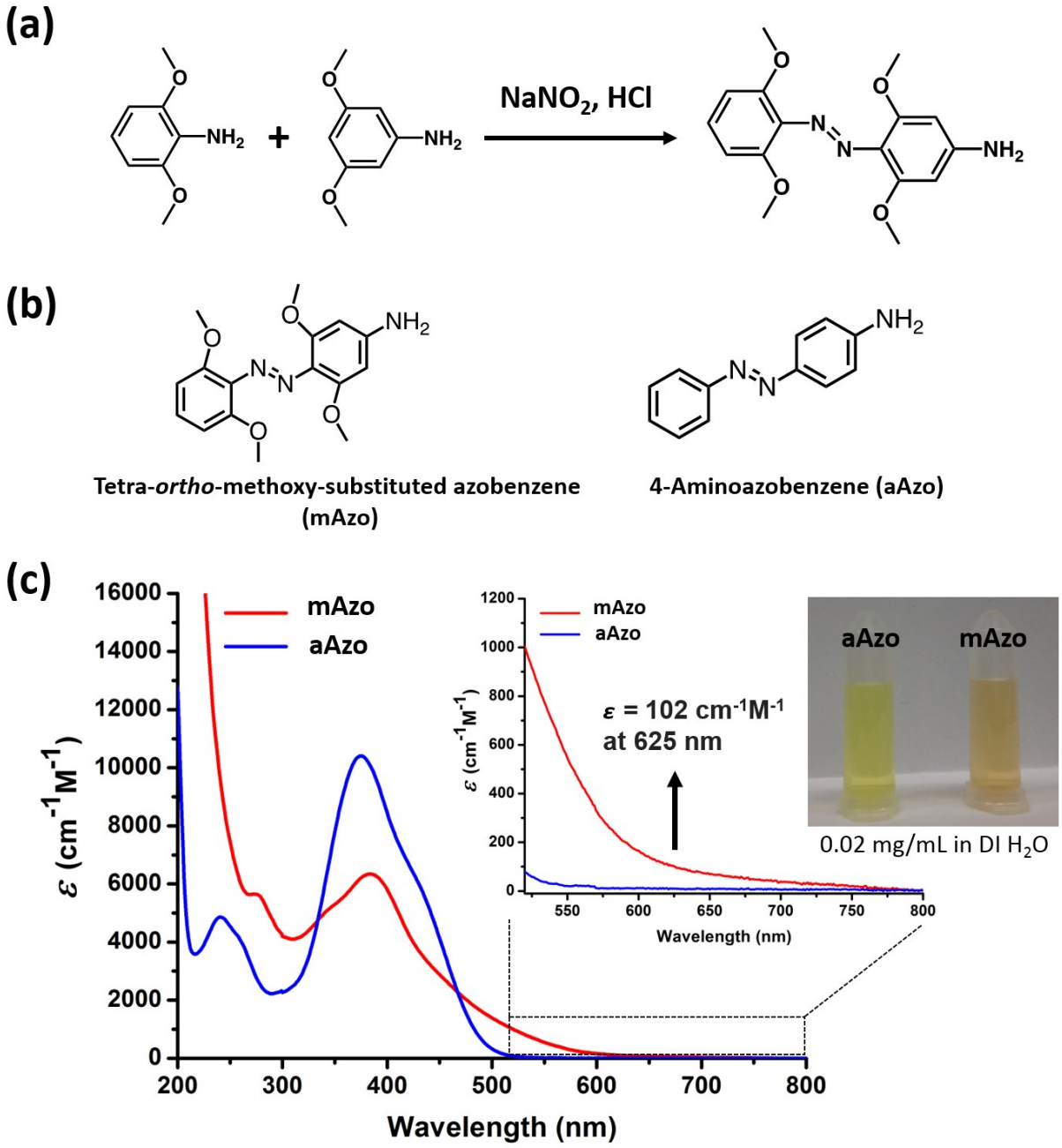

Fig. S7 (a) The synthetic reaction of mAzo. (b) Molecular structures of tetra-*ortho*-methoxy-substituted azobenzene (mAzo) and 4-aminoazobenzene (aAzo). (c) UV-Vis spectra of mAzo and aAzo dissolved in D.I. H_2O. Inset shows the enlarged spectra and the photo of mAzo and aAzo dissolved in D.I. H_2O.

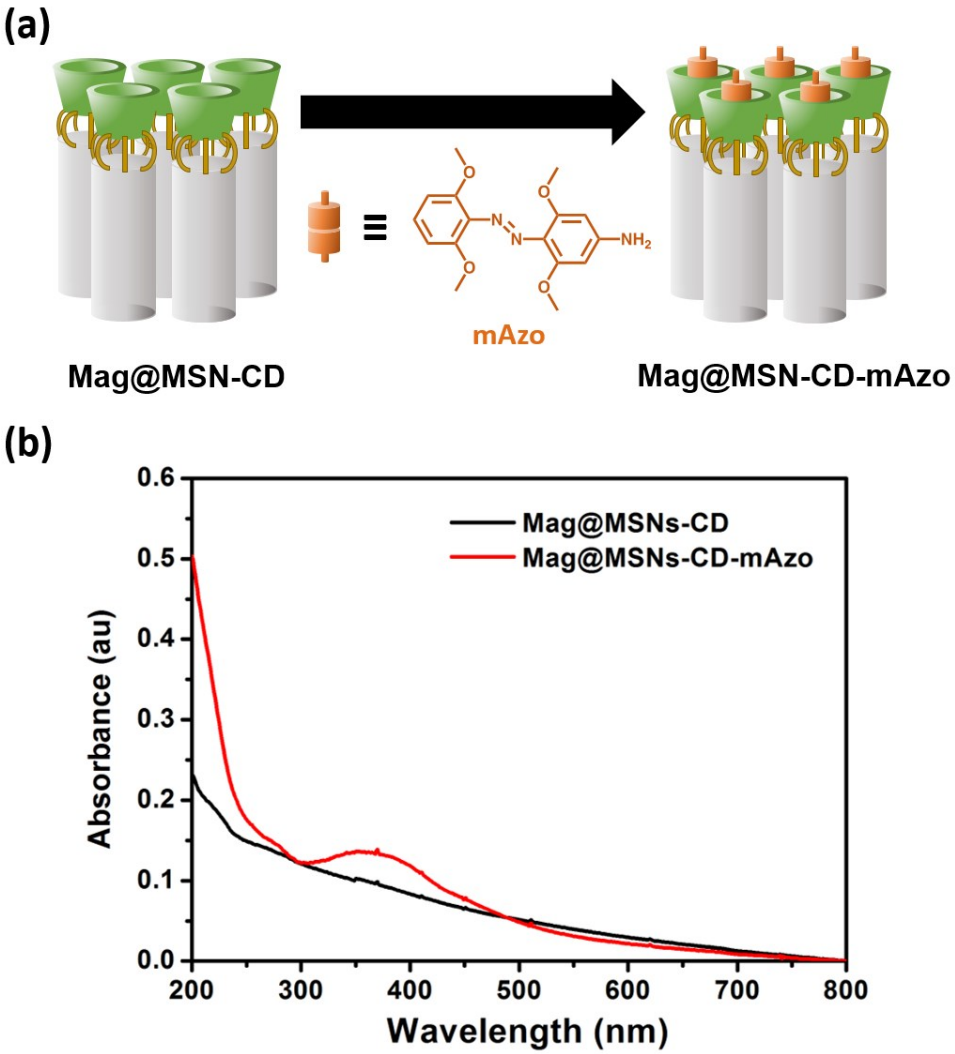

Fig. S8 The synthesis of Mag@MSNs-CD-mAzo. (a) mAzo was plugged in the cavities of CD rings of Mag@MSNs-CD. (b) UV-Vis spectra of Mag@MSNs-CD, and Mag@MSNs-CD-mAzo in D.I. H_2O.

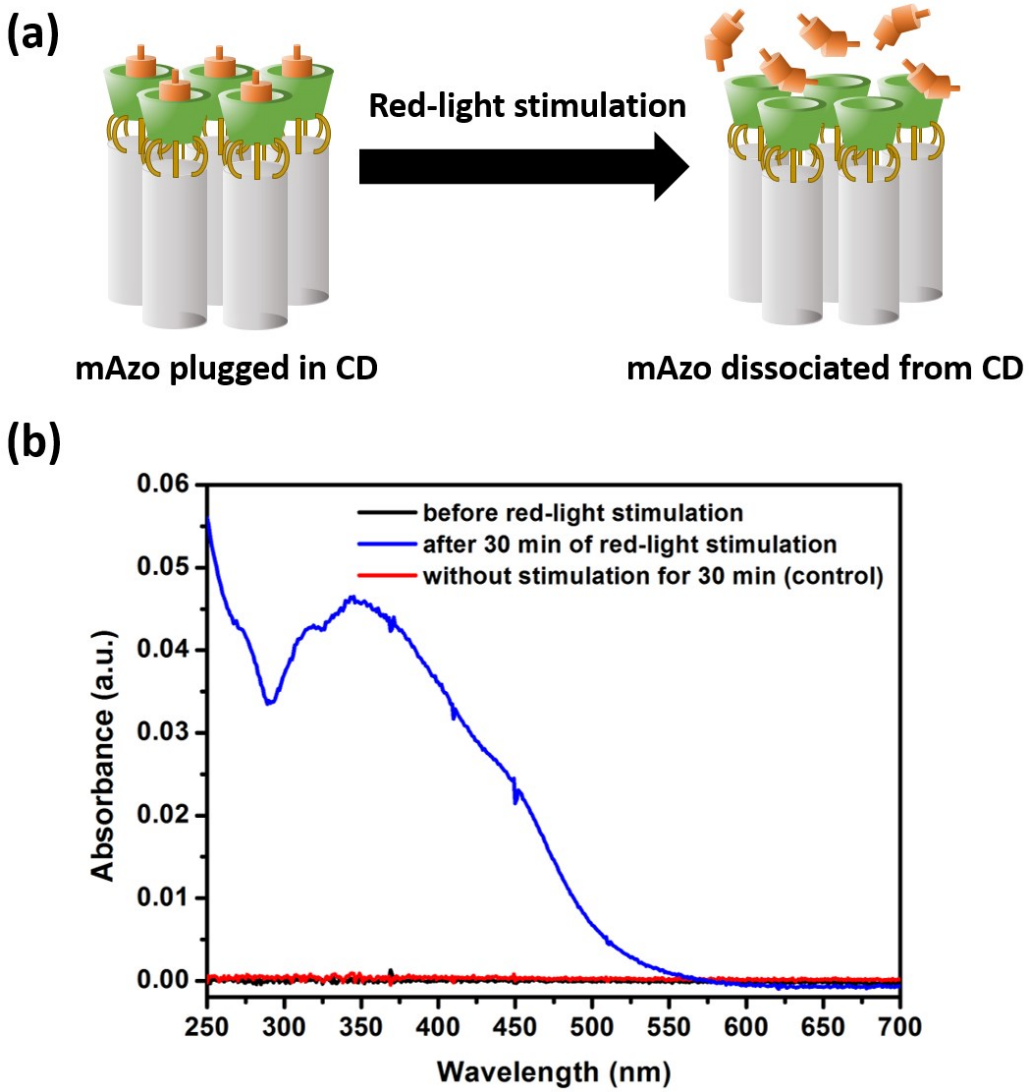

Fig. S9 (a) Disassembly of mAzo from CD stimulated by a red light. (b) UV-Vis spectra of the supernatant of Mag@MSNs-CD-mAzo before the red-light stimulation (black), after 30 min of the red-light stimulation (blue), or under dark as the control (red).

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