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Supporting Information

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1. Experimental Section

1.1. General

Doxorubicin (Dox) was purchased from LC laboratories and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received without further purification unless otherwise noted. 1-Adamantanamine (Ad) hydrochloride and β-cyclodextrin (β-CD) were purchased from TCI America (San Francisco, CA). N-hydroxysuccinimide (SCM) and maleimido (MAL) hetero-functionalized poly(ethylene glycol) (SCM-PEG-MAL, MW = 5 kD) were obtained from NANOCS Inc (New York, NY). Ad-grafted polyamidoamine dendrimers (Ad-PAMAM), β-CD-grafted branched polyethylenimine (CD-PEI), DOTA-grafted CD-PEI (CD-PEI-DOTA), and Ad-functionalized polyethylene glycol (Ad-PEG) were prepared via the method reported previously by our group.[1] Oleic acid, oleylamine, and hexane were purified by distillation under an argon atmosphere. Phosphate-Buffered Saline (PBS), and penicillin/streptomycin were obtained from Invitrogen (Carlsbad, CA). DLD-1 colorectal adenocarcinoma cell line and RPMI-1640 medium were purchased from the American Type Culture Collection (Manassas, VA). Fetal Bovine Serum (FBS) was obtained from Lonza Walkerrsville Inc (Walkerrsville, MD). 96-well BD Falcon culture plates were purchased from Fisher Scientific. CCK-8 assay (cell counting kit-8) was purchased from Dojindo Molecular Technology.

1.2. Synthesis

Synthesis of 6-nm Zinc-doped magnetite nanoparticles (MNP)

6-nm Zinc-doped iron oxide nanoparticles were prepared using a simple one-pot reaction under an argon atmosphere.[2] Zinc chloride (0.5 g, 3.67 mmol) and Iron(III) acetylacetonate (1.77 g , 5.00 mmol) were placed in a 250 ml three-neck round bottom flask in the presence of oleic acid (8.95 g, 31.69 mmol), oleylamine (23.18 mg ,86.65 mmol), and hexane (10 ml,76.00 mmol). The mixture was heated up from room temperature (rt) to 250 °C for 6 h and maintained at 250 °C for 1 h. After removing the heat source, the mixture was cooled down to rt under an ambient condition and ethanol was added to obtain 6-nm Zinc doped iron oxide nanoparticles (MNP), black precipitates. The MNP were then isolated by centrifugation and dispersed in toluene. Characterizations of MNP were carried out by transmission electron microscope (TEM), energy-dispersive X-ray spectroscopy (EDS), and X-ray diffraction (XRD).

Synthesis of Ad-grafted 6-nm Zinc-doped magnetite nanoparticles (Ad-MNP)

The surface of MNP was modified with tetramethylammonium hydroxide (TMAOH) for the phase transfer from toluene to aqueous phase. MNP were collected by centrifugation in the presence of excess ethanol and were allowed to react with 1M TMAOH/n-BuOH solution for 30 min under sonication. The MNP were isolated by centrifugation with 3000 rpm for 5 min and then re-dispersed in water. To introduce the carboxylic functional group on the surface of MNP, bovine serum albumin (BSA) was bio-

conjugated with MNP. Subsequently, BSA molecules were cross-linked to each other with 2,2'-(ethylenedioxy)bis(ethylamine) by 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC)-mediated coupling reactions in the presence of N-hydroxysulfosuccinimide (Sulfo-NHS). Finally, excess amounts of adamantylamine were conjugated to MNP with the same EDC/NHS coupling method. The product was purified by ultrafiltration and re-dispersed in water to yield Ad-grafted 6-nm Zinc-doped magnetite nanoparticles (Ad-MNP).

Synthesis of Dox-encapsulated supramolecular magnetic nanoparticles (Dox⊂SMNPs)

A 12-μL DMSO solution containing various amounts of Ad-PAMAM (0.06, 0.13, and 0.26 mg) and Dox (0.16 mg) was added into a 500-μL PBS mixture of Ad-MNP (0.1 mg), Ad-PEG (3.15 mg), and CD-PEI (2 mg) under vigorous stirring. The mixture was kept at 70 °C for 30 min, followed by annealing at rt for 2 h, yielding three different sizes of Dox-encapsulated supramolecular magnetic nanoparticles (70-nm, 100-nm, and 160-nm Dox⊂SMNPs).

Synthesis of 70-nm supramolecular magnetic nanoparticles (SMNPs, drug-free vector)

A 12-μL DMSO solution containing 0.06mg of Ad-PAMAM was added into a 500-μL PBS mixture of Ad-MNP (0.1 mg), Ad-PEG (3.15 mg), and CD-PEI (2 mg) under vigorous stirring. The mixture was kept at 70 °C for 30 min, followed by annealing at rt for 2 h, yielding the drug-free vector, i.e., 70-nm supramolecular magnetic nanoparticles (70-nm SMNPs, **Figure S1**).

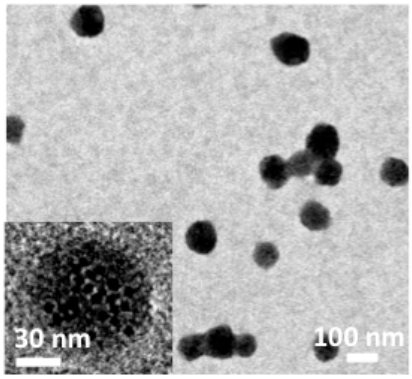


Figure S1. TEM image of 70-nm SMNPs

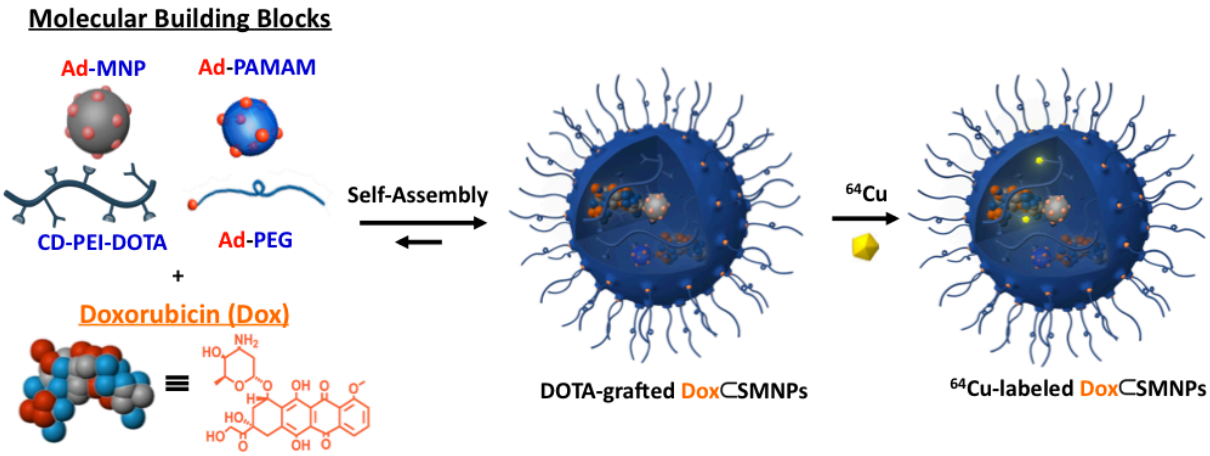
Synthesis of DOTA-grafted Dox⊂SMNPs

A 12-μL DMSO solution of Ad-PAMAM with three different concentrations (0.06, 0.13, and 0.26 mg) was slowly injected under vigorous stirring into a 270-μL PBS mixture of Ad-MNP (0.1 mg) and Ad-PEG (3.15 mg), and followed by an addition of 230-μL CD-PEI-DOTA (2mg) into the mixture. The mixture was incubated at rt for 20 min and then heated to 70 °C for another 30 min. The resulting DOTA-grafted

DoxCSMNPs with three sizes (70 nm, 100 nm, and 160 nm) were obtained when the solution was cooled to rt.

^{64}Cu labeling of DOTA-grafted DoxCSMNPs

All liquids were pretreated with Chelex-100 (Bio-Rad, Herchules, CA) to remove trace amounts of metal contaminants. The ^{64}Cu chloride (Washington university at St. Louis) was mixed with NH_4OAc buffer (pH 5.5, I = 0.1 M); DOTA-grafted DoxCSMNPs in 800-fold excess were added to the solution. The mixture was incubated for 1 h at 60 °C. The ^{64}Cu-labeled DoxCSMNPs product was purified by a molecular weight cut off filter (Centricon YM10, Billerica, MA) at 10,000 × *g* for 10 min. The labeling yield (> 95%) was determined by measuring the radioactivity in the filter, the filtrate, and the retentate, respectively. The ^{64}Cu-labeled DoxCSMNPs **(Scheme S1)** were re-suspended in saline for *in vivo* injections.



Scheme S1. Schematic representations of a self-assembled approach for producing DOTA-grafted DoxCSMNPs from Ad-MNP, Ad-PAMAM, CD-PEI-DOTA, Ad-PEG, and Dox. By covalently attaching a chelation ligand (i.e., DOTA) onto CD-PEI, a radioisotope (i.e., $^{64}Cu^{2+}$) can be incorporated to give ^{64}Cu-labeled DoxCSMNPs.

2. Dynamic light scattering (DLS)

DLS experiments were performed with a Zetasizer Nano instrument (Malvern Instruments Ltd., United Kingdom) equipped with a 10-mW helium-neon laser (λ = 632.8 nm) and thermoelectric temperature controller. Measurements were taken at a 90° scattering angle. The results for the three DoxCSMNPs were 150±0.7, 188±1.1, and 280±1.5 nm.

3. Transmission electron microscope (TEM)

The morphology and size of DoxCSMNPs were examined on a Philips CM 120 transmission electron microscope (TEM), operating at an acceleration voltage of 120 kV. The TEM samples were prepared by drop-coating 2-μL of DoxCSMNPs solution onto carbon-coated copper grids. Excess amounts of droplets

were removed with filter paper after 45 s. The size and the distribution of the three Dox⊂SMNPs are 70±9, 100±7, and 160±80 nm.

4. Zeta potential (ζ) measurements

Zeta potential of Dox⊂SMNPs was determined by photon correlation spectroscopy using a Zetasizer Nano instrument (Malvern Instruments, Malvern, Worcestershire, UK). The measurements were performed at 25 °C with a detection angle of 90° and the raw data was subsequently correlated to Z average mean size by using a cumulative analysis of the Zetasizer software package. The zeta potential of the resulting three sizes of Dox⊂SMNPs (70-nm, 100-nm, and 160-nm) was 18, 22, and 28 mV, respectively.

5. Drug encapsulation efficiency

A collection of Dox⊂SMNPs with various loading amount of Dox (0.01-0.86 mg) was obtained according to the above protocol. Non-encapsulated Dox was removed from Dox⊂SMNPs by centrifugation of Dox⊂SMNPs solution at 1300 rpm for 30 min using centrifugal filter devices (3000 NMWL). After recovering the filtrate containing non-encapsulated Dox, the Dox concentration was analyzed by ultraviolet absorption at a wavelength of 490 nm. The measurements were performed in triplicate. The amount of the Dox encapsulated in the SMNPs was then calculated by subtracting the free Dox in the filtrate from the total loading amount of Dox. Drug encapsulation efficiency (EE) of the resulting Dox⊂SMNPs was obtained as the amount of the Dox encapsulated in the SMNP vector divided by the total loading amount of Dox, and summarized in **Figure S2**.

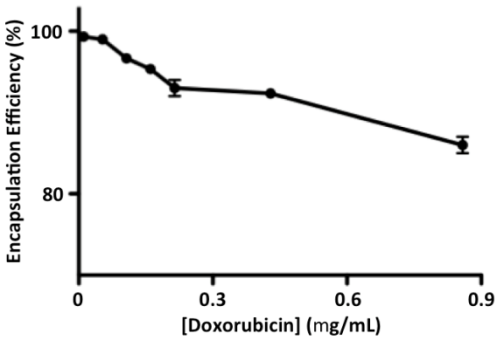


Figure S2. Dox encapsulation efficiency of a collection of Dox⊂SMNPs with different Dox loading amounts

6. Quenching effect of Dox⊂SMNPs

The photophysical properties of Dox⊂SMNPs were studied using fluorescence spectroscopies. The fluorescence emission spectra were recorded on a fluorescence spectrometer, FluoreMax-3 (Horiba Jobin Yvon, New Jersey). As illustrated in **Figure S3**, around 97% of the Dox⊂SMNPs fluorescence intensity is quenched when compared to free Dox with the same concentration, strongly suggesting that

Dox molecules are self-organizing inside SMNP vectors, which results in their fluorescence quenching.[3] This quenching phenomenon can be restored upon the release of the Dox from the SMNP vectors. Together, this environmentally sensitive fluorescent property of Dox can be exploited for visualization of drug release kinetics.

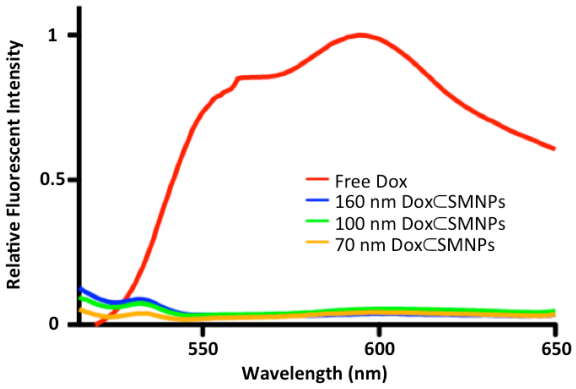


Figure S3. Fluorescence spectra of free Dox and three different sizes of DoxCSMNPs. Dramatically quenched fluorescence (approximately 97 %) were observed ($\lambda_{ex.}$= 490 nm).

7. Stability of DoxCSMNPs in culture medium

DoxCSMNPs were synthesized in PBS solution according to above protocol and their sizes were characterized by TEM. Three different sizes of DoxCSMNPs were then added into 5% FBS containing culture medium for 2 days to study the stability of DoxCSMNPs and their size changes were summarized in **Figure S4**. The measurements were repeated three times.

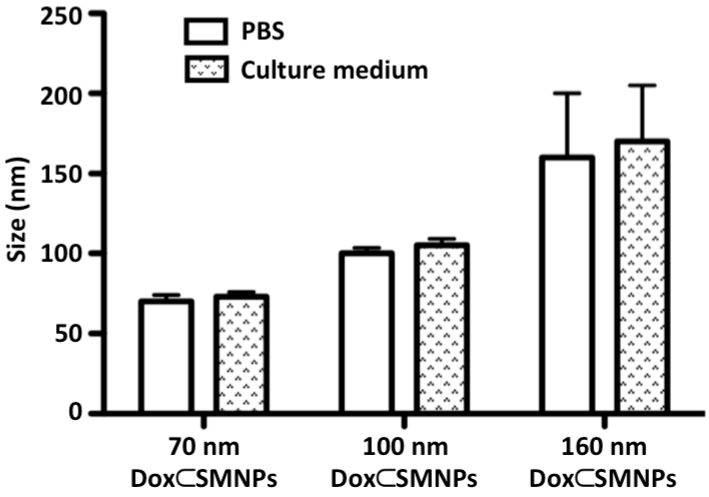


Figure S4. Stability study of DoxCSMNPs in both PBS and 5% FBS containing culture medium.

8. Micro-PET imaging

NU/NU mice were purchased from DLAM Breeding Colony Services (Los Angeles, CA). All animal manipulations were approved by the University of California at Los Angeles Animal Research Committee

(ARC protocol# 2006-135-12). Imaging studies were performed in the Crump Preclinical Imaging Technology Center, using equipment, techniques, and SOPs developed for quantitative mouse imaging.[4] NU/NU mice were injected subcutaneously, using sterile techniques, in the right flank with DLD-1 cells suspended in a 1:1 mixture of HBS buffer and matrigel (BD Biosciences, Franklin Lakes, NJ). Prior to use in tumor induction, DLD-1 cells were cultured in RPMI-1640 medium. Tumors were grown for 9 days until tumors reach to the size of 7 mm x 7 mm and the animals (n=3) were injected with 100 µL of silane containing either 70-nm, 100-nm, or 160-nm ^{64}Cu (300 µCi) labeled Dox⊂SMNPs via tail vein. Micro-PET imaging of the mice occurred at different time points (10 h, 24 h, 36 h, and 48 h post injection) and was performed with a micro-PET FOCUS 220 PET scanner (Siemens Preclinical Solutions, Knoxville, TN). The mice were anesthetized by using 1.5–2% isoflurane in a heated (36 °C) induction chamber fifteen minutes prior to imaging. The mice were then transferred to a heated isolation/imaging chamber for imaging.[5] 10 minute static micro-PET scans were acquired and images created using filtered back-projection without scatter or attenuation correction. To determine the estimated percent bioavailable injected dose of tumor retained Dox⊂SMNPs, 3D-ellipsoid regions of interest were placed around the tumor and a whole body region was used to determine the total bioavailable PET dose using Amide software.[6] Similarly, PET images were used to determine biodistribution data of the estimated percent bioavailable injected dose at all four time points for all three nanoparticle sizes and is shown in **Figure S5**. Clearance data of 70-nm Dox⊂SMNPs out of various organs is shown in **Figure S6** at various time points.

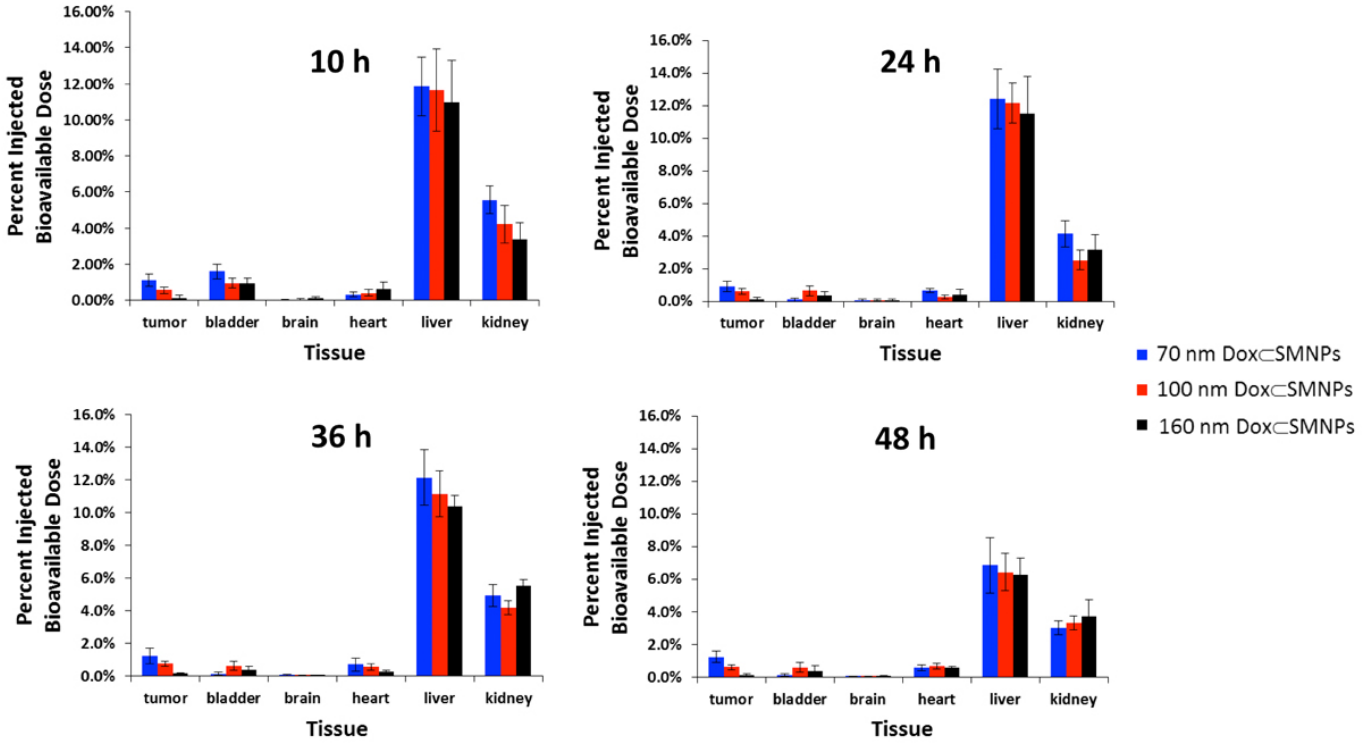


Figure S5. Biodistribution data of the 70-nm, 100-nm, and 160-nm radio-labeled Dox⊂SMNPs at 48 h post injection quantified by using PET data.

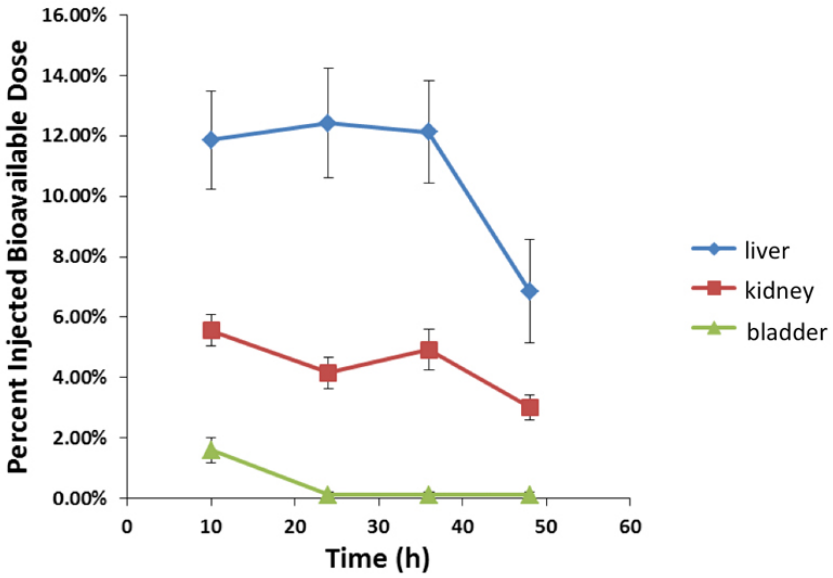


Figure S6. The percent injected bioavailable dose of the 70-nm Dox⊂SMNPs as it clears various organs over 48 hours.

9. Drug release profile

a. Drug release performance of Dox⊂SMNPs as a function of AMF duration

A high-radiofrequency heating machine (HF 10K, Taeyang System Co., Korea) that radiates an alternative magnetic field (AMF) with the strength of 37.4 kA/m at the frequency of 500 kHz was applied to the nanoparticle sample. 70-nm Dox⊂SMNPs dispersed in water (3 mg/ml) were placed in the center of a water-cooled magnetic induction coil (diameter, 5 centimeters) insulated with Styrofoam. The change in fluorescent intensity caused by the release of encapsulated Dox upon AMF was measured by a photoluminescence spectrometer (Ex 490 nm and Em 593 nm, FP-6500, Jasco, U.S.A) in accordance with an application time (0, 1, 2, 5, 10, 20, 30, and 50 min) of AMF. The results (**Figure S7a**) show increasing Dox fluorescence from SMNP vector upon AMF application; >90% of drug release occurs at 10 min of AMF duration. Temperature of the solution does not change during the measurement (**Figure S7b**).

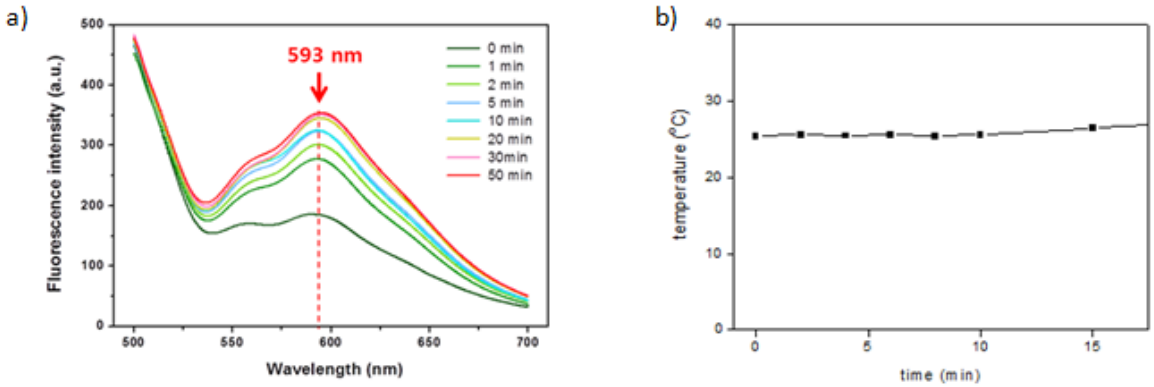


Figure S7. a) Dox release profiles of Dox⊂SMNPs as a function of AMF duration. b) Solution temperature profile during the AMF application.

b. Doxorubicin release profile measurement with single- and multiple-pulsed AMF

Single- and multiple-pulsed AMF were applied to the 70-nm Dox⊂SMNPs. The experimental condition of sample concentration and the strength of AMF was kept the same as in **Section 9a**. For single-pulse measurement, AMF was applied to the sample only once for 2 min. For multiple-pulse measurement, AMF was applied twelve times with 2 min pulse duration and 8 min intermittence. The released Dox was excited at 490 nm and the emission of fluorescence was observed at 593 nm using photoluminescence spectroscopy (FP-6500, Jasco, U.S.A).

10. *In vitro* experiment

The colon cancer cell line, DLD-1, was chosen for the *in vitro* on-demand drug release studies. DLD-1 cells (1.5 x 10^4) were treated with 70-nm Dox⊂SMNPs at a concentration of 200 μg/ml and the cells were then kept in the incubator for 24 h for the transfection. After washing the cells with PBS for three times, AMF of 37.4 kA/m, 500 kHz was applied to the cells for 10 min. The cell death caused by released Dox was observed in morphological view with optical/fluorescent microscope (**Section 10a**) and was also quantified by CCK-8 assay (**Section 10b**).

a. Observation of morphological changes in cancer cells

The formation of apoptotic bodies after AMF treatment was observed with a bright field and fluorescent microscope (Nikon, Eclipse Ti, Melville, New York, USA). The cell nuclei were stained with DAPI (blue) and the change of DAPI fluorescence distribution was observed, suggesting the formation of nucleus fragmentations. Furthermore, a significant increase in fluorescent intensity from Dox (red) and sustained accumulation in the nuclei was also observed, indicating a successful release of Dox from Dox⊂SMNPs upon AMF.

b. Quantitative analysis of cell death with CCK-8 assay

CCK-8 assay was used for the quantification of cell viability after AMF treatment. The cells were mixed with the CCK-8 reagent and kept in the incubator for 3 h according to provider's instruction. The absorption spectrum for the supernatant of culture medium was measured by using UV-Vis spectrometer at 450 nm.

11. *In vivo* experiment

NU/NU mice (female) were purchased from Narabiotech co. (Korea). Food and water was available *ad libitum*. The animal study protocol was reviewed and approved by the Animal Care and Use Committee of University of Yonsei. Female NU/NU mice, 4-5 weeks old, were anesthetized, shaved, and prepared for implantation of the tumor cells. DLD-1 cells were collected from cell culture, and 5×10^6 cells suspended in a 1:1 mixture of PBS buffer and matrigel were then injected subcutaneously into the right flank of a mouse (n = 3). When the tumor volume reached to 100 mm^3, 150 μg/kg of 70-nm Dox⊂SMNPs were administered to the mouse intravenously via tail vein. After 36 h post injection, the mouse was placed into a water-cooled magnetic induction coil and applied with AMF of 37.4 kA/m at 500 kHz to the tumor region for 10 min. The change of tumor volume was monitored over the course of treatments (The termination point of the experiment occurred on day 15 or when the tumor volume reached 1500 mm^3). For the double injection group, 70-nm Dox⊂SMNPs were injected after 7 days from the 1st treatment. For comparison, control groups studies were carried out as followed: the group treated with only 70-nm Dox⊂SMNPs without the application of AMF, the group applied with only AMF without the injection of 70-nm Dox⊂SMNPs, the group treated with SMNPs (drug-free vectors) upon an application of AMF, and negative control group (PBS only). The *in vivo* results of drug-free SMNPs show the same tumor growth behavior as the other control groups (**Figure S8**) indicating no hyperthermic effect caused by the heat generated from magnetic nanoparticles in SMNPs.

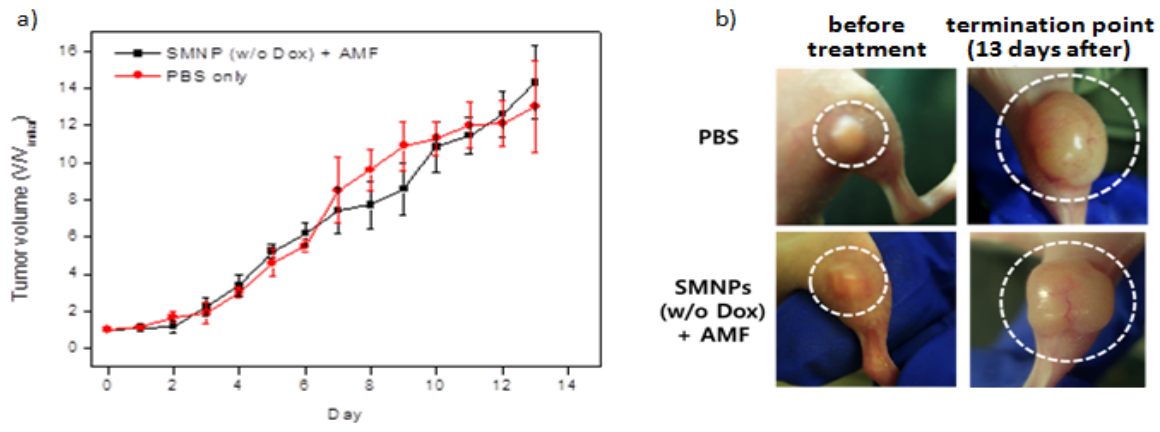


Figure S8. a) *In vivo* tumor growth profile with drug-free SMNPs and PBS injected mice (n=3). b) Images of tumors taken at before treatment and termination point (day 13) with drug-free SMNP vectors and PBS injected mice.

	System	Dox Amount/injection	Treatment Protocol	References
In vivo	Our system: **Dox⊂SMNPs**	2.8 μg/kg **Dox** (150 ug/kg **Dox⊂SMNPs**)	Two-dose treatment with applied AMF 36h post-injection	
In vivo	**Dox**-loaded iron oxide	640 μg/kg **Dox**	3 times a week until 19 days	*Angew. Chem. Int. Ed.* 2008, 47, 5362 –5365
In vivo	PEI-CD/Ad-**Dox**/pTRAIL SNP	300 μg/kg **Dox**	Twice a week for 2 weeks	*Biomaterials* 2012, 33, 1428-1436
In vivo	**Dox**-loaded thermosensitive liposome and gold nanorods	2500 μg/kg **Dox**	Inject once with NIR irradiation 48 h post-injection until 30 days	*ACS Nano* 2011, 5 (6), 4919–4926
In vivo	**Dox**-loaded pegylated nanographene oxide	10000 μg/kg **Dox**	Inject once with NIR light irradiation 24 h post-injection until 40 days	*Biomaterials* 2011, 32, 8555-8561
In vivo	**Dox**-SWNT	5000 μg/kg **Dox**	Once per week for 2 weeks	*Angew. Chem. Int. Ed.* 2009, 48, 7668 –7672
Clinical	Doxorubicin HCL - Adriamycin®	60-75 mg/m² ≈ 1300-1700 μg/kg **Dox**	Intravenous injection administered at 21-day intervals	Guidelines from Pfizer Inc
Clinical	DOXIL® (Pegylated liposomal **Dox**)	50 mg/m² ≈ 1100 μg/kg **Dox**	Intravenous injection once a month	Guidelines from Johnson & Johnson

Table S1. Comparison of Dox amount per injection and treatment protocol of our system, Dox⊂SMNPs, to several clinical and *in vivo* drug delivery systems.

Reference

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