Supplementary Information

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Multifunctional Self-Fluorescent Polymer Nanogel for Label-free Imaging and Drug Delivery

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1. Experimental

Materials. Abietic acid (AA, ~85%, ACROS), oxalyl chloride (98%, Alfa Aesar), 3-chloro-1-propanol (98%, Alfa Aesar), *N, N*-dimethylaminoethyl methacrylate (DMAEMA, TCL), poly(ethylene glycol) diacrylate ($PEG_{13}DA$, Aldrich), and doxorubicin hydrochloride (DOX, Aldrich) were used as received unless otherwise noted. All other solvents and agents were analytical grade, and used without further purification.

Characterization. The absorption spectrum of abietic acid after heat treatment was recorded using Shimadzu UV-2450 spectrometer. The emission spectrum was recorded using the SLM Aminco-Bowman series 2 luminescence spectrometer. The average particle size and the zeta potential of the nanogels were measured by using Zetasizer Nano-Zs (Malvern Instruments, U. K.) at 25 ^{o}C. Transmission electron microscopy (TEM) images were measured with Hitachi H8000 TEM (Japan) by placing one drop of the samples on copper grids coated with carbon. Atomic force microscopy (AFM) images were measured with Nanoscope V (VEECO, U. S.) by placing one drop of the samples on the surface of clean mica and dried on the vaccum oven. The AFM observation was performed in tapping mode.

Synthesis of DMAEMA-g-3-choloropropyl-abietate (abietane-based monomer). Abietic acid (10.00 g, 3.3 × 10^{-2} mol) was heated for 4 h at 180 ^{o}C under nitrogen protection and then dissolved in tetrahydrofuran (THF, 100 mL). Oxalyl chloride (6.23 g, 5.0 × 10^{-2} mol) was slowly added to the solution. After the solution was stirred at 0 ^{o}C for 3h, excessive oxalyl chloride was removed by distillation. Triethylamine (0.36 g, 3.6 × 10^{-3} mol) and 3-chloro-1-propanol (15.6 g, 1.6 × 10^{-1} mol) were subsequently added. The reaction mixture was stirred at 0 ^{o}C overnight and the solvent was evaporated. The product 3-choloropropyl-abietate was further purified by silica gel chromatography (ethyl acetate/hexane: 1/9 (v/v)) to produce a brown oil product (8.0 g) at a yield of ~ 65%.

The abietane-based monomer was synthesized by quaternization of DMAEMA using 3-choloropropyl-abietate: 3-choloropropyl-abietate and DMAEMA with molar feed ratios 3:1 were dissolved in 5 mL acetonitrile. The mixture was refluxed at 50 ^{o}C for 72 h. After the completion of the reaction, the solvent was evaporated. The product was then dissolved in chloroform and

precipitated in hexane three times. The obtained solid product dried in a vacuum oven until constant weight.

Synthesis of abietane-based nanogels. Abietane-based nanogels were synthesized by using a seed emulsion copolymerization. In a typical run, 13.4 mg (2.5×10^{-5} mol) of abietane-based monomers were dissolved in 5 mL distilled water containing 0.4% (w/w) $PEG_{13}DA$. 60 μL of ammonium sulfate (6.0×10^{-6} mol) was added slowly into solution under stirring at room temperature. Opalescent suspension occurred, indicating the formation of seed particles. Then 100 μL of 1% ascorbic acid solution and 100 μL of 5% hydrogen peroxide aqueous solution were added into the reaction system to initiate polymerization of abietane-based monomers and crosslinking at 50 ^{o}C. The reaction was allowed to proceed at 50 ^{o}C for 3h. The resultant suspension was dialyzed against distilled water for 24 h to remove residual monomers and small molecules.

DOX loading and releasing. 5 mL of Doxorubicin hydrochloride aqueous solution (0.1 mg/mL) was added into 5 mL of nanogel solution (1 mg/mL) to reach a final concentration of nanogel at 0.5 mg/mL. After stirring in the dark for 12h, the pH of the mixture was adjusted to 7.4. Then the mixture was stirred in the dark for another 24h. The free doxorubicin in the solution was separated from the nanogels by centrifugation (12,000 rpm/min for 20min). The amount of DOX was determined by using an UV absorbance at 490 nm according to a calibration curve.

The release of the DOX from the abietane-based nanogels in phosphate buffered saline solution at 37 ^{o}C was evaluated by the dialysis method. The DOX-loaded abietane-based nanogel solution of known DOX concentration was placed inside a dialysis bag (MWCO=3,000) and dialyzed against phosphate buffered saline at 37 ^{o}C. The released DOX outside of the dialysis was collected at predetermined periods and determined by UV-vis spectrometry at 490 nm based on

the linear calibration curve. Cumulative release was expressed as the total percentage of drug released through the dialysis membrane over time.

Synthesis of folic acid-decorated DOX-loaded abietane-based nanogels. Folic acid was first dissolved in NaOH (0.01 mol/L) solution with concentration of 1 mg/mL as stock solution. 10 μL stock solution was added into the nanogels solution (1mg/mL). After stirring at room temperature for 3h, the abietane-based solution was centrifuged at 12,000 rpm for 20 min to remove free folic acid. It is worthy to note that for folic acid-decorated nanogels, the loading efficiency and loading content of drug were at 63% and 3.4% respectively.

Cytotoxicity of abietane-based nanogels. The cytotoxicity of nanogels against MDA-MB-231 breast cancer cells was assessed by MTT assay. In brief, MDA-MB-231 cells were seeded in 96-well plates at a density of 4×10^4 cells/mL. After 24h incubation, the cells were exposed to a series of doses of abietane-based nanogels, DOX-loaded abietane-based nanogels, and DOX at 37 ^{o}C. After 24h incubation, the sample wells were washed twice with PBS buffer, and 100 μL of freshly prepared MTT solution (0.5 mg/mL) in culture medium was added into each sample well. The MTT medium solution was carefully removed by centrifugation after 4h incubation in the sample wells. Dimethyl sulfoxide (100 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all precipitates formed. The absorbance of individual wells at 570 nm was then detected. The absorbance of MTT in the sample wells was determined by the difference between the absorbance of the sample wells and that of the corresponding control wells. The cell viability was expressed by the ratio of the absorbance of MTT in the sample wells to that of the cells incubated with culture medium only.

Cellular imaging. The cellular images were acquired with a Laser Confocal Scanning Microscope (LCSM, ZEISS, LSM 510 Meta, Germany). Cells (MDA-MB-231or MCF-7) (4 ×

10^4 cells/well) were seeded on a 6-well plate at 37 ^oC for 24h. After that, DOX-loaded abietane-based nanogels or Folic acid-decorated, DOX-loaded abietane-based nanogels with a concentration of 100μg/mL were added to the cell wells. Folic acid was also added to the cell well with a final concentration of 20 mM. After further incubation with a predetermined time, these nanogel-loaded cells were washed with PBS three times to remove the free nanogels attached on the outer surface of cell membrane. The cellular uptake was detected on LCSM (Zeiss LSM 410, Germany) under excitation wavelength of 360 nm and 488 nm for fluorescent abietane-based nanogels and DOX, respectively.

2. Results

(1) UV-vis absorption spectra of abietic acid before and after thermal treatment

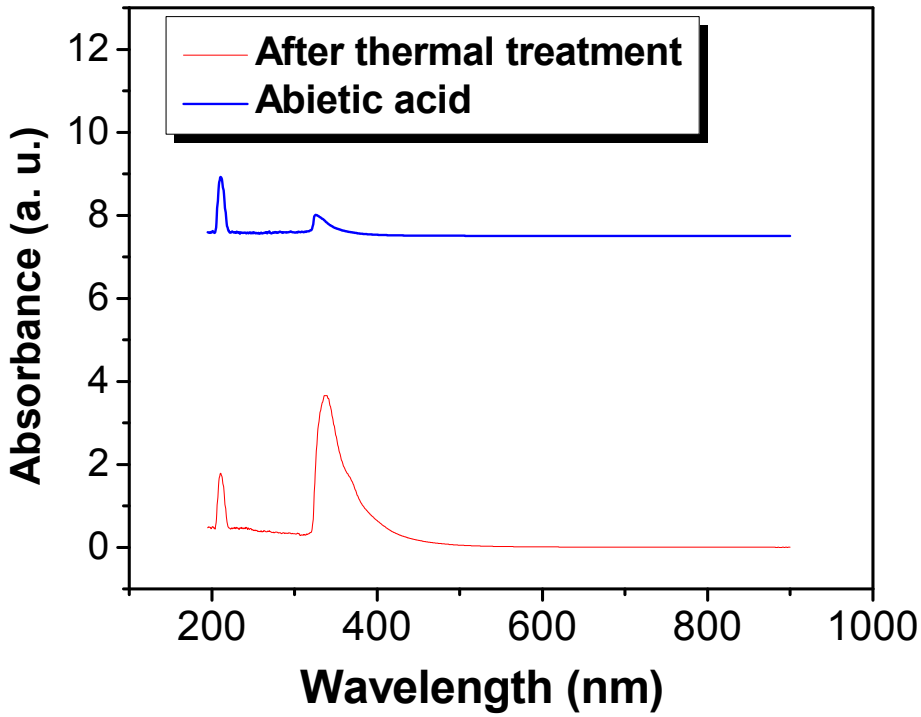

Fig. S1. UV-vis absorption spectra of abietic acid before and after thermal treatment.

(2) Size and Zeta -potential of abietane-based nanogels

Table S1. Dependence of feed ratio of [abietane monomer] / [ammonium sulfate] on size and Z-potential of abietane-based nanogels.

Sample	Abietane Monomer (mM)	Ammonium Sulfate (mM)	Crosslinker (%, w/w)	Size (Dh) (nm)	Z-potential (mV)
1	5	0.5	0.4	134.4	16.5
2	4	0.5	0.4	105.3	16.1
3	3	0.5	0.4	91.3	18.4
4	2	0.5	0.4	118.6	17.3
5	1	0.5	0.4	1620	-

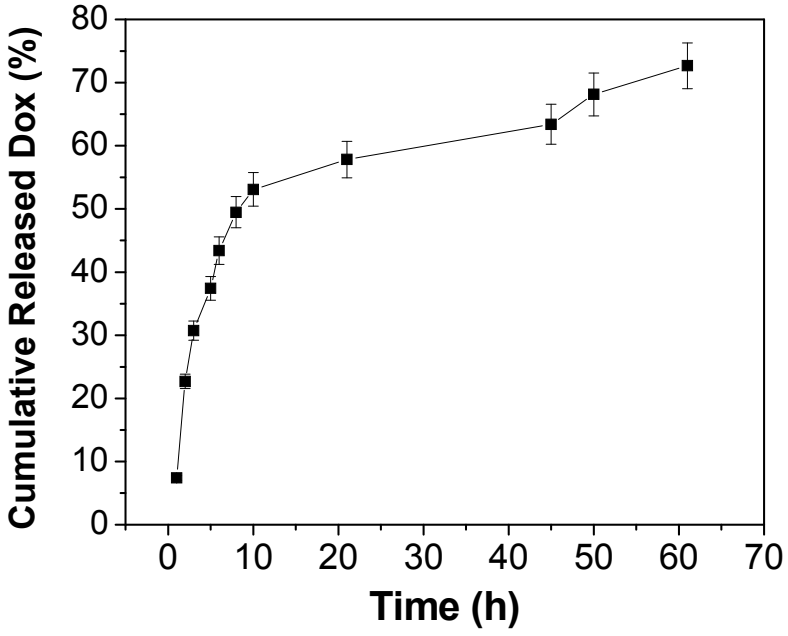

Fig. S2. Release profile of DOX from the abietane-based nanogels in PBS solution (pH=7.4) at 37 ^{o}C.

(3) *In vitro* release of DOX-loaded abietane-based nanogels

Fig. S2 shows the release profile of DOX from abietane-based nanogels in PBS solution (pH=7.4) at 37 ^{o}C. A burst release with about 53% of the total loaded DOX occurred in the first 10 hour. This portion of drug was probably deposited at the region near the surface of nanogels and can diffuse to the aqueous medium in a short time. After the initial burst release, the nanogels released about 72% (accumulated) of entrapped DOX in 61h. These results indicated that the abietane-based nanogels showed slow *in vitro* release of the drug at the conditions with physiological pH and temperature.

(4) Morphology of folic acid-decorated abietane-based nanogels

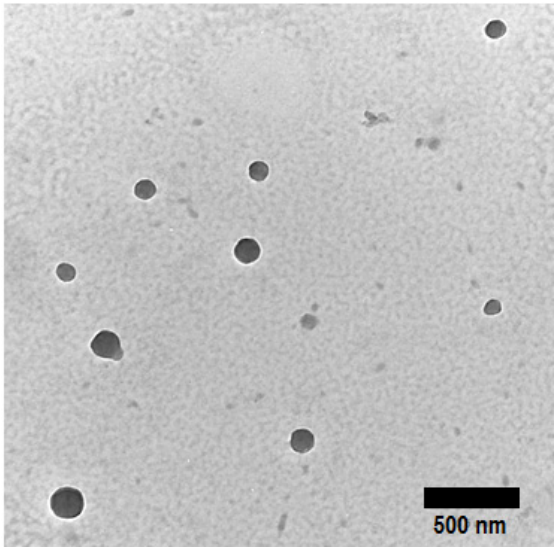

Fig S3. TEM image of folic acid-decorated abietane-based nanogels.

(5) Effect of folic acid concentration on size and Zeta-potential of abietane-based nanogels

Table S2 shows the clear effect of folic acid concentration on size and Zeta-potential of abietane-based nanogels. The diameter increase and zeta-potentials decrease of nanogels was observed as the folic acid concentration increased (0-20 μg/mL). After folic acid decoration, the

positive charge on the nanogels surface weakened because of electrostatic interaction between the folic acid and nanogels. It was further noted that when the folic acid concentration was 40 μg/mL, the nanogels precipitated out of the solution, suggesting that the surface charge was completely shielded by folic acid. All these data confirmed that the folic acid was incorporated into the nanogels.

Table S2. Effect of folic acid concentration on size and Zeta-potential of abietane-based nanogels (prepared by the feed ratio of [abietane monomer] : [ammonium sulfate] = 6:1)

Folic acid (μg/mL)	**0**	**10**	**20**	**40**
Diameter (nm)	91.8	117.2	182.1	>1000 (aggregate)
Zeta Potential (mV)	23.7	21.4	19.1	____

(6) Target delivery of folic acid-decorated abietane-based nanogels

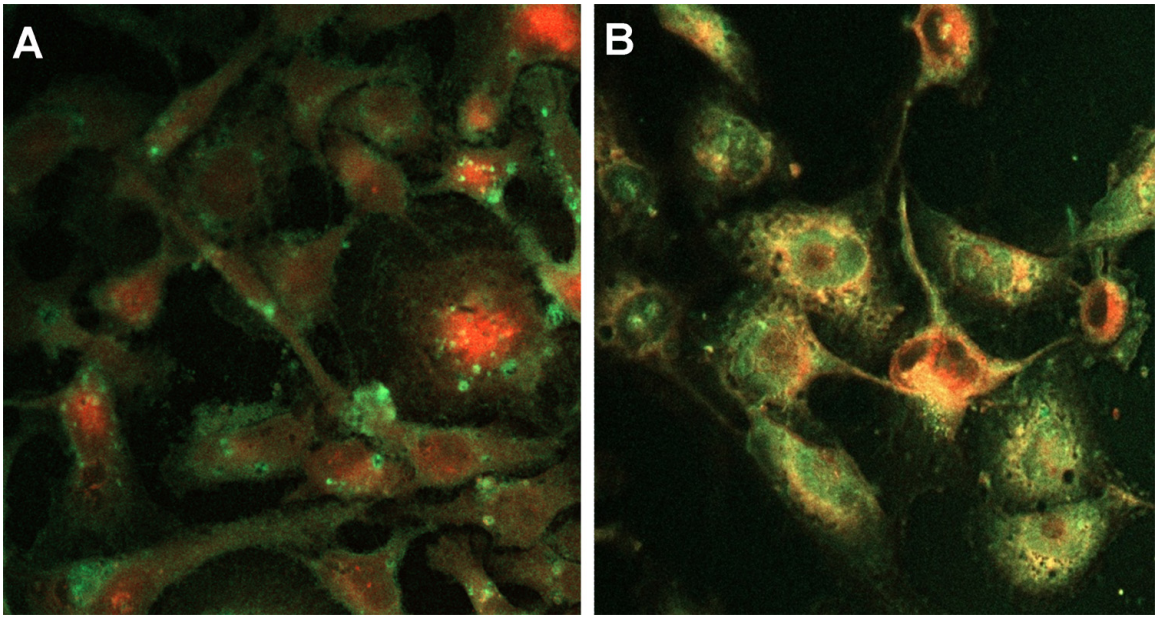

Figure S4. LCSM images of MDA-MB-231 cells incubated 4h with (A) DOX-loaded abietane-based nanogels and (B) folic acid-decorated, DOX-loaded abietane-based nanogels.

(7) *In vitro* cytotoxicity of abietane-based nanogels

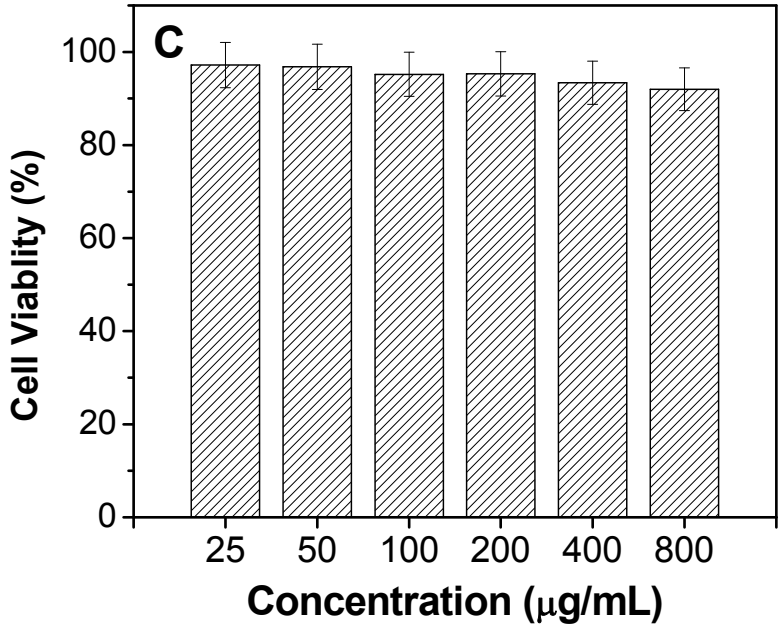

Fig. S5. *In vitro* cytotoxicity of abietane-based nanogels without DOX after 24h incubation with MDA-MB-231cells.

(7) The effect of folic acid-decorated abietane-based nanogels on cell uptake of FR-negative cancer cells

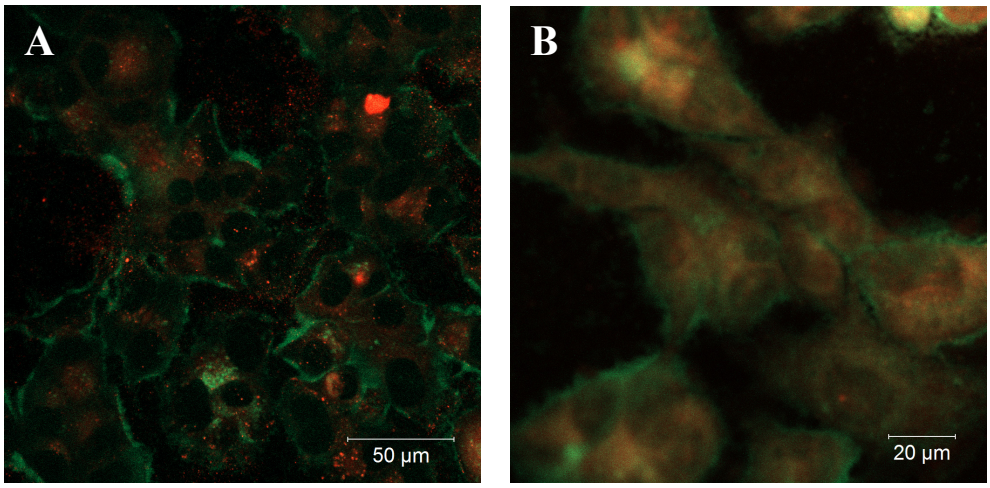

Figure S6. LCSM images of MCF-7 cells incubated for 2h with (A) DOX-loaded abietane-based nanogels and (B) folic acid-decorated, DOX-loaded abietane-based nanogels