Electronic Supplementary Information for

Folate decorated hollow spheres of microporous organic network as drug delivery materials

June Young Jang,[‡a] Huu Thuy Trang Duong, [‡b] Sang Moon Lee,[c] Hae Jin Kim,[c]
Yoon-Joo Ko,[d] Ji Hoon Heong,[e] Doo Sung Lee,*[b] Thavasyappan Thambi,*[b] and Seung Uk Son*[a]

[a]*Department of Chemistry, Sungkyunkwan University, Suwon 16419, Korea*
E-mail:sson@skku.edu
[b]*School of Chemical Engineering, Theranostic Macromolecules Research Center,*
Sungkyunkwan University, Suwon 16419, Korea
E-mail: dslee@skku.edu, thambi1983@gmail.com
[c]*Korea Basic Science Institute, Daejeon 350-333, Korea*
[d]*Laboratory of Nuclear Magnetic Resonance, National Center for Inter-University Research Facilities(NCIRF),*
Seoul National University, Seoul 08826, Korea
[b]*School of Pharmacy, Theranostic Macromolecules Research Center,*
Sungkyunkwan University, Suwon 16419, Korea
[‡] *These authors contributed equally to this work.*

Experimental Sections

Scanning and transmission electron microscopies (SEM & TEM) were conducted using a FE-SEM (JSM6700F) and a JEOL 2100F, respectively. N_2 adsorption-desorption isotherm curves were obtained at 77K using a BELSORP II-mini analyzer. The pore size distribution was analyzed by the density functional theory (DFT). UV-visible absorption spectroscopy was conducted using a JASCO V-630 and Optizen 3220UV (Mecasys Co., Ltd, Daejeon, Korea) spectrometers. Infrared absorption spectroscopy (IR) was conducted using a Bruker VERTEX 70 FT-IR spectrometer. Powder X-ray diffraction (PXRD) patterns were obtained using a Rigaku MAX-2200 (filtered Cu-Ka radiation). Solid state ^{13}C nuclear magnetic resonance spectroscopy was conducted in the cross-polarization (CP)-total suppression of side bands (TOSS) mode using a 500 MHz Bruker ADVANCE II NMR spectrometer at the NCIRF of Seoul National University. A 4 mm magic angle spinning probe was used. The spinning rate was 5 kHz. Elemental analysis was conducted using a CE EA1110 instrument. Water contact angles were measured using a Theta Optical Tensiometer model (KSV instruments, Ltd.) and electrooptics comprising a CCTV camera connected to a computer (software Attension Theta).

Synthetic procedure for H-MON and H-MON-FA

For using templates, silica nanospheres (average diameter: 189 ± 6 nm) were prepared by the synthetic procedures in the literature.[1] In a 250 mL round bottomed flask, ethanol (200 mL), distilled water (8 mL), and ammonia solution (28~30% aqueous solution, 5 mL) were added. The solution was stirred at room temperature for 30 min. After tetraethyl orthosilicate (TEOS, 14 mL, 63 mmol) was added, the solution was stirred (rpm 1150) at room temperature for 18 hours. After the reaction mixture was transferred to a 500 mL Erlenmeyer flask, a

mixture of hexane (200 mL) and methylene chloride (30 mL) was added. The silica spheres were separated by centrifugation, washed with a mixture of methanol (20 mL) and acetone (20 mL) four times, and dried under vacuum.

For the synthesis of SiO_2@MON, silica spheres (0.60 g), $(PPh_3)_2PdCl_2$ (17 mg, 0.024 mmol), CuI (4.6 mg, 0.024 mmol) were added to a mixture of toluene (20 mL) and triethylamine (40 mL) in a flame-dried 100 mL two neck Schlenk flask. After the mixture was sonicated for 1 hour, tetrakis(4-ethynylphenyl)methane[2] (0.10 g, 0.24 mmol) and 1,4-diiodobenzene (0.16 g, 0.48 mmol) were added under argon. The reaction mixture was heated at 90°C for 24 hours. After being cooled to room temperature, the powder was retrieved by centrifugation, washed with a mixture of methylene chloride (15 mL), methanol (15 mL) and acetone (15 mL) five times, and dried under vacuum. The obtained SiO_2@MON was added a mixture of aqueous HF solution (48~51% solution, 7.5 mL), methanol (20 mL), and water (15 mL). *Caution: the HF solution is a very toxic to human being and thus, should be handled with extreme care (specific gloves and good hood).* After the reaction mixture was stirred for 2 hours at room temperature, the powder was retrieved by centrifugation and washed with a mixture of methanol (20 mL) and water (20 mL) five times. The excess HF solution was neutralized by saturated NaOH solution. After the washed solution became neutral (checking by a pH paper), the powder (H-MON) was further washed with acetone by three times and dried under vacuum.

For the post-modification of H-MON with cysteines, we referred to the literature.[3] In our study, the following procedures were applied. H-MON (0.10 g), L-cysteine (97 mg, 0.80 mmol), and azobisisobutyronitrile (AIBN, 88 mg, 0.54 mmol) were added to a distilled toluene (6.5 mL) to a flame-dried two neck Schlenk tube under argon. The reaction mixture was heated at 100°C for 24 hours. The powder (H-MON-CYS) was retrieved by centrifugation, washed with a mixture of ethanol (30 mL) and water (15 mL) four times and then with acetone (40 mL) three times, and dried under vacuum.

For the coupling of H-MON-CYS with folic acids, we referred to the literature.[4] Folic acid (0.10 g, 0.23 mmol) was dissolved in distilled dimethylsulfoxide (DMSO, 10 mL) in a flame-dried two neck Schlenk flask under argon. After N,N'-dicyclohexylcarbodiimide (DCC, 60 mg, 0.29 mmol) was added, the reaction mixture was stirred for 1 hour at room temperature. After H-MON-CYS (50 mg) was added, the reaction mixture was stirred for 24 hours at room temperature. The powder (H-MON-FA) was retrieved by centrifugation, washed with a mixture of ethanol (30 mL) and water (15 mL) four times and then with acetone (40 mL) three times, and dried under vacuum.

Preparation procedures of DOX/H-MON and DOX/H-MON-FA

For the release and cell tests, DOX/H-MON and DOX/H-MON-FA were prepared by the dialysis method. H-MON or H-H-MON-FA (10 mg) was dispersed in a 1:1 mixture of DMSO/water (9 mL). DOX (2 mg) in a 1:1 mixture of DMSO/water (1 mL) was added to the H-MON or H-MON-FA solution and the mixture was stirred for 3 h. Then, the resulting solution was dialyzed against distilled water using a dialysis tube (MWCO = 3,500 Da) for one day to remove unloaded DOX, followed by lyophilization to obtain the DOX-loaded MONs (DOX/H-

MON and DOX/H-MON-FA. The loading efficiency and content of DOX in MONs were determined using a UV-vis spectrophotometer at 485 nm (Optizen 3220UV, Mecasys Co., Ltd, Daejeon, Korea). For measuring the loading efficiency and the content of DOX in MONs, DOX-loaded MONs were vigorously sonicated in a 1:1 mixtrue of DMSO/DMF, and measured with the calibration curve obtained using a 1:1 mixture of DMSO/DMF solutions with different DOX concentrations. The loading efficiency and content of DOX were calculated using the following formula: Loading efficiency (%) = (weight of loaded drug/weight of drug in feed) × 100%, Loading content (%) = (weight of loaded drug/weight of MONs) × 100%.

Experimental procedures of in vitro release behaviors of DOX from DOX/H-MON and DOX/H-MON-FA

In vitro release behaviors of DOX from DOX/H-MON or DOX/H-MON-FA were investigated by the following procedures. DOX/H-MON or DOX/H-MON-FA (1 mg/ml) was dispersed in a phosphate-buffered saline (PBS, pH 7.4), and the solution was transferred to cellulose membrane tubes (MWCO = 3,500 Da). The dialysis tube was then immersed in 30 ml of PBS (pH 7.4). Each sample was gently shaken at 100 rpm in a water bath with a temperature of 37 ºC. The medium was refreshed at predetermined time intervals, and the DOX concentration was determined using UV-vis spectroscopy at 485 nm.

The structural stability of DOX/H-MON-FA was observed after the DOX release for one week. The DOX-loaded H-MON-FA containing cellulose membrane tubes were retrieved, immersed in 30 ml of water, and gently shaken at 100 rpm in a water bath at 37 ºC for 12 h. Thereafter, the salt free solution was lyophilized and the powder was subjected to SEM analysis. (Refer to Fig. S4 in the ESI)

Experimental procedures of in vitro cytotoxicity and intracellular drug release

A549 human lung carcinoma cells, MDA-MB-231 breast cancer cells, and 293T human embryonic kidney cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were propagated in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco BRL), and 1% (v/v) penicillin-streptomycin (Gibco, Grand Island, NY, USA) at 37 ℃ in a humidified 5% CO_2/95% air atmosphere.

To examine the *in vitro* cytotoxicity, the cells were detached using trypsin and were seeded at a density of 1×10^4 cells/well. One day after the cell growth, the medium was replaced and exposed to different concentrations of free MONs and DOX-loaded MONs, whereas fresh RPMI-1640 medium was used as control and was referred as 100% cell viability. The cells were incubated for 24 h or 48 h, washed twice with PBS and fresh culture medium was added. Subsequently, 20 μL of MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, 5 mg/mL) solution was added to each well and the plate was further incubated for 3 h to observe the reductive cleavage of yellow MTT to formazan (purple crystals) by mitochondrial dehydrogenase. Then, the medium was removed and DMSO was added to each well to dissolve insoluble purple formazan crystals. The viability of the cells is calculated by measuring the absorbance of colored solution at 490 nm (Multiskan Go, Winooski, VT, USA).

To observe the intracellular drug release behavior, the cells were incubated for 3 and 6 h with DOX-loaded MONs or free DOX. In all cases, the quantity of DOX was fixed to 5 μg. The cells were then washed twice with PBS (pH 7.4) and fixed with 4% formaldehyde solution. For nuclear staining, the cells were incubated with 4,6-diamino-2-phenylinodole (DAPI) for 10 min at room temperature, followed by washing with PBS (pH 7.4). The intracellular localization of DOX was observed using a confocal laser scanning microscopy Zeiss LSM 510 (Carl Zeiss MicroImaging GmbH, Oberkochen, Germany). The statistical significance of differences ($p < 0.01$) between MON and MON-FA groups were determined using one-way ANOVA. For competitive inhibition studies, the cells were first exposed to serum-free medium containing free FA (5 mg/ml) for 60 min.

References

1 W. Stöber, A. Fink and E. Bohn, *J. Colloid Interface Sci.* 1968, **26**, 62-69.

2 S. Yuan, S. Kirklin, B. Dorney, D. –J. Liu and Luping Yu, *Macromolecules* 2009, **42**, 1554-1559.

3 (a) B. Kiskan and J. Weber, *ACS Macro Lett.,* 2012, **1**, 37–40. (b) X. Han, M. Xu, S. Yang, J. Qian and D. Hua, *J. Mater. Chem. A,* 2017, **5**, 5123-5128.

4 S. Karamipour, M. S. Sadjadi and N. Farhadyar*, Spectrochim. Acta A* 2015, **148**, 146-155.

Fig. S1 PXRD pattern of H-MON, H-MON-CYS, and H-MON-FA.

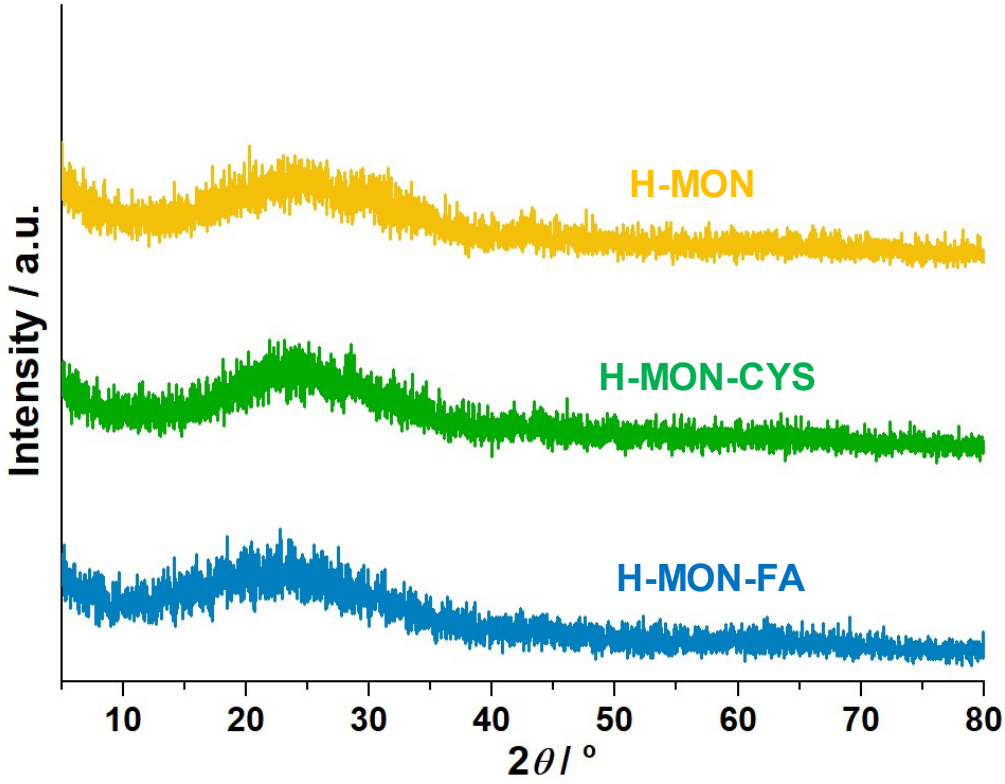


Fig. S2 Solid state ^{13}C NMR spectra of H-MON, H-MON-CYS, and H-MON-FA.

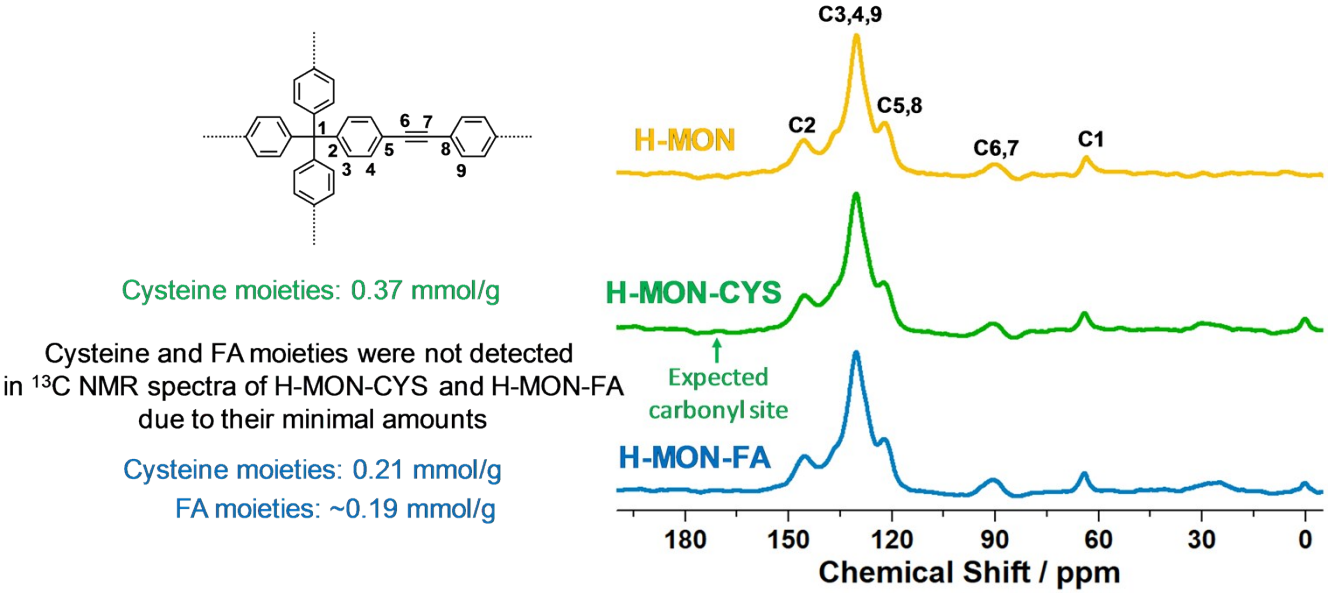


Fig. S3 Cell viability of cancer cells (A549 cell line) against H-MON-FA and H-MON (without DOX).

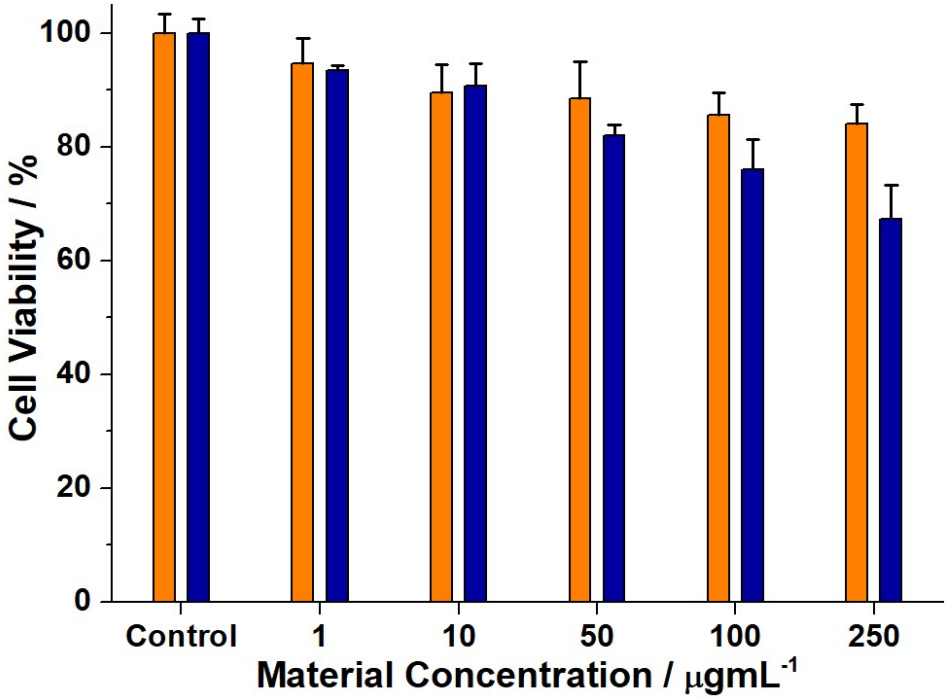
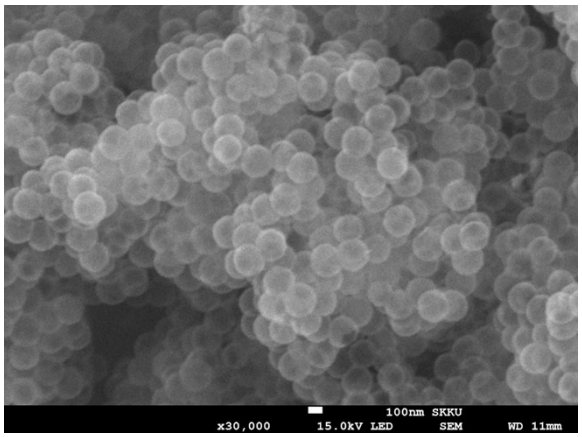


Fig. S4 SEM images of original H-MOF-FA and the H-MON-FA recovered after DOX release test for one week. (The DOX-loaded H-MON-FA containing cellulose membrane tubes were retrieved, immersed in 30 ml of water, and gently shaken at 100 rpm in a water bath at 37 ºC for 12 h. Thereafter, the salt free solution was lyophilized and the powder was subjected to SEM analysis.)

Original H-MON-FA



H-MON-FA recovered after DOX release test for one week

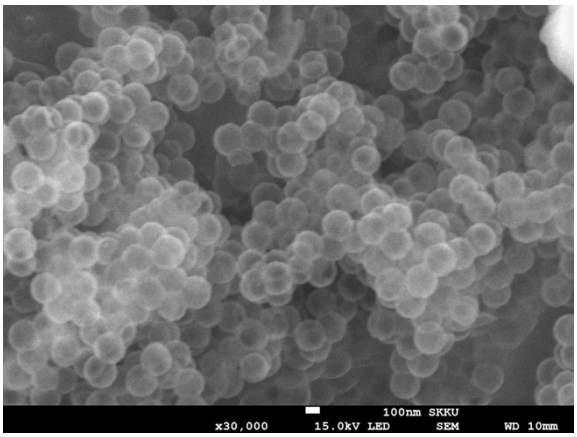


Fig. S5 DOX delivery tests (CLSM images) for normal cells (293T cell line) using free DOX, DOX/H-MON-FA and DOX/H-MON.

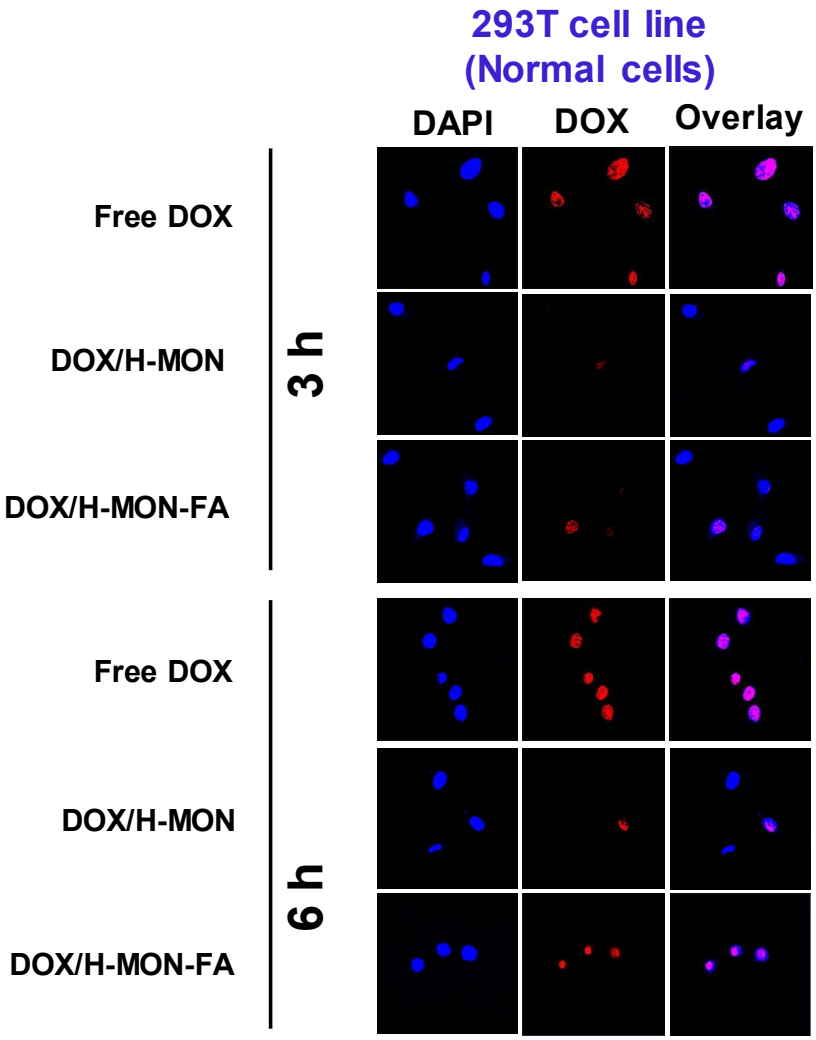


Fig. S6 DOX delivery tests (CLSM images) for breast cancer cells (MDA-MB-231 cell line) using DOX/H-MON-FA, DOX/H-MON, and free FA.

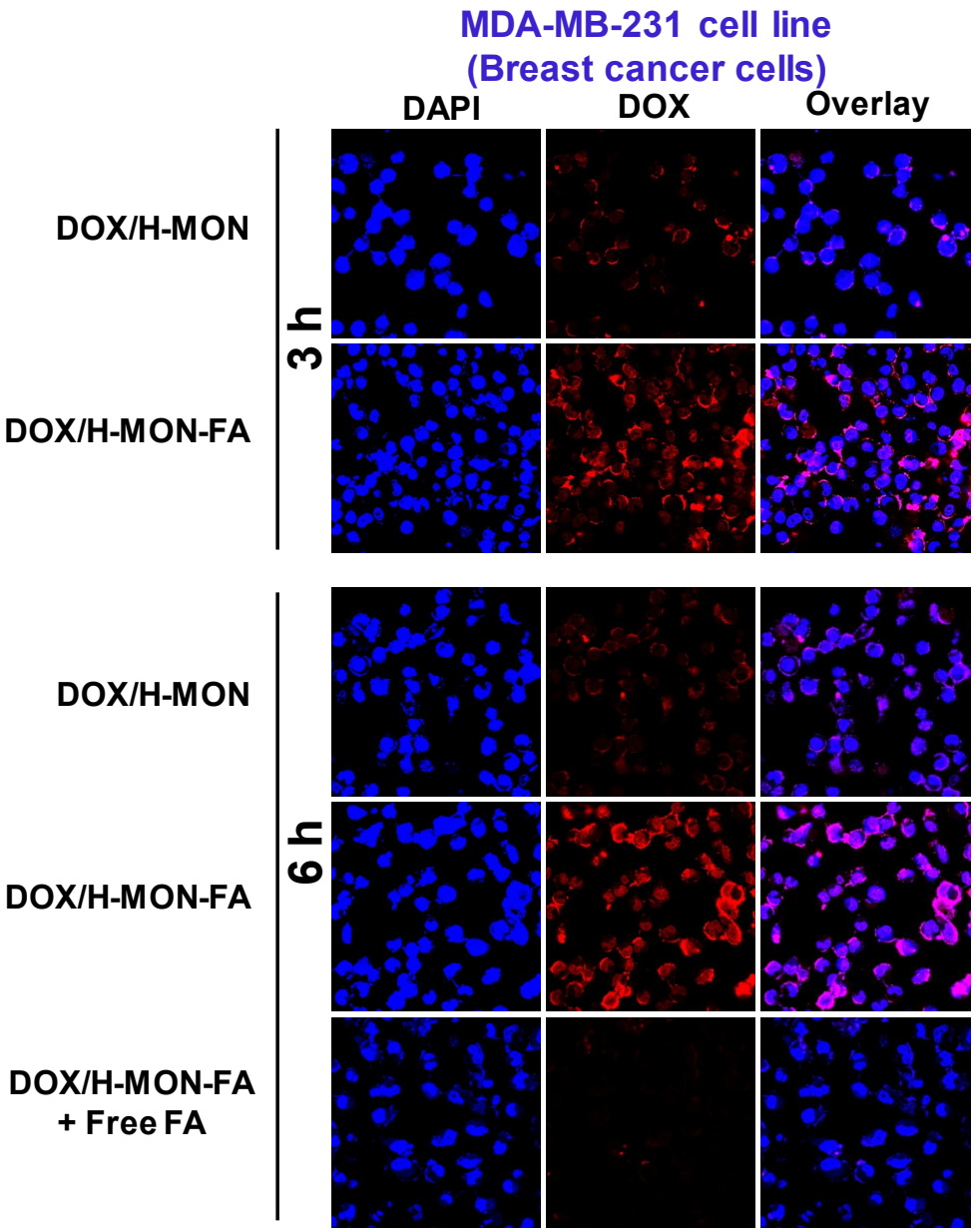


Fig. S7 Illustration of a DOX delivery to cancer cells by H-MON-FA.