Supporting Information

Modular Metal–Organic Polyhedra Super-Assembly: From Molecular-Level Design to Targeted Drug Delivery

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Section S1. General information

Reagents. All chemicals and reagents were used as received. 4-Pyridylboronic acid pinacol ester, tetrakis(triphenylphosphine) palladium(0), potassium phosphate, 1,4-dioxane, 2,5-dibromo-3-dodecylthiophene, chloroform, $Pd(BF_4)_2$, ethyl acetate, diethyl ether, polyoxyethylene (6) lauryl ether, polyoxyethylene (10) lauryl ether, polyoxyethylene (23) lauryl ether, dimethyl sulfoxide (DMSO), doxorubicin (DOX), sulforhodamine B, Mn(III)tetra (4-sulfonatophenyl) porphyrin, 6-aminocoumarin, fluorescein isothiocyanate, CdSe/ZnS quantum dots (QDs), Ham's F-12K (Kaighn's) medium, Iscove's modified Dulbecco's media (IMDM), and formaldehyde solution (36.5–38% in H_2O) were purchased from Sigma-Aldrich. Au NPs were synthesized according to the reported literature.[1] Epidermal growth factor (EGFR)-biotin and NeutrAvidin were purchased from Thermo Fisher Scientific. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] (ammonium salt)) (DSPE-PEG-2000-biotin) and 1,2-distearoyl-sn-glycero-3-phosphoeth-anolamine-N-[methoxy (polyethyleneglycol)-2000] (ammonium salt) (DSPE-PEG-2000) were purchased from Avanti Polar Lipids. Heat-inactivated fetal bovine serum (FBS), 10× phosphate-buffered saline (PBS), 0.5% trypsin-ethylenediaminetetraacetic acid (EDTA) solution, and penicillin-streptomycin (PS) were purchased from Gibco (Logan, UT). Dulbecco's modification of Eagle's medium (DMEM) was obtained from Corning Cellgro (Manassas, VA). Absolute (200 proof) ethanol was obtained from Pharmco-Aaper (Brookfield, CT). CellTiter-Glo 2.0 assay kit was purchased from Promega (Madison, WI). Hoechst 33342 were obtained from Thermo Fisher Scientific (Rockford, IL). 1× PBS, Alexa Fluor 488 phalloidin, and rhodamine phalloidin were purchased from Life Technologies (Eugene, OR). Milli-Q water with a resistivity of 18.2 MΩ cm was obtained from an inline Millipore RiOs/Origin water purification system.

Characterization. The morphology of the samples was characterized by field-emission gun scanning transmission electron microscopy (STEM, JEOL 2010F) at 200 kV and transmission electron microscopy (TEM, Hitachi H-7650) at 200 kV. Argon adsorption−desorption isotherms were obtained using a Quantachrome ASiQ2 instrument

at 87 K. ^{1}H NMR spectra were obtained using a JEOLJNM-ECA300 at 300 MHz. Atomic force microscopy (AFM) images were acquired using an Asylum Research MFP-3D™ AFM. UV-Vis absorption spectra were recorded using a Perkin-Elmer UV/vis Lambda 35 spectrometer. The fluorescence emission measurements were carried out using a fluorescence spectrometer (Perkin-Elmer LS55). Fluorescence images were acquired using a Zeiss LSM510 META (Carl Zeiss MicroImaging, Inc.; Thornwood, NY, USA) operated in channel mode of the LSM510 software. The software used for the optimization of the structure of $Pd_{24}L_{48}$-C_{12} MOP was Materials Studio 8.0. Due to the large coordination structure of $Pd_{24}L_{48}$-C_{12} MOP (more 3,000 atoms), only molecular mechanics (MM) simulation was used.

Section S2. Ligand 1 synthesis

For the synthesis of ligand 1, 2.10 g 4-pyridylboronic acid pinacol ester (9.80 mmol), 0.407 g tetrakis(triphenyl- phosphine)palladium(0) (0.352 mmol), and 5.95 g potassium phosphate (28.0 mmol) were first added into a three-neck flask. Under the protection of argon atmosphere, 70 mL 1,4-dioxane and 1.121 mL 2,5-dibromo-3-dodecylthiophene (3.61 mmol) were then added, and the suspension was stirred at 90 °C for 3 days. After cooling down, the residue was filtered and washed with chloroform. The filtrate was further purified by silica gel column chromatography to give a yellow solid.

Section S3. $Pd_{24}L_{48}$-C_{12} MOP synthesis

For the preparation of $Pd_{24}L_{48}$-C_{12}, 31.0 mg ligand 1 (76.4 μmol) was mixed with 8.80 mg $Pd(BF_4)_2$ (38.2 μmol) in a mixture of acetonitrile and chloroform (3:1 v/v) and reacted at 70 °C for 24 h. After cooling down, an excess amount of a mixture of ethyl acetate and diethyl ether (1:1 v/v) was added to the solution to promote precipitation. The precipitate was centrifuged (10,000 rpm, 10 min) and dried in vacuum to give the desired cage as a light yellow solid.

Section S4. MOP super-assembly

For the synthesis of single MOP@micelle, 10 mg mL^{-1} of polyoxyethylene (6) lauryl ether-based micelle was first prepared. Then, a small drop of MOP solution (0.038 mM in DMSO) was added into the micelle solution followed by sonication to promote encapsulation. For the synthesis of MOP_{sa}@micelle with different sizes, 1 mg mL^{-1} of polyoxyethylene (6) lauryl ether-based micelle was prepared and then different amounts of $Pd_{24}L_{48}$-C_{12} MOP (0.17 mM in DMSO) were added, followed by sonication to promote super-assembly. To vary the length of PEG outside the micelle, an alternative assembly unit, i.e. polyoxyethylene (10) lauryl ether or polyoxyethylene (23) lauryl ether, was used.

Section S5. Guest loading number calculation

The loading number of guest molecules inside $Pd_{24}L_{48}$-C_{12} MOP was determined by UV-vis spectroscopy. The fitting of the absorbance versus the concentration of the guest molecules and $Pd_{24}L_{48}$-C_{12} MOP was first carried out. The characteristic absorption wavelengths of $Pd_{24}L_{48}$-C_{12} MOP and the guest molecules (DOX, sulforhodamine B, and Mn(III)tetra (4-sulfonatophenyl) porphyrin) were found to be 365, 503, 561, and 467 nm, respectively. As shown in Figure 3a and Figure S6, the MOP of $Pd_{24}L_{48}$-C_{12} displayed no absorption at the characteristic absorption peak positions observed for the guest molecules. From the UV-vis spectra of the guest molecule-loaded MOP_{sa}@micelles, the concentration of various guest molecules can be determined. After subtraction of the absorption at 365 nm, originating from the guest molecules, the residual absorption at 365 nm that is attributed to the adsorption of $Pd_{24}L_{48}$-C_{12} cage can be used further for cage concentration fitting. Based on the above couple of steps, the loading number of the guest molecules inside $Pd_{24}L_{48}$-C_{12} MOP can be determined.

Section S6. Separation membrane fabrication

A microtube that contains porous polypropylene membrane was used. 100 μL $Pd_{24}L_{48}$-C_{12} MOP@micelle (1 mg mL^{-1}) solution was added into the microtube and then

centrifuged at 10,000 rpm for 10 min. This coating process was repeated for a couple of times until the desired thickness was achieved. During each coating, the membrane was washed by water once.

Section S7. Hemolysis assay

The purified red blood cells (RBCs) were incubated with different concentrations of particles at room temperature for 3 h in continuous rotating state. Double distilled (DI) water and 1× PBS containing purified RBCs were used as the positive and negative controls, respectively. Finally, the mixtures were centrifuged at 300 g for 3 min, and 100 μL supernatant of all samples was transferred to a 96-well plate. The absorbance of hemoglobin in the supernatant was measured by a BioTek microplate reader (Winooski, VT) at 540 nm. The hemolysis percentage of each sample was determined using the reported equation.[2] Percent hemolysis (%) = 100 × (Sample $Abs_{540\ nm}$ – Negative control $Abs_{540\ nm}$)/(Positive control $Abs_{540\ nm}$ – Negative control $Abs_{540\ nm}$).

Section S8. Drug release

For the drug release studies, 2 mg of DOX-loaded MOP_{sa}@micelle in 1.2 mL PBS buffer solution (pH 7.4 or 5.5) was loaded into a small tube at room temperature. During each time interval, the nanoparticles were centrifuged (20,000 rpm, 10 min), and half of the supernatant solution was withdrawn, followed by the addition of 0.6 mL fresh PBS buffer. The content of DOX was determined by UV-vis titration.

Section S9. Cell culture

Cell culture was performed using standard procedures (atcc.org). For adherent cells, HeLa (CCL-2) and A549 (CCL-185) were obtained from American Type Culture Collection (ATCC) and respectively stored in DMEM and F-12K media containing 10% FBS at 37 °C and 5% CO_2. Cells were passaged at approximately 80% confluency. For

coating purposes, living adherent cells (HeLa and A549) were removed from plate bottom using Trypsin-EDTA (0.25%) and then suspended in culture media.

Section S10. Cell viability testing

Cell viability of the constructed nanocarriers was assessed by CellTiter-Glo 2.0 assay. Briefly, single-MOP@micelle or MOP_{sa}@micelle nanocarriers were first diluted to a concentration of 50000 cells mL^{-1}. Then, 100 μL of the samples was added into a white 96-well plate. Subsequently, 100 μL CellTiter-Glo 2.0 reagent was dispensed into each well. The luminescence was recorded 10 min after addition of CellTiter-Glo 2.0 reagent by a BioTek microplate reader. Cell viability was calculated as a percentage of mammalian cells in the absence of nanocarriers.

Section S11. Anti-EGFR modification

First, polyoxyethylene (23) lauryl ether, DSPE-PEG-biotin, and DSPE-PEG were mixed at mol% ratio of 92:4:4, and then dried under high vacuum to remove the organic solvent. Then, the dried film was hydrated in 1× PBS, and the bath was sonicated for 30 min to obtain a micelle solution at a concentration of 1 mg mL^{-1}. Then, 150 μL fluorescent dye labeled $Pd_{24}L_{48}$-C_{12} MOP in DMSO was added to the micelle solution, followed by sonication to promote MOP super-assembly. The obtained MOP_{sa}@micelle-based nanocarriers were washed with 1× PBS twice. For anti-EGFR modification, 200 μL neutravidin protein (3 mg mL^{-1}) was added to the nanocarriers. After incubation for 30 min, the particles were centrifuged (20,000 rpm, 10 min), and the supernatant was removed. After redispersion in 1× PBS, 200 μL biotin-EGFR (0.2 mg mL^{-1}) was added and incubated at room temperature for 30 min. After washing with 1× PBS twice and redispersion in 100 μL PBS, the antibody-conjugated nanocarriers can be directly used for in vitro targeting experiments.

Section S12. In vitro targeting

For the studies, 2×10^5 A549 (CCL-185, ATCC) cells in 6-well plates with 2 mL F-12K media containing 10% FBS and 1% PS were seeded and then incubated at 37 °C in 5% CO_2-humidified atmosphere. After 24 h, the media was removed and replaced with 1 mL fresh complete cell culture media supplemented with 50 μg mL^{-1} of EGFR-modified or unmodified MOP_{sa}@micelles for different times at 37 °C under 5% CO_2-humidified atmosphere. After incubation, the media was removed, and the cells were gently washed twice with 1× PBS. For imaging purposes, the treated cells were fixed in 4% paraformaldehyde (in 1× PBS) at room temperature for 15 min, washed with 1× PBS twice, and then stored in 1 mL 1× PBS. The cell nuclei and F-actin were stained with 1 mL Hoechst 33342 (3.2 μM in 1× PBS) for 30 min and 200 μL Alexa Fluor488 phalloidin (20 nM in 1× PBS) for 45 min, respectively. After staining, the cells were washed with 1× PBS twice and stored in 1× PBS prior to fluorescence microscope imaging.

Section S13. Pharmacokinetics and biodistribution studies

All the animal procedures complied with the guidelines of the University of New Mexico Institutional Animal Care and Use Committee. The experiments were performed on female Albino C57BL/6 mice (6 weeks). To evaluate the circulation half-life, 150 μL of CdSe/ZnS QD (627 nm)-labeled MOP_{sa}@ micelles (1 mg/mL) were injected into the eye of the mice. The blood was collected at 0.5, 1, 2, 6, 12, 24, and 48h following the injection. Each time point group contained three mice. The collected blood samples were diluted with the same amount of 1X PBS before fluorescence measurement. Particle retention in circulation at these time points was determined by measuring the fluorescence on a BioTek microplate reader (Winooski, VT). Pharmacokinetics parameters were calculated to fit a non-compartment model.

To study the related biodistribution in various tissues, 150 μL of CdSe/ZnS QD (627 nm)-labeled MOP_{sa}@ micelles (1 mg/mL) were retro-orbital injected to mice. At each of the 6, 12, 24, and 48 h time points following the NP injection, three mice were randomly

selected and euthanized. Their spleen, liver, heart, lung, and kidneys were collected. The collected organs were examined with an IVIS fluorescence imaging system (Xenogen, Alameda, CA), and the fluorescence intensity of the MOP particles in different organs was further semi-quantified by the IVIS imaging software.

Section S14. Supplementary Figures

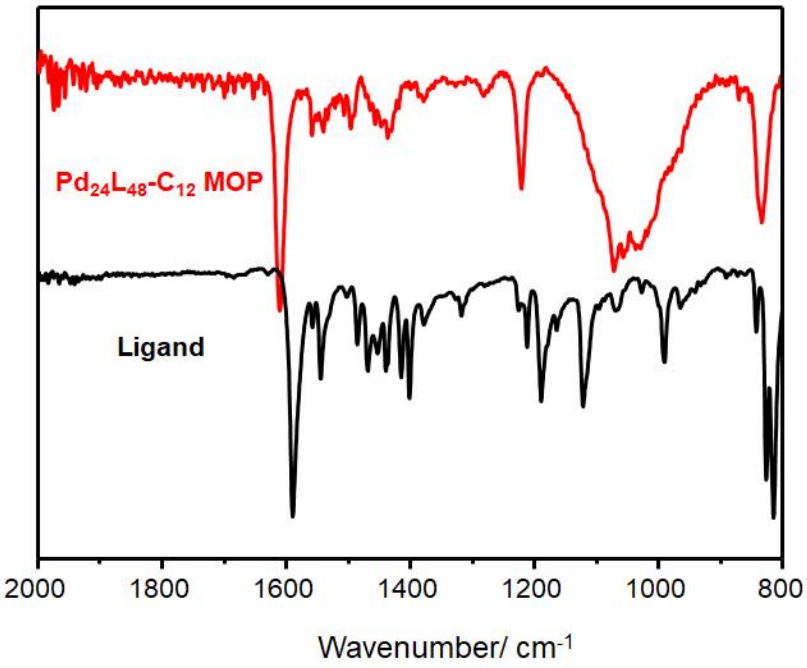

Figure S1 | Fourier transform infrared spectrophotometry (FT-IR) of organic ligand and the formed $Pd_{24}L_{48}$-C_{12} MOP.

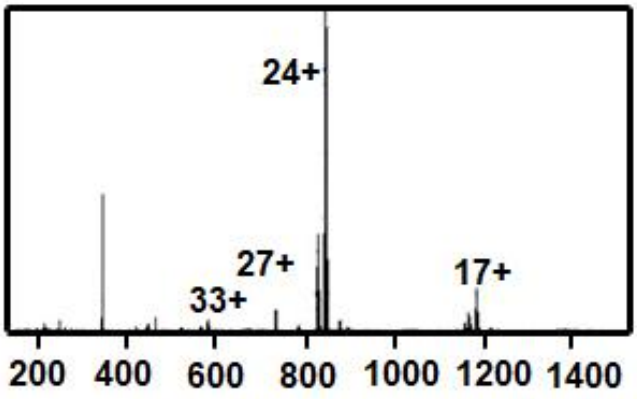

Figure S2 | Electrospray ionization mass spectrometry pattern of the molecular cage $Pd_{24}L_{48}$ with no hydrophobic alkane chains decoration.

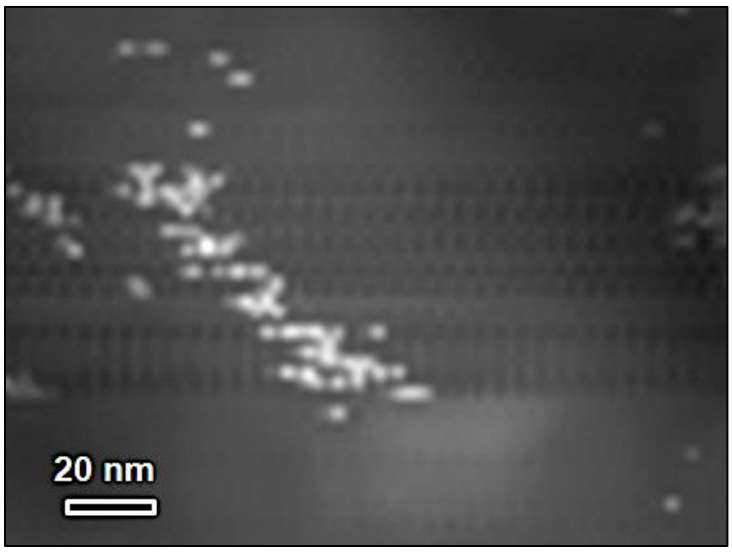

Figure S3 | AFM image of the molecular cage $Pd_{24}L_{48}$-C_{12} MOP on silicon substrate.

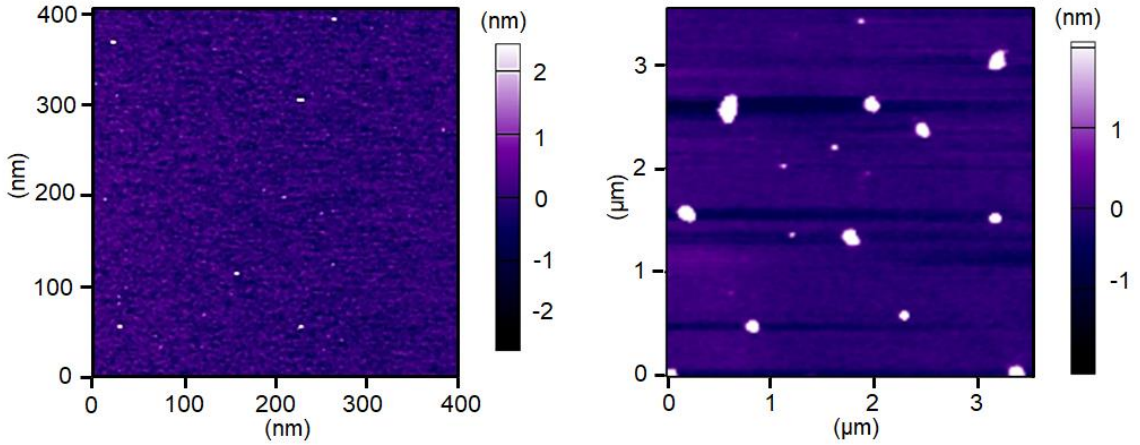

Figure S4 | AFM images of MOP_{sa}@micelles with different sizes.

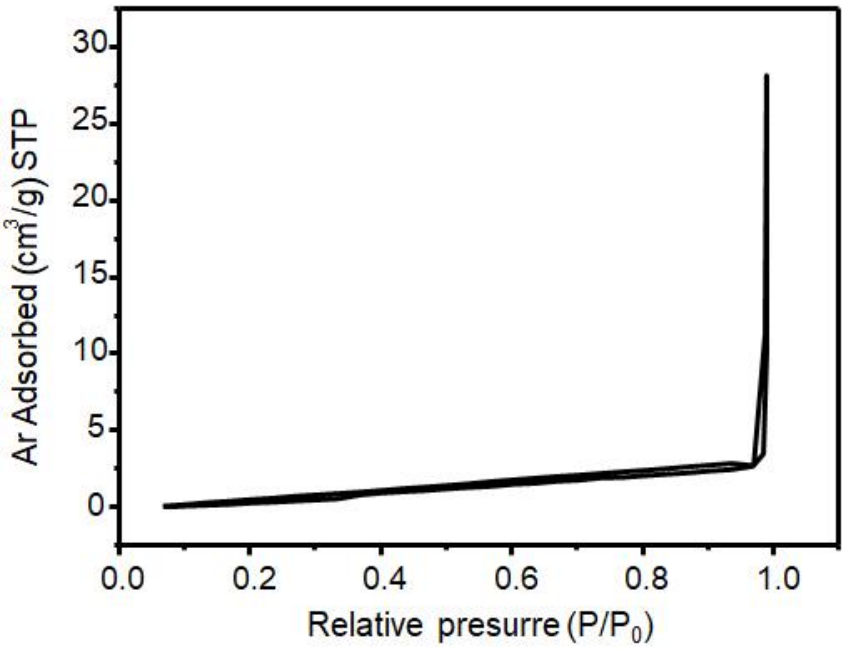

Figure S5 | Ar sorption isotherm of the MOP_{sa}@micelle.

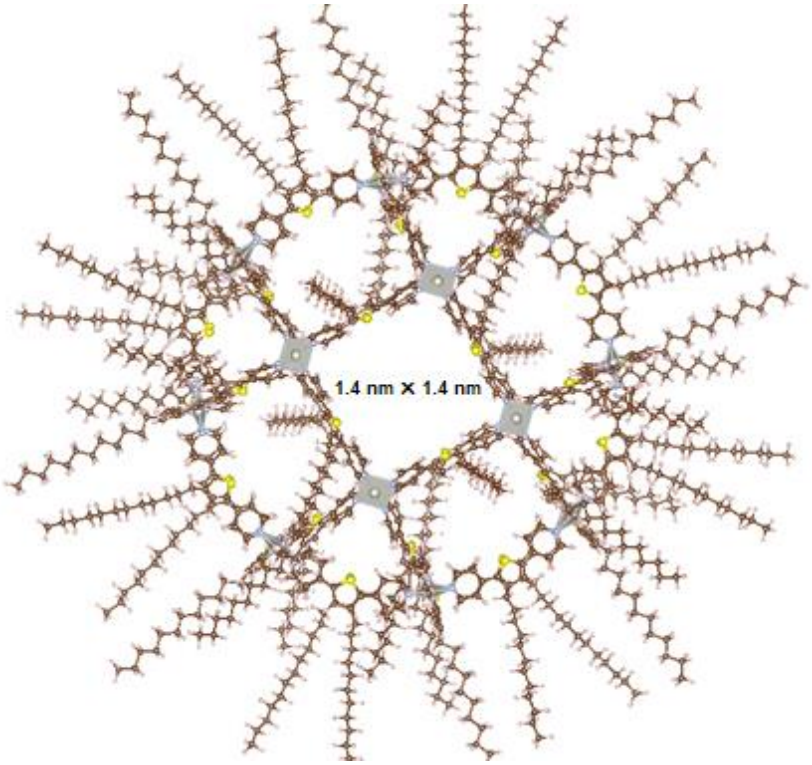

Figure S6 | Optimized structure of $Pd_{24}L_{48}$-C_{12} MOP based on molecular mechanics calculation with a relative large pore window of 1.4 nm × 1.4 nm.

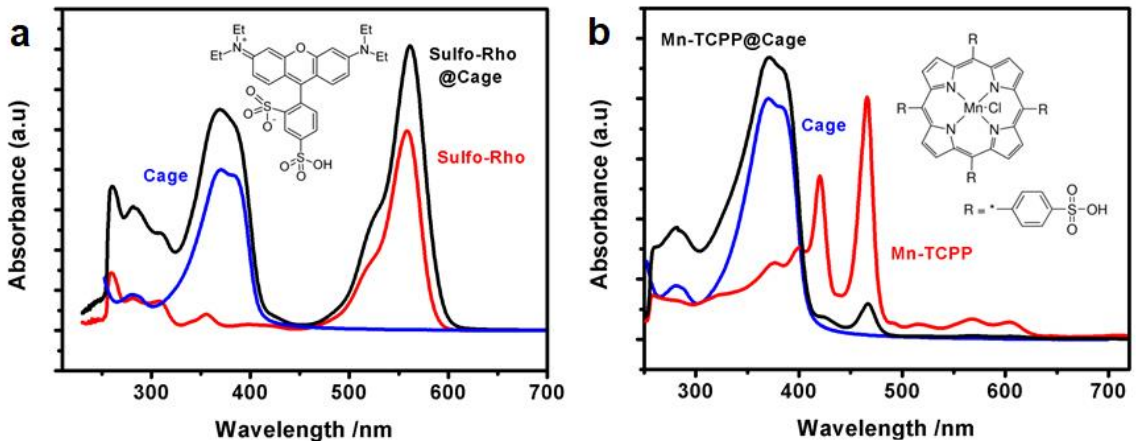

Figure S7 | UV-Vis spectra of the dye-loaded $Pd_{24}L_{48}$-C_{12} MOP and free dyes: (a) sulforhodamine B and (b) Mn-TCPP.

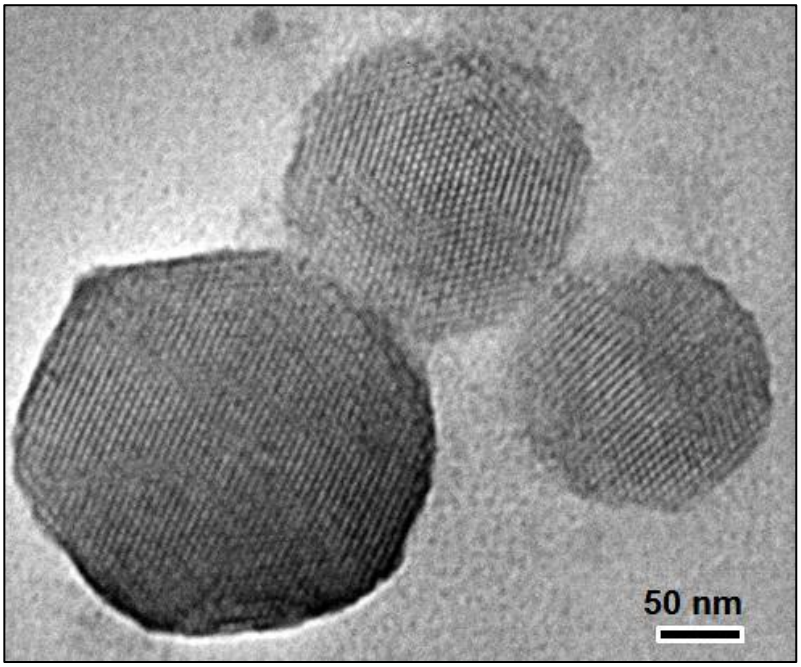

Figure S8 | TEM image of DOX-loaded MOP_{sa}@micelle.

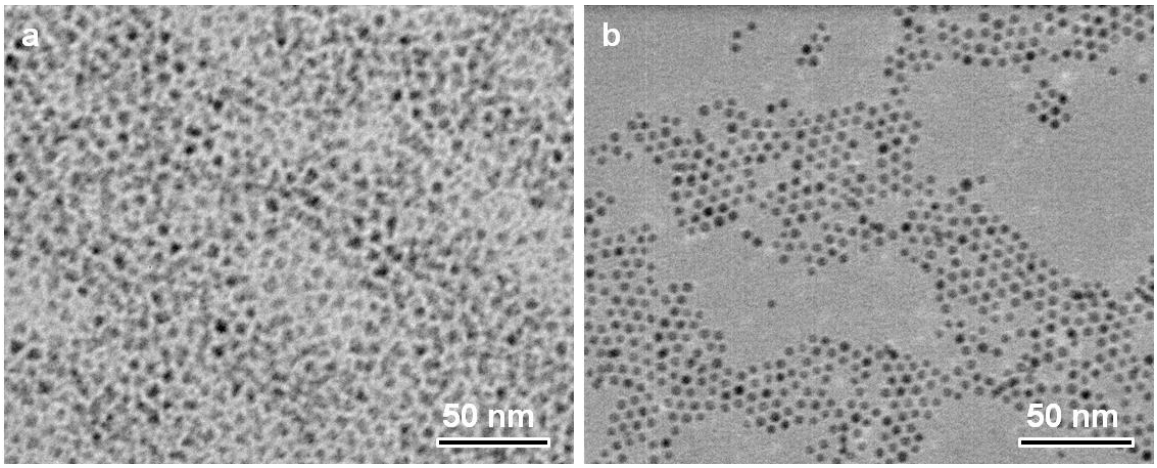

Figure S9 | TEM image of the commecial CdSe/ZnS quantum dots (a) and the synthesized Au NPs (b).

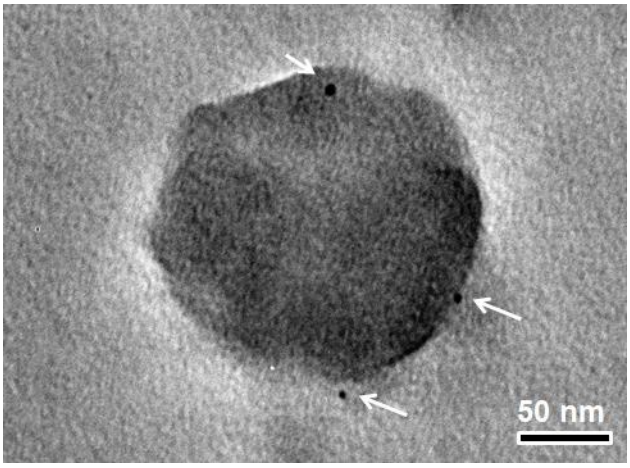

Figure S10 | TEM image of the Au NPs@MOP_{sa}@micelle. The Au NPs were pointed out by arrows.

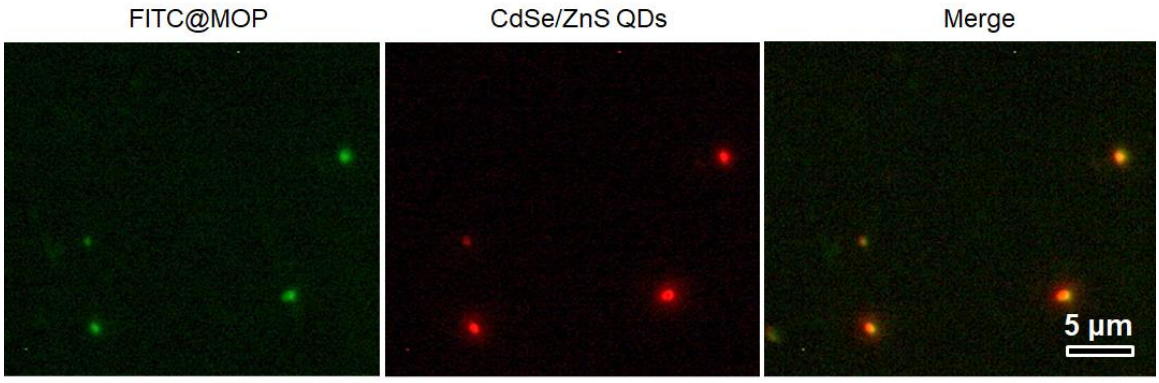

Figure S11 | Fluorescent image of the CdSe/ZnS quantum dot@ MOP_{sa}@ micelles with fluorescein isothiocyanate dye previously loaded in MOP nanocavities. The overlapping of the fluorescent points from different channels confirms the successful doping of quantum dots in MOP_{sa}@micelles.

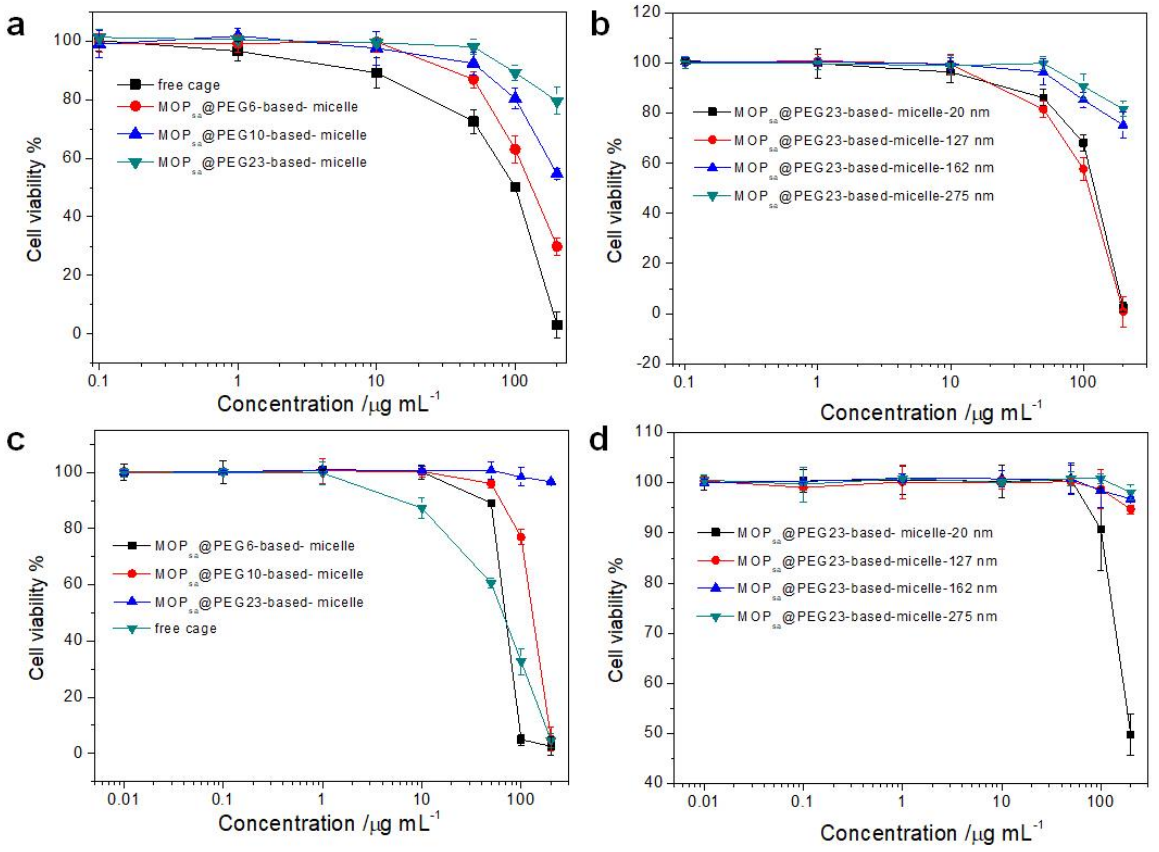

Figure S12 | Cytotoxicity profiles of single MOP-supported micelle and MOP super-assembly-supported micelles with different PEG chain lengths and sizes against (a,b) A549 and (c,d) HeLa cells.

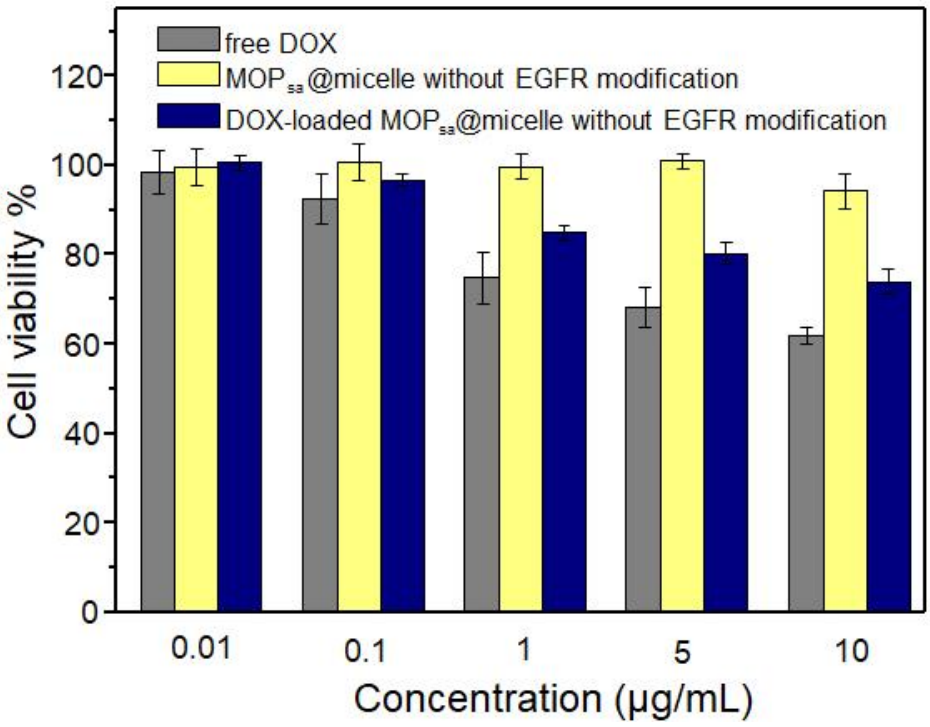

Figure S13 | Sustained viability of A549 cells after incubation of free DOX, MOP$_{sa}$@micelle without EGFR modification, and DOX-loaded MOP$_{sa}$@micelle without EGFR modification for 2 h.

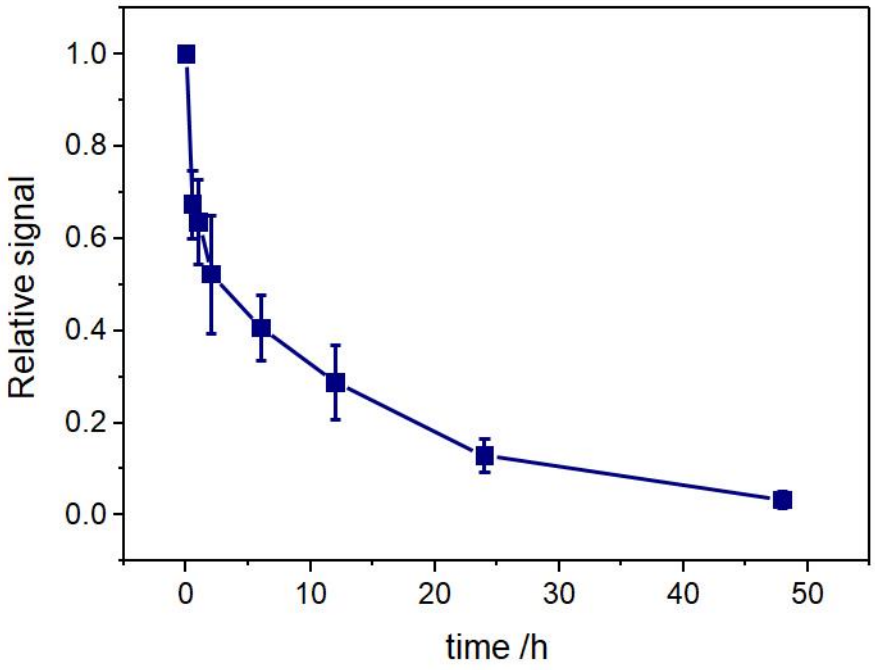

Figure S14 | The circulation of the created MOP_{sa}@ micelle NPs in mice.

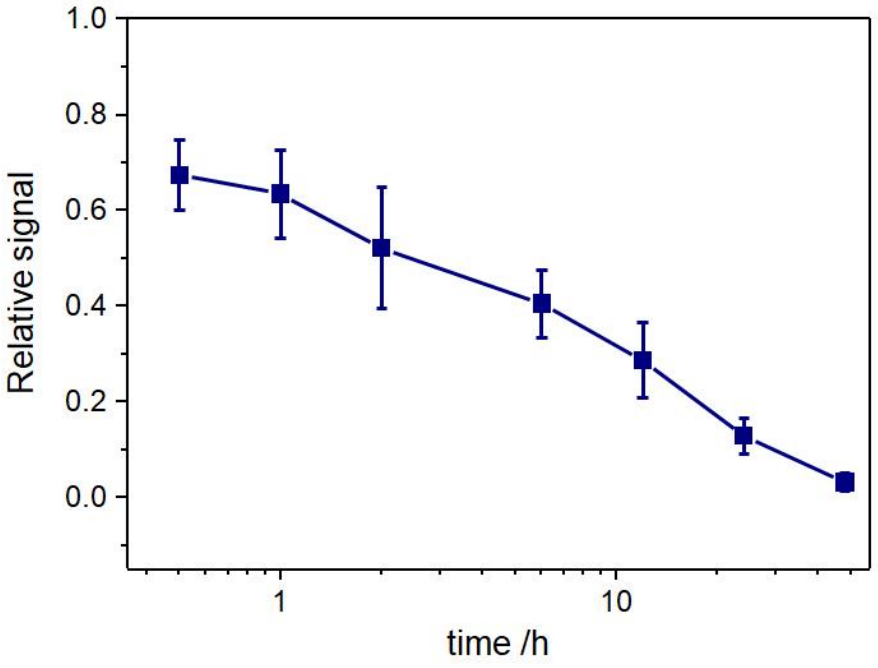

Figure S15 | Semilog plot of the circulation of the created MOP_{sa}@ micelle NPs in mice.

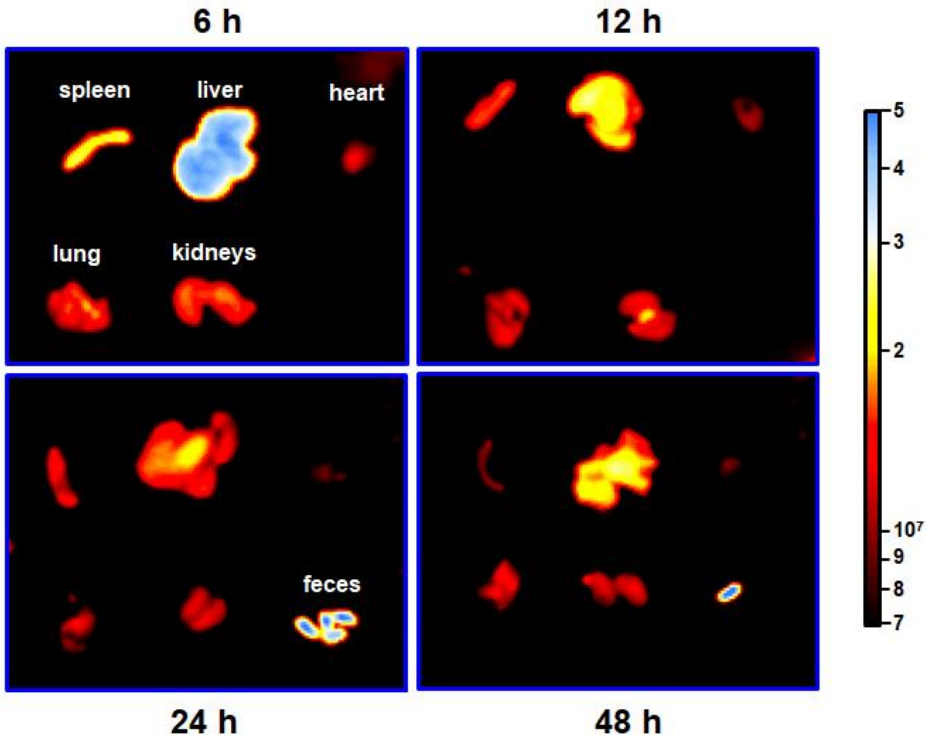

Figure S16 | Fluorescence images of different organs at 6, 12, 24, and 48 h after intravenous administration of the MOP_{sa}@ micelle NPs.

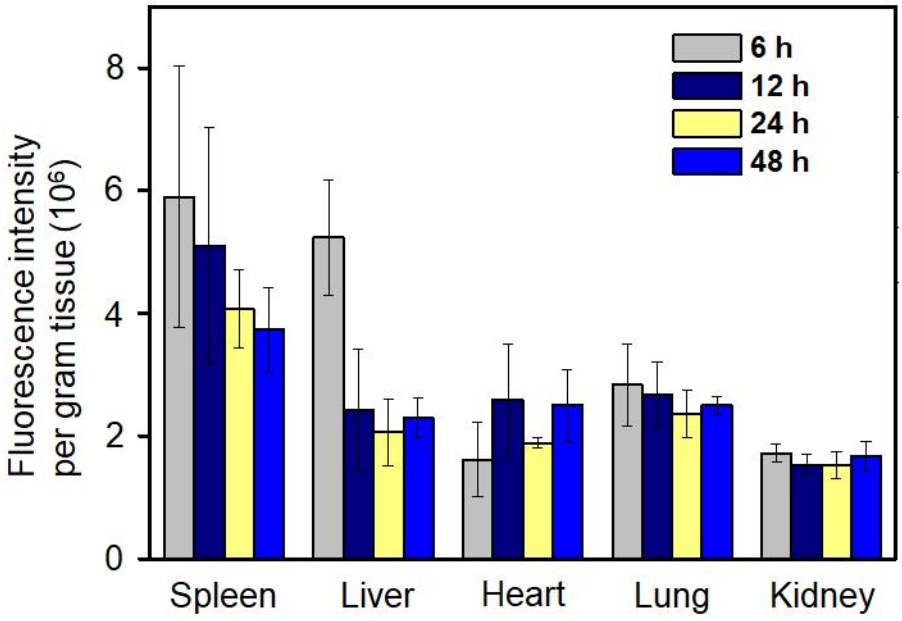

Figure S17 | Fluorescence intensity per gram of tissue at 6, 12, 24, and 48 h after intravenous administration of the MOP_{sa}@ micelle NPs.

Section S15. Supplementary references

[1]. S. Xiong, D. R. Dunphy, D. C. Wilkinson, Z. Jiang, J. Strzalka, J. Wang, Y. Su, J. J. Pablo, C. J. Brinker, *Nano Lett.* **2013**, *13*, 1041.

[2]. Y. Lin, C. L. Haynes, *J. Am. Chem. Soc*. **2010**, *132*, 4834.