Supporting Information

Ligand-Switchable Micellar Nanocarriers for Prolonging Circulation Time and Enhancing Targeting Efficiency

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Methods

Synthesis of Block Copolymers.

PEG-*b*-PCL. PEG-*b*-PCL was synthesized as shown in Figure S1. The degree of polymerization (DP) of CL was estimated to be 44 by calculating the peak integration ratio of -OCH_2CH_2- protons of PEG at 3.59 ppm and –COCH_2- protons of PCL at 2.30 ppm. The polymer dispersity index (PDI) is about 1.15 (GPC using THF as eluent).

RGD-PEG-*b*-PCL. HOOC-PEG-*b*-PCL was synthesized as shown in Figure S1. In brief, HOOC-PEG-OH (0.5 g, 0.1 mmol) and ε-CL(0.60 g, 5.3 mmol) were dissolved in 15 mL of toluene, followed by addition of one drop of $Sn(Oct)_2$ into the solution. After freeze-degas-thaw cycles for three times, the reaction mixture was stirred at 110 °C for 12 h. Then the solvent was precipitated into excess diethyl ether to obtain the crude product. The precipitate was dried under vacuum. As shown in Figure S3a, DP of CL was estimated to be 44 by calculating the peak integration ratio of -OCH_2CH_2- protons of PEG at 3.59 ppm and –COCH_2- protons of PLys at 2.30 ppm. The PDI is about 1.21. HOOC-PEG_{5k}-*b*-PCL_{5k} (100 mg, 10 μmol) was dissolved in 5 ml of DMSO, and then EDC·HCl (10 mg) and NHS (2.5 mg) were added into the solution. After stirring at room temperature for 2 h, the mixture was added into the solution of cRGDfk (15 mg, 25 μmol) and TEA (10 μL) in 3 ml of DMSO. The reaction solution was stirred at room temperature for 24 h. And then the whole solution was dialyzed against deionized water for 2 days, followed by lyophilisation to obtain the RGD-PEG-PCL. As shown in Figure S3b, the characteristic peak of RGD was at 7.20-7.30 ppm. The degree of modification was calculated to be 80% comparing with the characteristic peak of PEG.

NHS-PAE-*b*-PCL. NHS-PAE-*b*-PCL was synthesized by a Michael-type addition polymerization of PCL monoacrylate, HDD and TDP as shown in Figure S2. To obtain PCL monoacrylate, 2-hydroxyethyl acrylate (0.093 g, 0.80 mmol) and ε-CL (5.0 g, 43.8 mmol) was dissolved in 15 mL of toluene, and then one drop of $Sn(Oct)_2$ was added into the solution. After freeze-degas-thaw cycles for three times, the reaction mixture was stirred at 110 °C for 12 h. Then the solvent was precipitated into excess diethyl ether to obtain the crude product. The PCL monoacrylate was dried under vacuum. According to the ^1H NMR result, the Dp of PCL monoacrylate was determined to be 53 by comparing of the peak integration of -C*H*=CH_2 protons of acrylate around 6.0 ppm and -CH_2CO protons of PCL at 2.30 ppm. Then, PCL monoacrylate (0.6 g, 0.1 mmol), HDD (0.678 g, 3 mmol) and TDP (0.682 g, 3.15 mmol) were dissolved in 8 ml of $CHCl_3$. After stirring at 55 °C for 3 days, NASI (25 mg, 0.15 mmol) was added in the reaction mixture. To obtain NHS-PAE-*b*-PCL, the whole solution was precipitated into excess diethyl ether. The precipitate was dried under vacuum. As shown in Figure S3. The DP of PAE was estimated to be 18. The PDI is about 1.42.

RGD-PAE-*b*-PCL. NHS-PAE-*b*-PCL (150 mg, 10 μmol) was dissolved in the solution of 3 ml of $CHCl_3$ and 5 ml of DMSO. And then cRGDfk (15 mg, 25 μmol) and TEA (20 μL) were added in the solution of NHS-PAE-PCL. After stirring at 35 °C for 12 h, the reaction temperature was adjusted to 55 °C and kept for 8 h. The whole solution was transferred to a dialysis bag (MWCO 3500) and dialyzed against distilled water for 3 days. RGD-PAE-*b*-PCL was obtained by lyophilisation. The degree of modification was calculated to be 50% as shown in Figure S3.

Cy5-PEG-*b*-PCL. BOC-NH-PEG-*b*-PCL was synthesized through ROP using BOC-NH-PEG-OH as the initiator. Briefly, BOC-NH-PEG_{2k}-OH (0.5 g, 0.25 mmol) and ε-CL(0.60 g, 5.3 mmol) were dissolved in 15 mL of toluene, followed by addition of one drop of $Sn(Oct)_2$ into the solution. After freeze-degas-thaw cycles for three times, the reaction mixture was

stirred at 110 °C for 12 h. Then the solvent was precipitated into excess diethyl ether to obtain the crude product. The precipitate was dried under vacuum. Afterward, a Boc deprotection reaction was performed by addition of 4 ml CF_3COOH to the solution of BOC-NH-PEG-*b*-PCL (0.4 g, 0.1 mmol) in CH_2Cl_2 (4 ml) for 24 h at 30 °C, then the solvent was removed under reduced pressure, the crude product was purified by dissolving in appropriate methanol and evaporating under vacuum twice at 25 °C. The product was dissolved in CH_2Cl_2 and precipitated into excess diethyl ether to obtain the NH_2-PEG-*b*-PCL. According to the ^1H NMR result (not shown), the Dp of PCL monoacrylate was determined to be 20. NH_2-PEG-*b*-PCL (30 mg, 6.9 μmol) and TEA (10 μL) were dissolved in 5 ml of DMSO, followed by the addition of Cy5 (3 mg, 4 μmol) in 1 ml of DMSO. After stirring at room temperature for 24 h, the whole solution was dialyzed against deionized water for 3 days in the dark. Cy5-PEG-*b*-PCL was obtained by lyophilisation.

Reversible and Rapid Hiding/Exposing of Targeting Ligand. The results of the second cycle for the micelles alternatively immersed in phosphate buffer at pH 7.4 and 6.5 were +3.81 mV at pH 6.5 and -7.82 mV at pH 7.4.

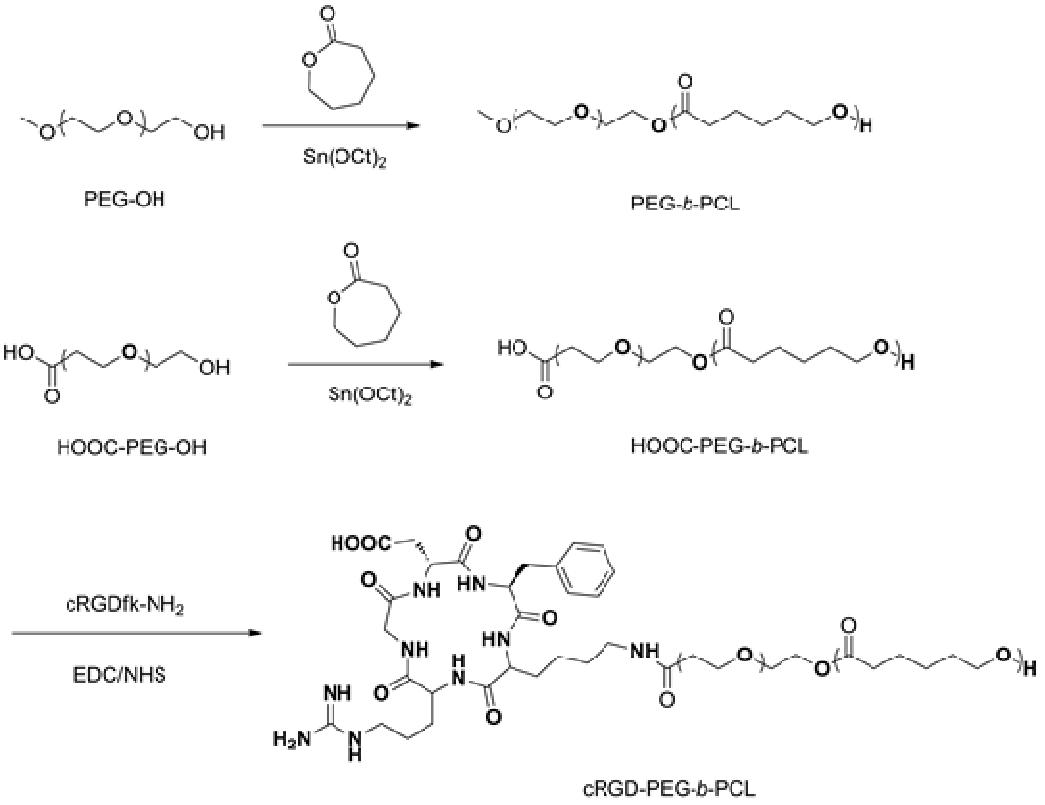

Figure S1. Synthesis of PEG-*b*-PCL and RGD- PEG-*b*-PCL.

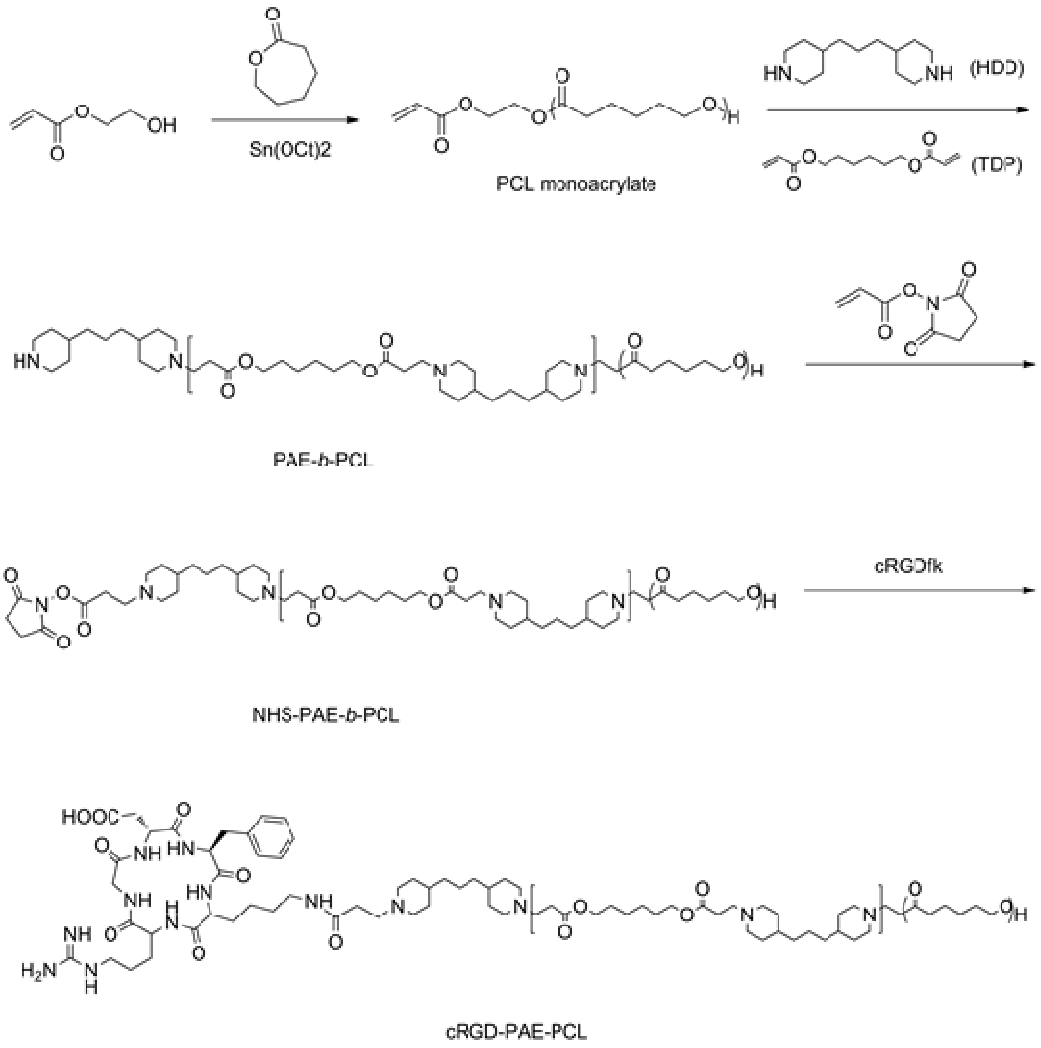

Figure S2. Synthesis of PAE-b-PCL and RGD-PAE-b-PCL.

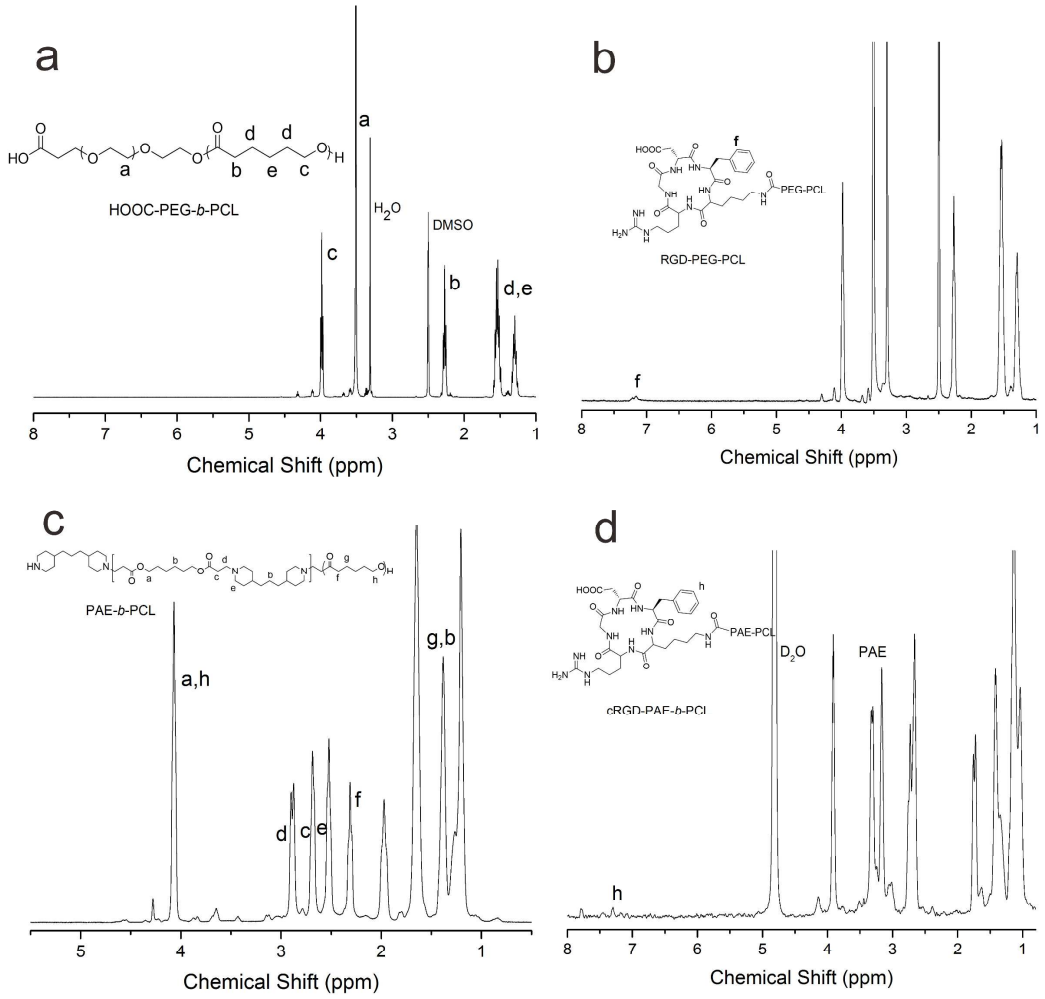

Figure S3. ^{1}H NMR spectra of polymers. (a) HOOC-PEG-*b*-PCL in DMSO-d_6. (b) RGD-PEG-*b*-PCL in DMSO-d_6. (c) PAE-*b*-PCL in $CDCl_3$. (d) RGD-PAE-*b*-PCL in D_2O with addition of DCl.

Table S1. Formulations of micelles.

micelle	PEG_{5k}-PCL_{5k} (mg)	$RGD_{0.8}$-PEG_{5k}-PCL_{5k} (mg)	$PAE_{7.6k}$-PCL_{6k} (mg)	$RGD_{0.5}$-$PAE_{7.6k}$-PCL_{6k} (mg)
SSPM	5.0	0	0	0
$SSPM^{RGD}$	4.5	0.5	0	0
MSPM	2.6	0	2.4	0
$MSPM^{RGD}$	2.1	0.5	2.4	0
$MSPM^{SRGD}$	2.6	0	1.4	1.0

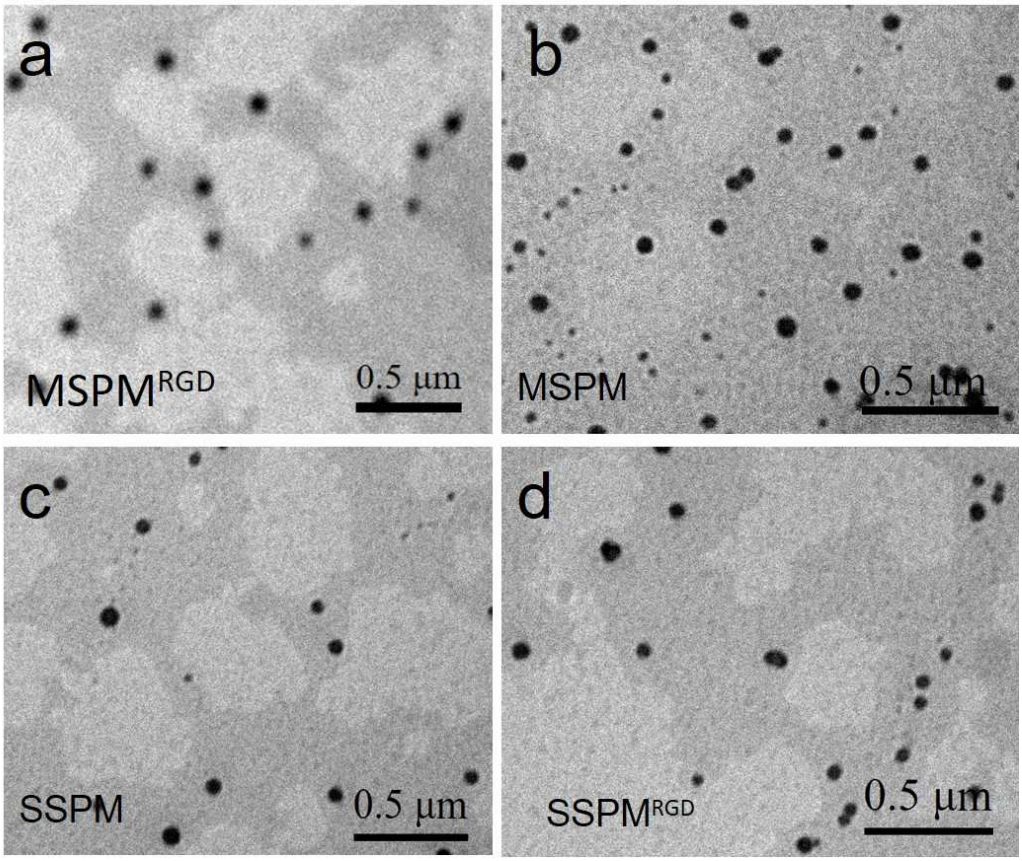

Figure S4. TEM image of different micelle formulations. (a) $MSPM^{RGD}$/pH 7.4. (b) MSPM /pH 7.4. (c) SSPM/pH 7.4. (d) $SSPM^{RGD}$/pH 7.4.

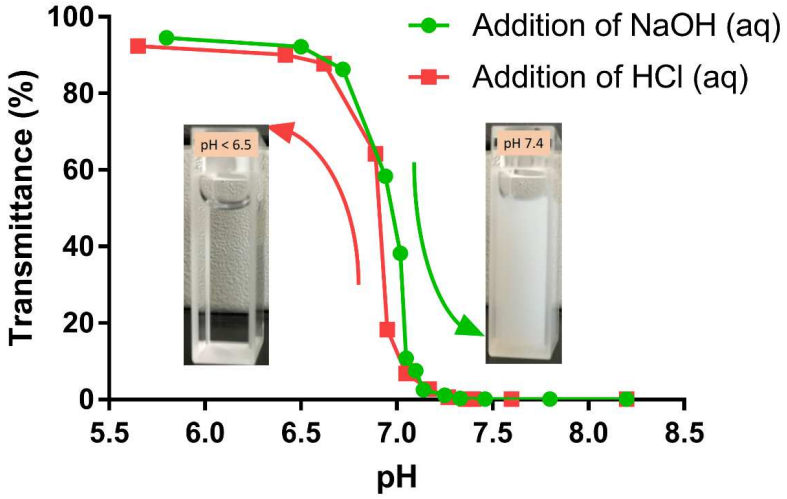

Figure S5. The transmittance of PAE solution with the addition of NaOH (0.25 M) and HCl aqueous solution.

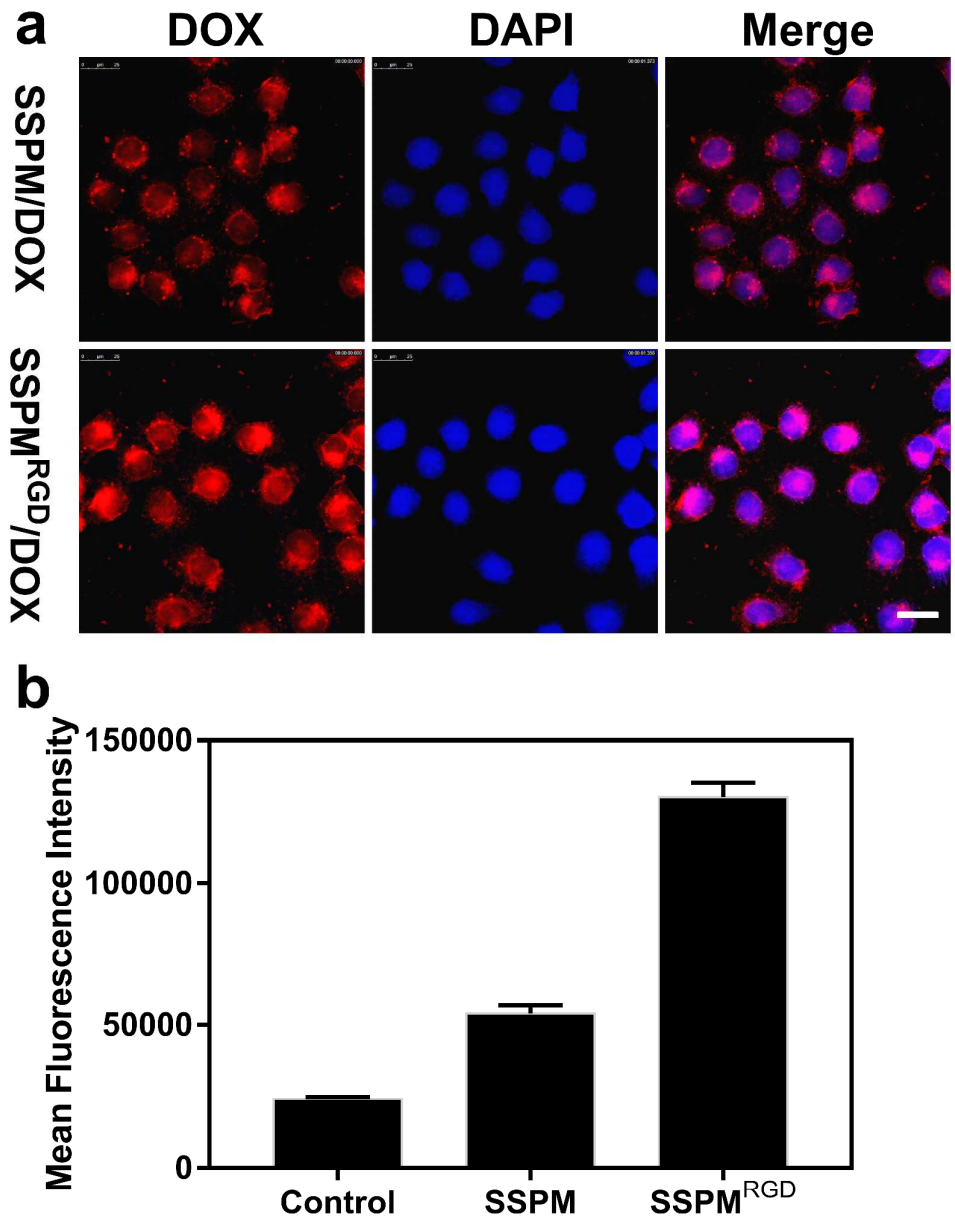

Figure S6. (a) Inverted fluorescent microscopy image of HepG2 cells after incubation with SSPM/DOX and SSPM^{RGD}/DOX at pH 7.4 for 2h. The cell nuclei was stained by DAPI (blue). (b) The flow cytometric analysis of SSPM/DOX and SSPM^{RGD}/DOX at pH 7.4.

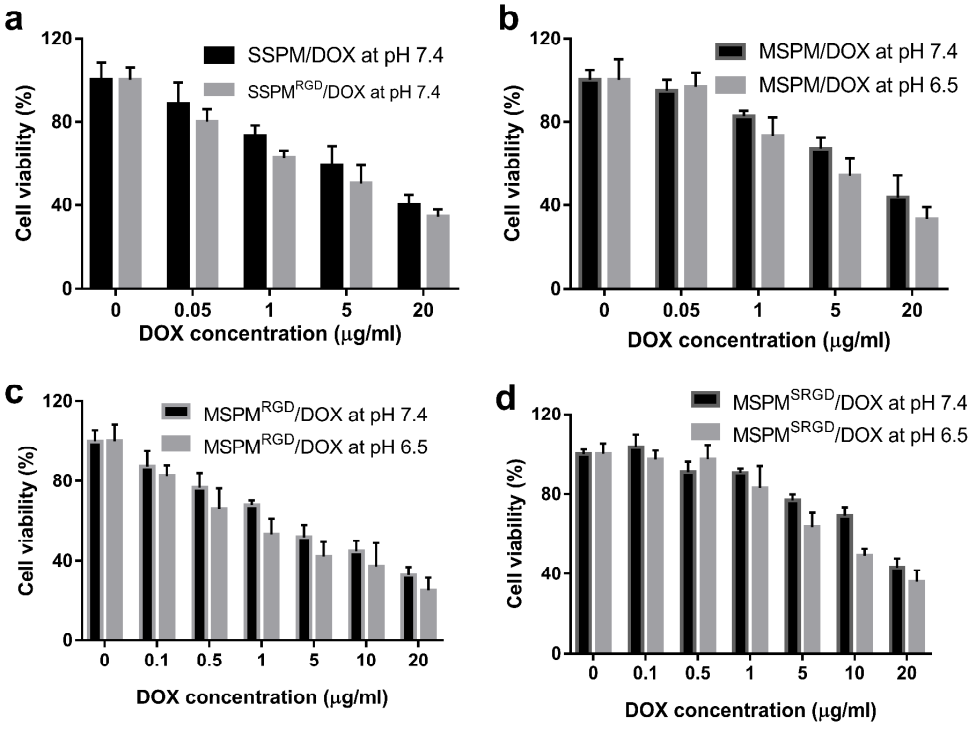

Figure S7. Cytotoxicity of DOX loaded micelles. (a) SSPM/DOX and SSPM^{RGD}/DOX incubated with HepG2 Cells at pH 7.4. (b,c,d) DOX loaded micelles incubated with HepG2 cells at pH 7.4 and 6.5 for MSPM/DOX (b), MSPM^{RGD}/DOX (c) and MSPM^{SRGD}/DOX (d).

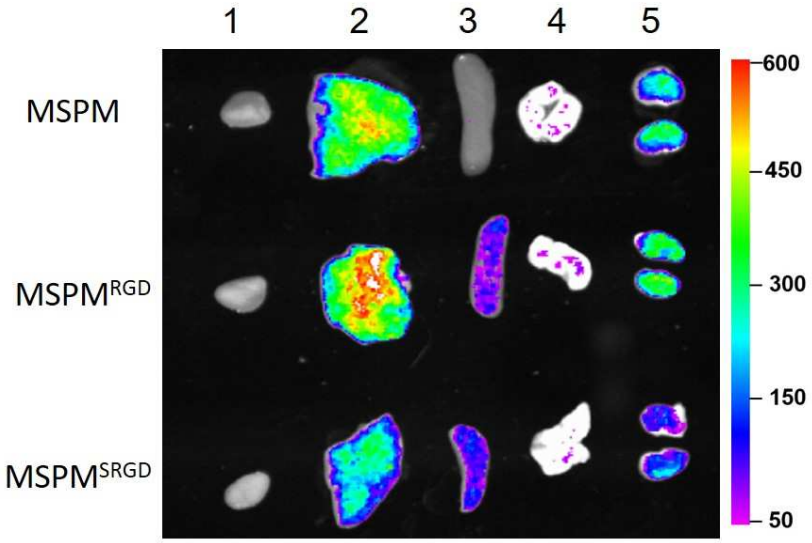

Figure S8. *Ex vivo* fluorescence imaging of major organs harvested from the HepG2 tumor-bearing nude mice at 6 h post-injection. The numeric label for each organ is as follows: 1, heart; 2, liver; 3, spleen; 4, lung; 5, kidney.

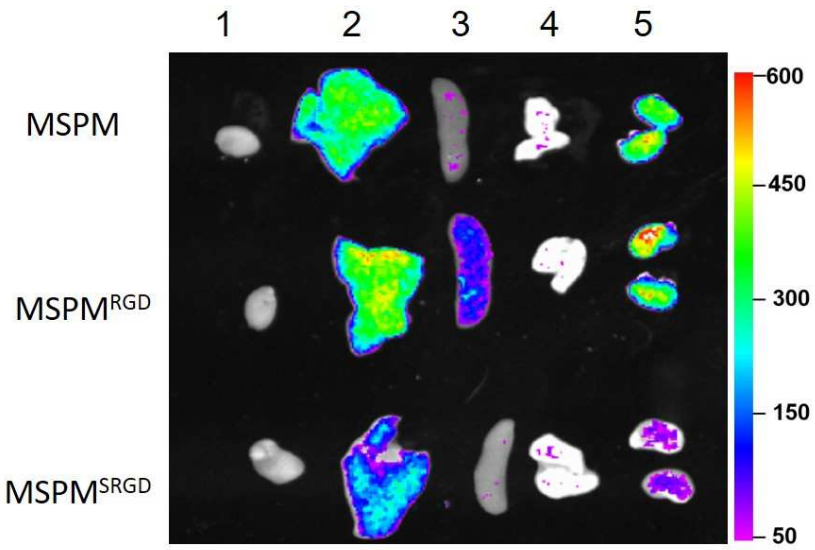

Figure S9. *Ex vivo* fluorescence imaging of major organs harvested from the HepG2 tumor-bearing nude mice at 24 h post-injection. The numeric label for each organ is as follows: 1, heart; 2, liver; 3, spleen; 4, lung; 5, kidney.

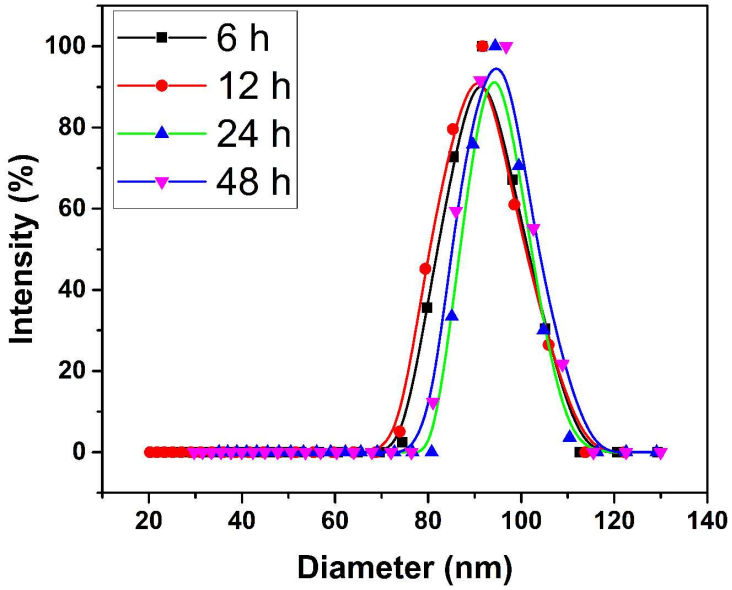

Figure S10. The stability of MSPM^{SRGD} under physiological condition.