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Supporting Information

ROS-Sensitive Micelles for Controlled Delivery of Antibiotics to Combat Intracellular *Staphylococcus aureus*-Associated Infections

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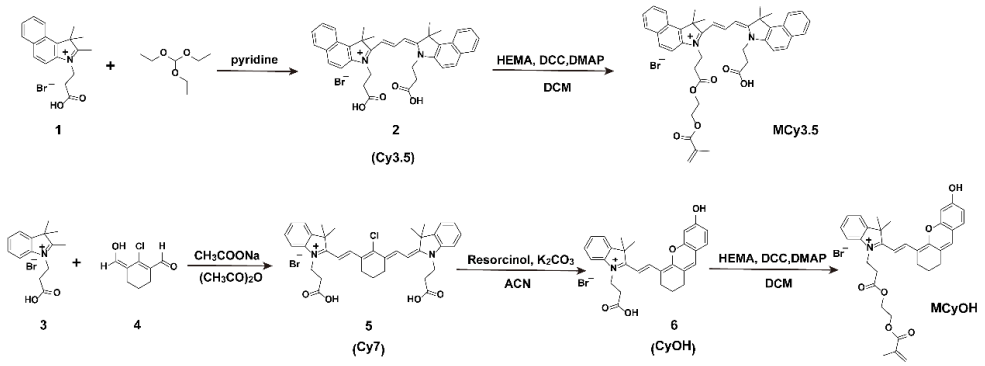
1. Materials and Instruments

Resorcinol, sodium acetate, orthoformic acid triethyl ester, hydroxyethylmethacrylate (HEMA), dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), Poly(ethylene glycol) methyl ether (Mn 5000), acryloyl chloride were purchased from Millipore-Sigma. 4-(hydroxymethyl) boronic acid and pinacol were obtained from TCI. Other chemicals were purchased from Fisher Scientific without purification.

^{1}H NMR spectra were recorded using a Bruker Advance 400 MHz spectrometer with deuterated chloroform ($CDCl_3$), deuterium dimethyl sulfoxide (d_6-DMSO) and Deuterium Oxide (D_2O) as the solvent. UV−Vis spectra were recorded with a Spectramax® Plus 384 Microplate Reader (Molecular Devices). Confocal laser scanning microscopy (CLSM) images of samples were captured with an LSM710 confocal microscope (Carl Zeiss). Transmission electron microscopy (TEM) was performed on a JEOL-JEM1011 instrument with an acceleration voltage of 120 kV, and scanning electron microscopy (SEM) was peformed on FEI Quanta 200 equipped with accelerating voltage (20 kV). Particle size and Zeta potential were measured with a Zeta-sizer Nano ZS instrument (Malvern).

Molecular weight distribution of the polymers was characterized via gel permeation chromatography (GPC) calibrated with respect to polystyrene standards on a Shimadzu LC-20 series liquid chromatography instrment. GPC data was analyzed with Shimadzu LCsolution GPC postrun software. DMF was used as the eluent for all polymers characterized (flow rate of 0.5 mL/min, room temperature).

2. Synthesis



Scheme S1. Synthetic route for preparing fluorescent monomers mCy3.5 and mCyOH.

2.1 Compound 2 (Cy 3.5)

Compound 1[1] (100 mg, 0.277 mmol) was dissolved in pyridine (1.0 mL) under a N_2 protective atmosphere, heated to 100° C, slowly combined dropwise with orthoformic acid triethyl ester (0.1 mL, 0.5 mmol), and heated for another 2 h at 100 ℃. After cooling to room temperature, the reaction mixture was precitated with diethyl ether and purified on a silica column using a mixed eluent of dichloromethane/methanol (10:1) to give compound 2 to as a purple solid.

2.2 Monomer mCy3.5

Compound 2 (1.3g, 2.0 mmol) and 2-hydroxyethyl methacrylate (HEMA, 312 mg, 2.4 mmol) were dissolved in dry DCM under an N_2 atmosphere and cooled to 0 ℃, and DCC (495 mg, 2.4 mmol) and DMAP (29.3 mg, 0.24 mmol) were added to the mixture. The reaction was allowed to continue at room temperature for 48 hours. The mixture was filtered and the liquid phase was washed three times with a saturated NaCl solution followed by drying over Na_2SO_4 overnight. After filtering and removing the solvent under vacuum, the crude product was purified by column chromatography on silica gel using dichloromethane /methanol (10:1) mixture as the eluant to yield mCy3.5 as the final solid.

2.3 Compound 5 (Cy 7)

Compound 3 [2](4.08 g, 12.0 mmol), compound 4 [3] (0.96 g, 5.44 mmol) and sodium acetate (0.47 g, 5.44 mmol) were dissolved in 30 mL acetic anhydride under nitrogen atmosphere. The mixture was stirred for 2 h at room temperature. Then the mixture solvent was removed under vacuum. The residuals were washed with ether to obtain a green solid. The crude product was purified by silica gel column chromatography using dichloromethane /methanol (10:1) as the eluent to give 5 as a green solid.

2.4 Compound 6 (CyOH)

A solution of resorcinol (220 mg, 2.0 mmol) and K_2CO_3 (276 mg, 2.0 mmol) in 15 mL acetonitrile (ACN) at room temperature under nitrogen atmosphere was stirred for 20

min. A solution of ACN (10 mL) containing compound 5 (660 mg, 1.0 mmol) was added to the above mixture solution via a syringe. The mixture solution was heated for 4 h at 50 °C under nitrogen. The solvent was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography using dichloromethane /methanol (10:1) as the eluent to give 6 as a blue solid.

2.5 Monomer mCyOH

CyOH (1.0 g, 2.0 mmol) and 2-hydroxyethyl methacrylate (HEMA, 312 mg, 2.4 mmol), were dissolved in dry DCM under an N_2 atmosphere. After cooling to 0 °C, DCC (495 mg, 2.4 mmol) and DMAP (29.3 mg, 0.24 mmol) were added to the mixture. The reaction was allowed to continue at room temperature for 48 hours. The mixture was filtered and the liquid phase was washed three times with a saturated NaCl solution followed by drying over Na_2SO_4 overnight. After filtering and removing the solvent under vacuum, the crude product was purified by column chromatography on silica gel with dichloromethane /methanol (10:1) mixture as the eluant to give CyOH as a solid.

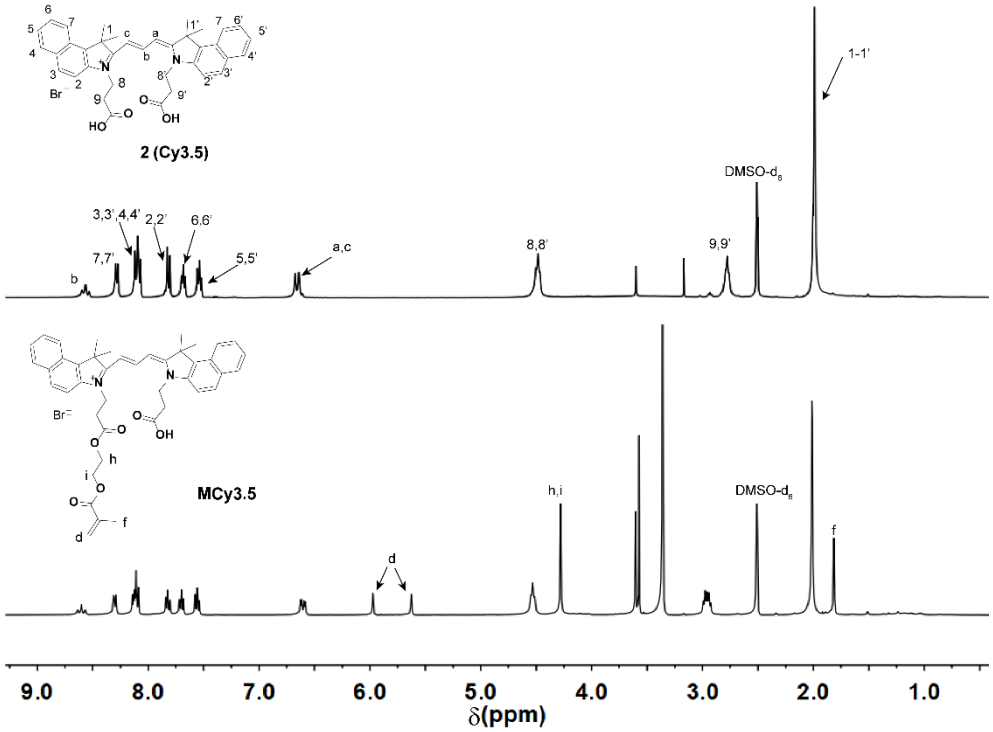


Figure S1. ^{1}H NMR spectra of Cy3.5 and monomer mCy3.5 in DMSO-d6.

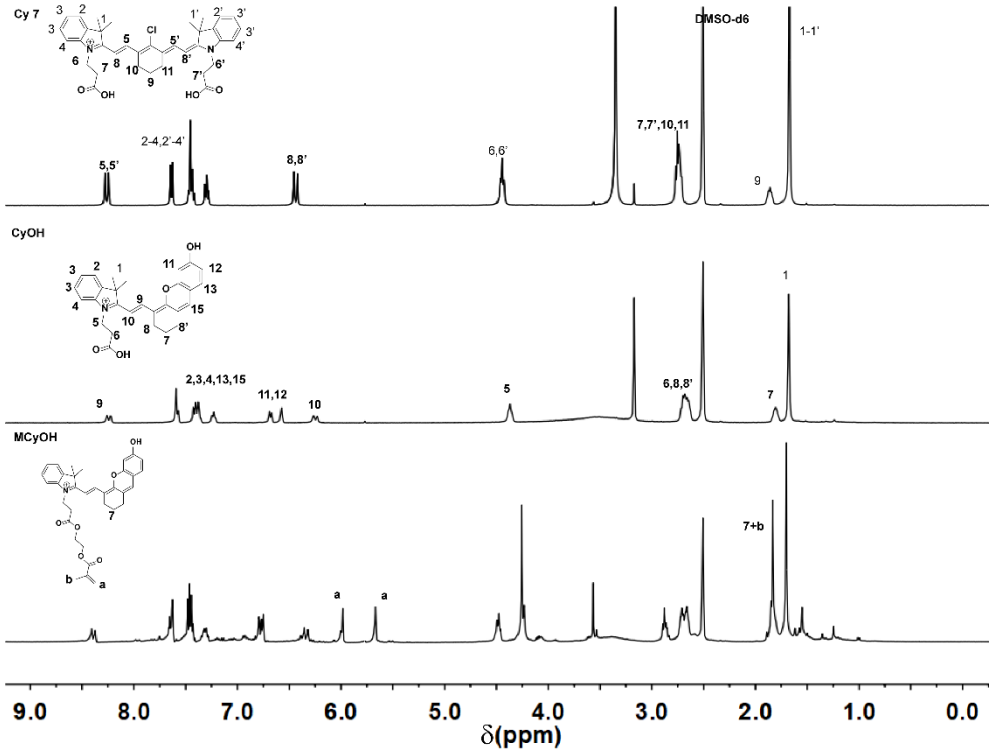
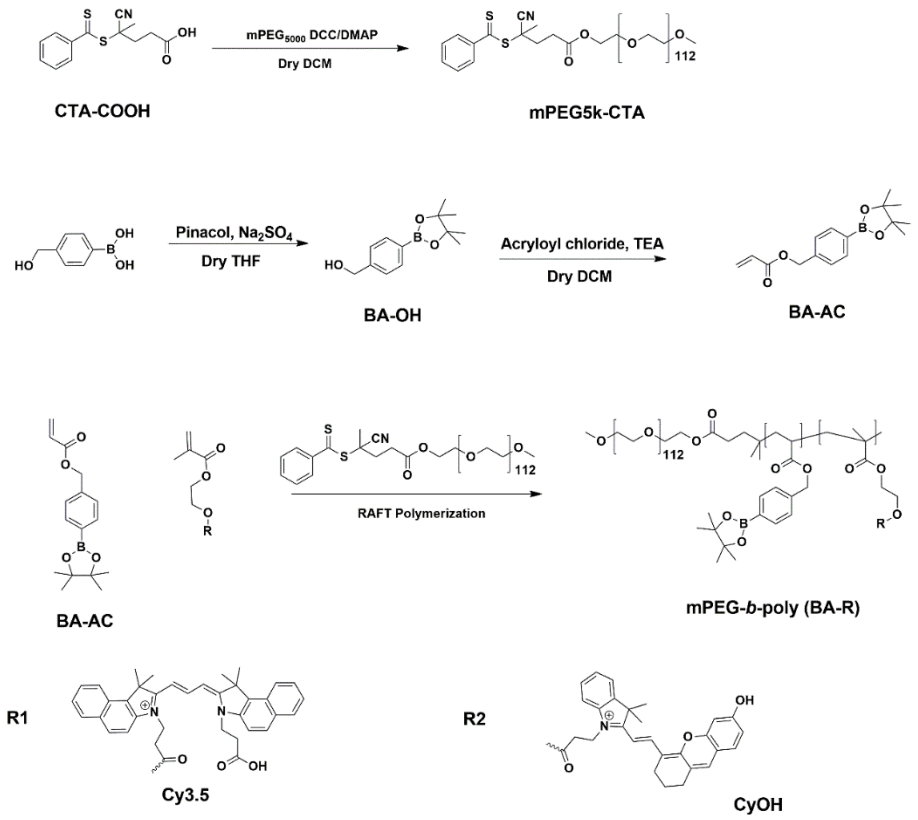


Figure S2. ^{1}H NMR spectra of Cy7 (top), CyOH (middle) and final monomer mCyOH (bottom) in DMSO-d6.



Scheme S2. Synthesis of the ROS-sensitive diblock copolymer for preparing P2 and preparing mixed MF micelles.

2.6 4-(hydroxymethyl)phenylboronic acid pinacol ester (BA-OH)

A suspension of 4-(hydroxymethyl) boronic acid (6.10 g, 40 mmol), pinacol (5.67 g, 48 mmol) and sodium sulfate (6.0 g, 42 mmol) in dry THF (60 mL) was stirred overnight under nitrogen at room temperature. The mixture was filtered, concentrated and re-dissolved in ethyl acetate, followed by washing with deionized water 3 times. The organic layer was concentrated and purified by column chromatography on silica gel using a mixture of hexane/EtOAc (2/1, v/v) as the eluent and evaporated as a transparent liquid. Yield:7.1 g (75%).

2.7 Synthesis of monomer mBA-AC

BA-OH (11.7 g, 50 mmol) was dissolved in anhydrous DCM (60 mL), followed by addition of 6.1 g TEA (60 mmol). After cooling to 0 ℃, 5.4 g (60 mmol) of acryloyl chloride in 10 mL dried DCM was added dropwise within 1 h. Then, the reaction

mixture was warmed to room temperature, stirred for 10 h, and filtered. The filtrate was concentrated on a rotary evaporator and diluted by ethyl acetate and washed with brine thrice. After drying over MgSO4, the organic solution was concentrated and purified by silica column chromatography using hexanes and ethyl acetate (v/v = 10/1) as the eluent. BA-AC was obtained as colorless crystal.

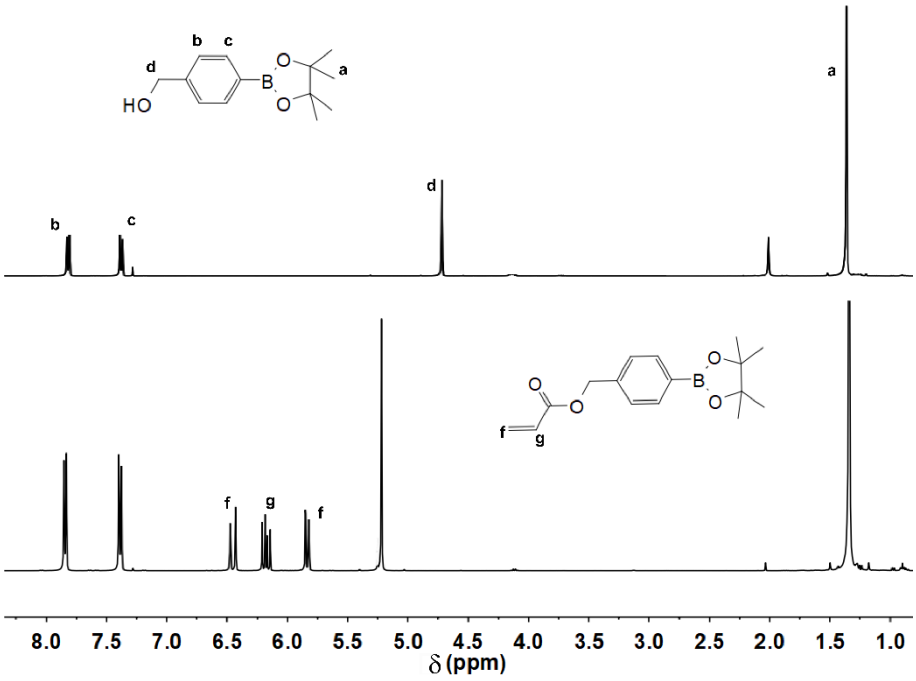


Figure S3. ^{1}H NMR spectra of BA-OH (top) and final monomer mBA-AC (bottom) in $CDCl_3$.

2.8 Synthesis of mPEG$_{5k}$ macro-RAFT agent (mPEG$_{5k}$-CTA)

The synthesis of mPEG$_{5k}$-CTA chain transfer agent was carried out by the reaction of Polyethylene glycol monomethyl ether (Mn 5000) with 4-cyno-4-((thiobenzoyl)sulfanyl)pentanoic acid, chain transfer agent (CTA) [4] with the assistance of 4-dimethylaminopyridine and dicyclohexyl-carbodiimide (DMAP) in methylene dichloride. Briefly, in a 250 mL one-neck round-bottom flask equipped with a magnetic stirring bar, CTA (0.41 g, 1.5 mmol) and mPEG–OH (5 g, 1.0 mmol) was dissolved in 50 mL of DCM. After the solution was homogenized by stirring, the flask was placed in an ice bath. Then, DCC (0.25 g, 1.2 mmol) and DMAP (0.015 g, 0.12 mmol) were added. After 30 min of stirring at 0 °C, the reaction temperature was raised to room

temperature and stirred for 2 days. The precipitated dicyclohexylurea (DCU) was removed by filtration. The filtrate was concentrated by rotary evaporator and precipitated into excess diethyl ether twice to remove the unreacted CTA, recrystallized twice in ethanol, and mPEG$_{5k}$ macro-CTA (light red color) was obtained by filtering and drying under vacuum at room temperature for 48 h.

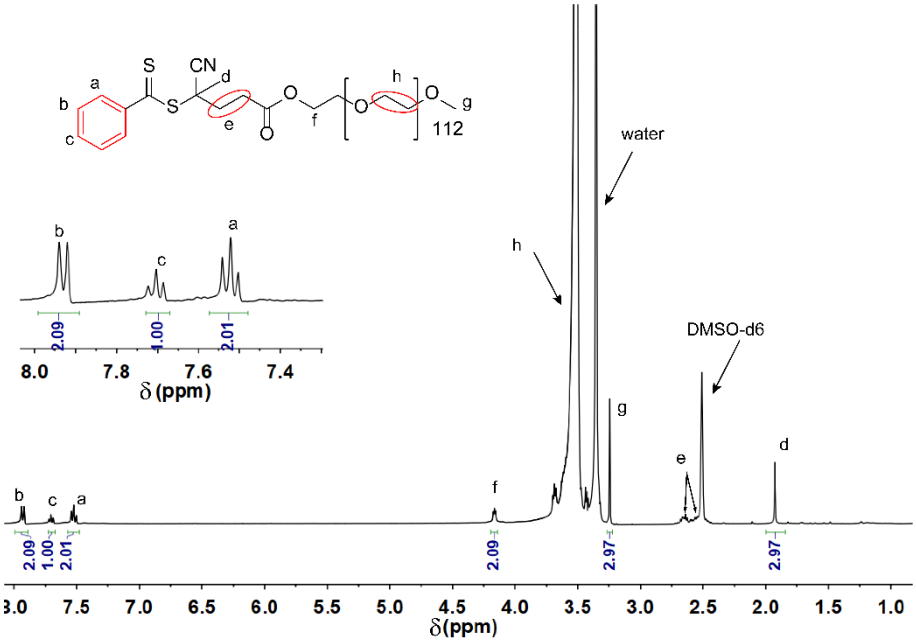


Figure S4. ^{1}H NMR spectra of mPEG$_{5k}$-CTA in DMSO-d$_6$.

2.9 RAFT polymerization of diblock copolymer.

Three amphiphilic block copolymers P1-P3 were prepared by the Reversible addition−fragmentation chain-transfer polymerization (RAFT) of monomer BA-AC and fluorescent monomer mCy3.5 and mCyOH. Typically, mPEG$_{5k}$-based macro-RAFT agent (113 mg, 0.02 mmol, 1 equiv.), BA-AC (345 mg, 1.2 mmol, 80 equiv.), AIBN (1.64 mg, 0.01 mmol, 0.2 equiv.), and 1,4-dioxane (2 mL) were charged into a Schlenk flask equipped with a magnetic stirring bar. The flask was carefully degassed by three freeze-pump-thaw cycles and sealed under N_2 protection. After heating at 70 °C in an oil bath and stirring for 12 h, the reaction flask was quenched by dipping into liquid nitrogen and opened; the mixture was then precipitated into an excess of n-hexane and the precipitate was dissolved with THF again. The above dissolution precipitation cycle was repeated three times. The final product was dried in a vacuum

oven overnight at room temperature, yielding a pink solid. The molecular weight and molecular weight distribution of mPEG$_{5k}$-*b*-PBA were determined by GPC using DMF as the eluent and polystyrenes as standards, revealing an Mn of 22.3 kDa and Mw/Mn of 1.18 (**Table 1**). The mean DP of PBA block for P2 was determined to be 60 by ^{1}H NMR analysis in $CDCl_3$ (**Figure S5**, mPEG5k$_{112}$-*b*-PBA$_{60}$). Following similar procedures, P1 or mPEG5k-*b*-PBA$_{25}$ and P3 or mPEG5k-*b*-PBA$_{104}$, control polymer mPEG5k-*b*-PS$_{55}$ and fluorescent polymers mPEG5k-*b*-p(BA$_{52}$-Cy3.5) and mPEG5k-*b*-p(BA$_{56}$-CyOH) (**Figure S6**) were also synthesized. The physical parameters of all synthesized block copolymers are summarized in **Table 1**.

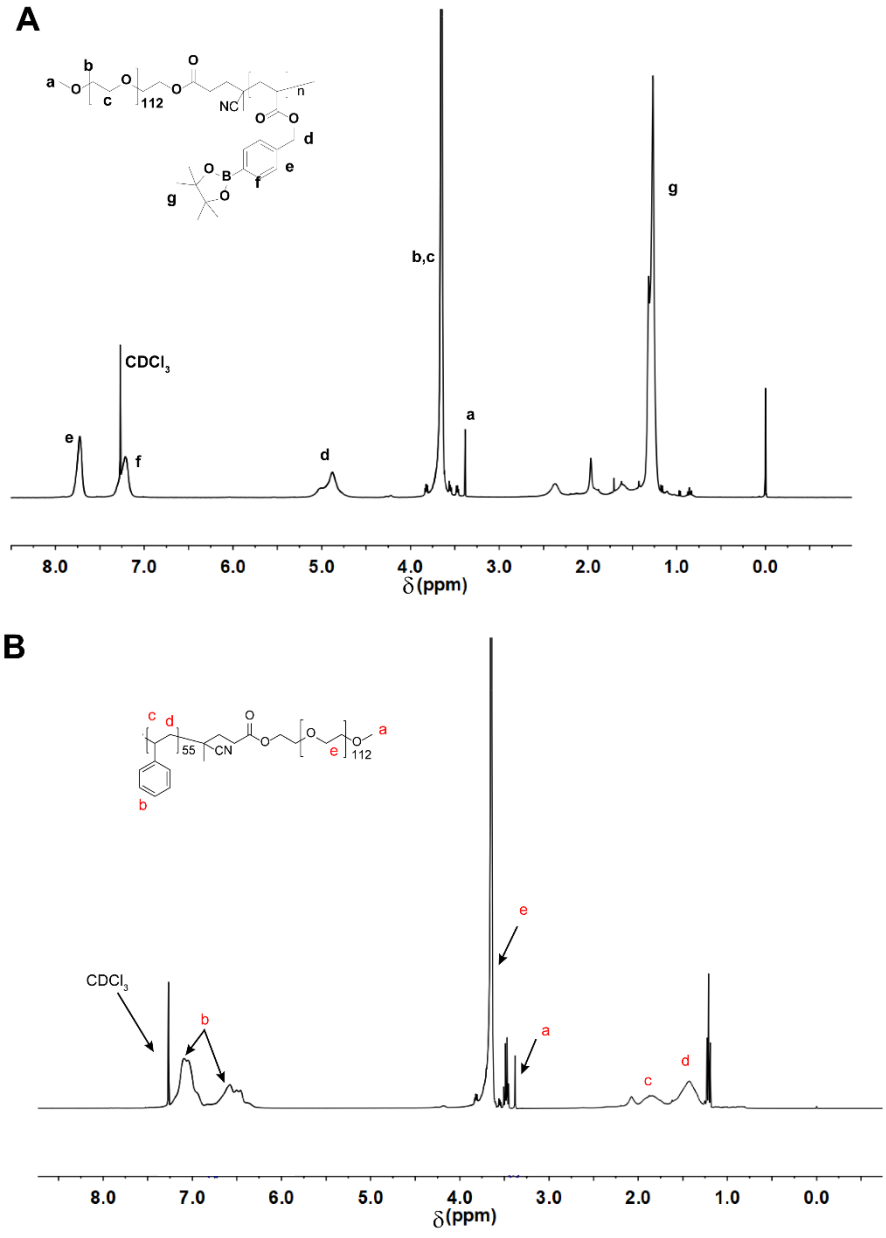


Figure S5. ^{1}H NMR spectra recorded for mPEG$_{5k}$-*b*-PBA$_{60}$ (A) and mPEG$_{5k}$-*b*-PS$_{55}$ (B) in $CDCl_3$. The average polymerization degree of PBA segments was approximately 60, as estimated from the integral ratio of peaks at 3.28 ppm (a, methyl of mPEG) and 7.78 ppm (e, proton of benzene). Similarly, the average polymerization degree of PS segments was approximately 55.

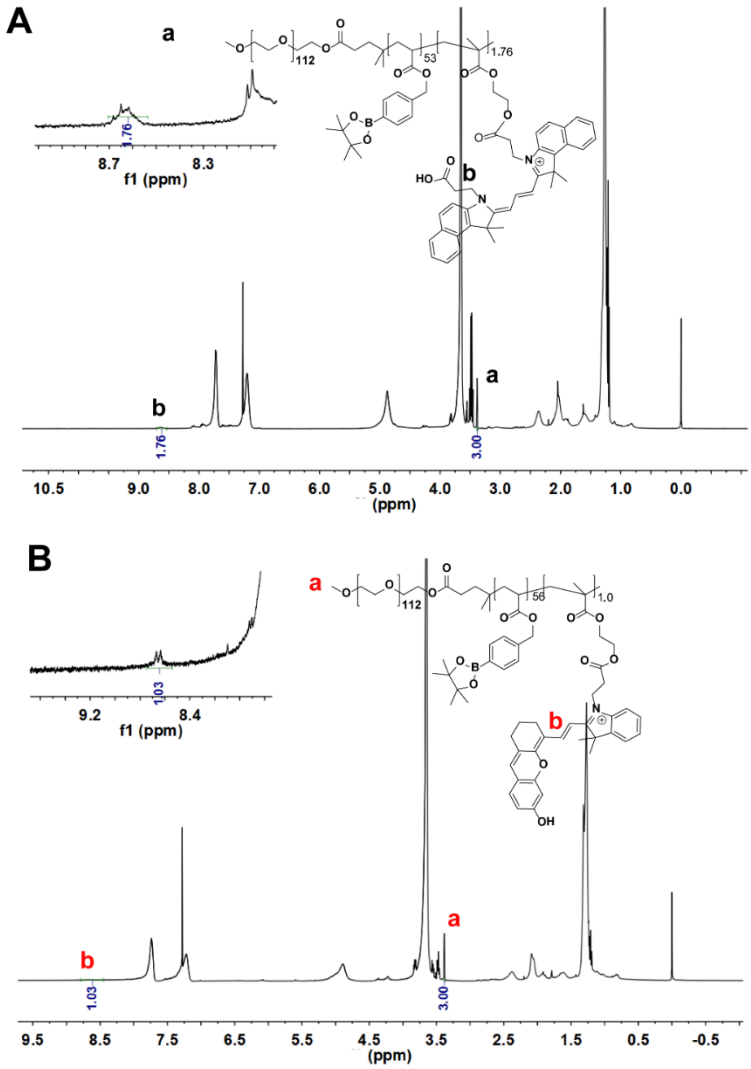


Figure S6. ^{1}H NMR spectra recorded for fluorescent polymers mPEG$_{5k}$-*b*-p(BA$_{52}$-Cy3.5) and mPEG$_{5k}$ *b*-p(BA$_{56}$-CyOH) in $CDCl_3$.

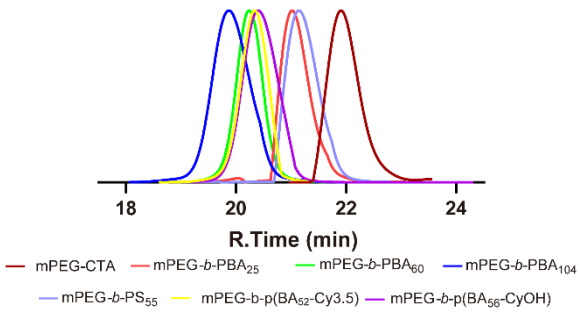


Figure S7. GPC spectra of all block copolymers synthesized.

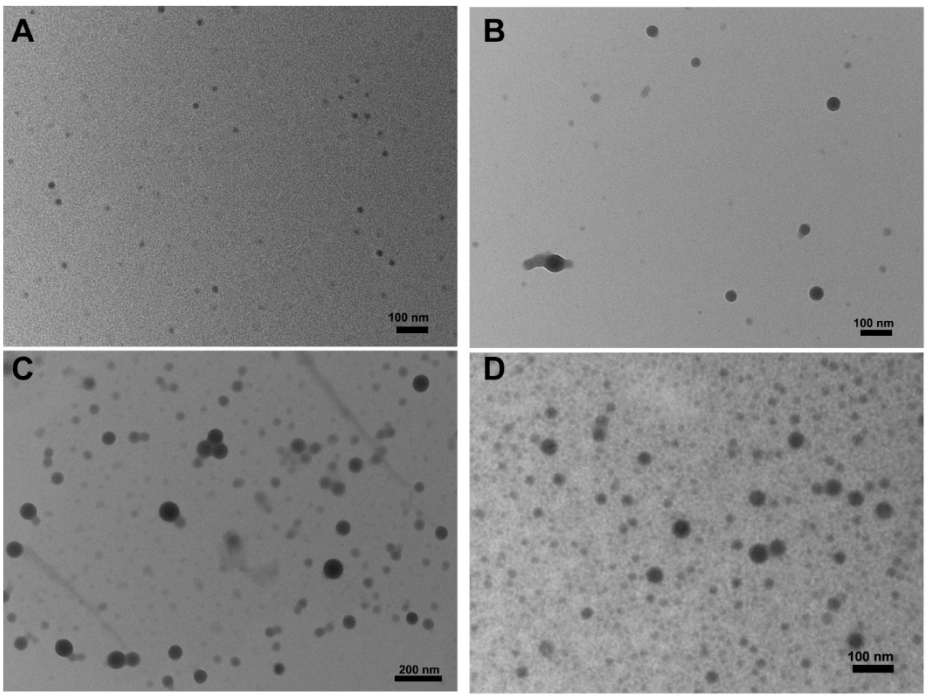


Figure S8. TEM photographs of P1 (A), P2 (B), P3 (C) and P4 (D) micelles (0.1 mg/mL) prepared in 10 mM PBS solution (pH 7.4, 37 ℃)

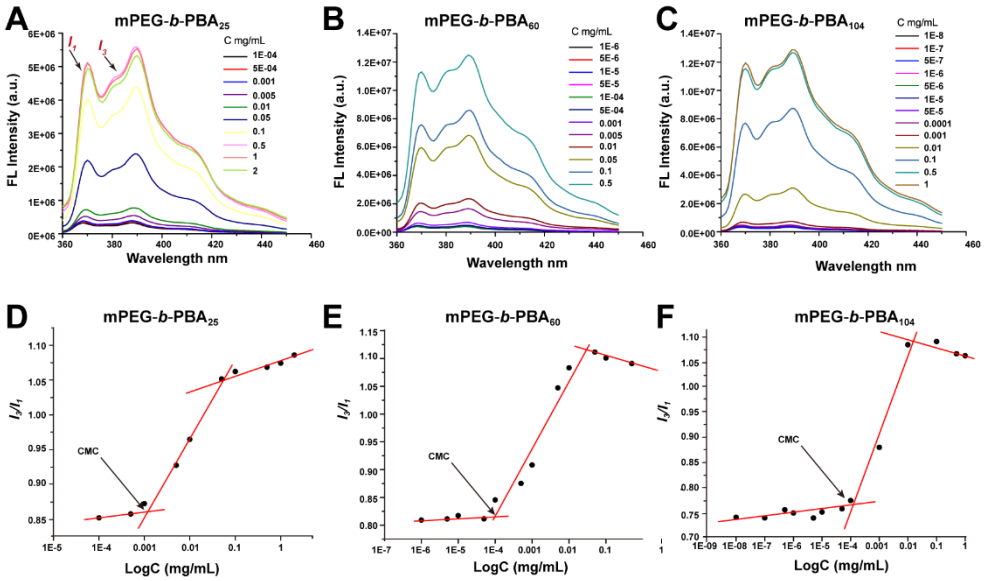


Figure S9. (A-C) The fluorescence emission spectra of pyrene as a function of copolymer concentration (mg/mL) in pH 7.4, 10 mM PB buffer. (D-F) The fluorescence intensity ratio of I_{384}/I_{373} (I_3/I_1) from pyrene emission spectra versus the log of the concentration (logC, mg/ml) to determine the CMC of different mPEG5k-*b*-PBA polymers prepared.

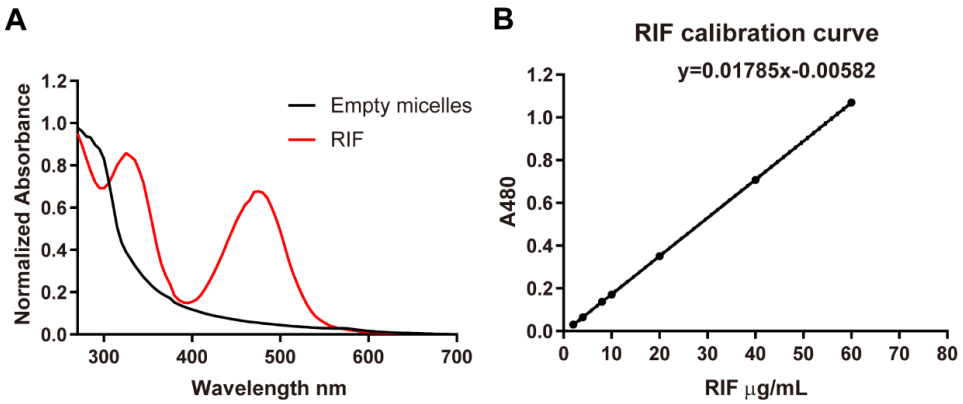


Figure S10. (A) Absorption spectra of empty micelles and free RIF in PB buffer (pH 7.4, 10 mM). (B) Calibration curve for RIF at 480 nm; all the measurements were conducted in triplicate.

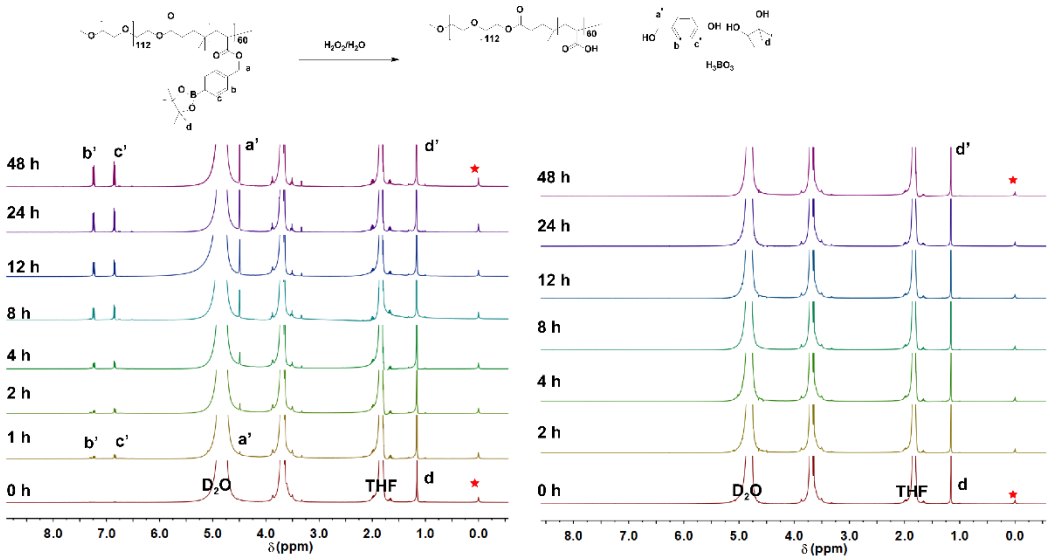


Figure S11. Time-dependent ^{1}H NMR spectra of micelle (5 mg/mL) in deuterated PB solution (pH 7.4, 10 mM, 37 °C) in the presence of 10 mM H_2O_2 (A) and without H_2O2 (B).

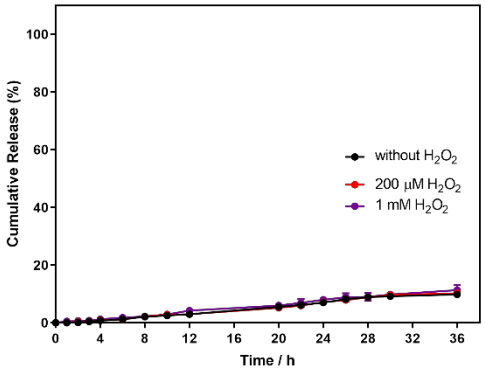


Figure S12. Cumulative release of RIF from control mPEG$_{5k}$-*b*-PS micelles after incubation with 200 μM and 1 mM H_2O_2 reveal less than 20% drug release at 36 h.

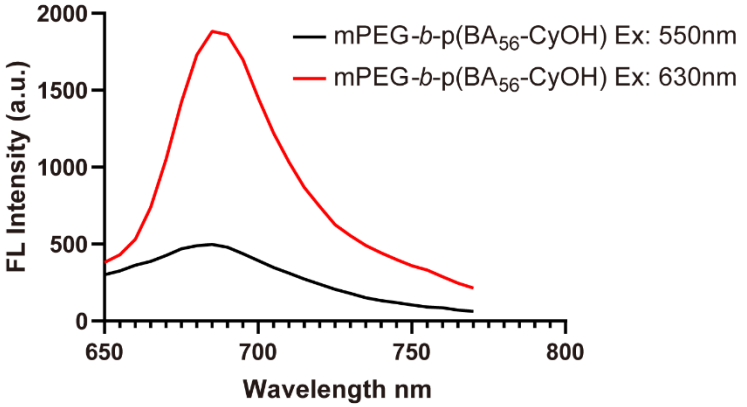


Figure S13. Fluorescent spectra of $mPEG_{5k}$-*b*-p(BA_{56}-*r*-CyOH) micelles excited at 550 nm and 630 nm.

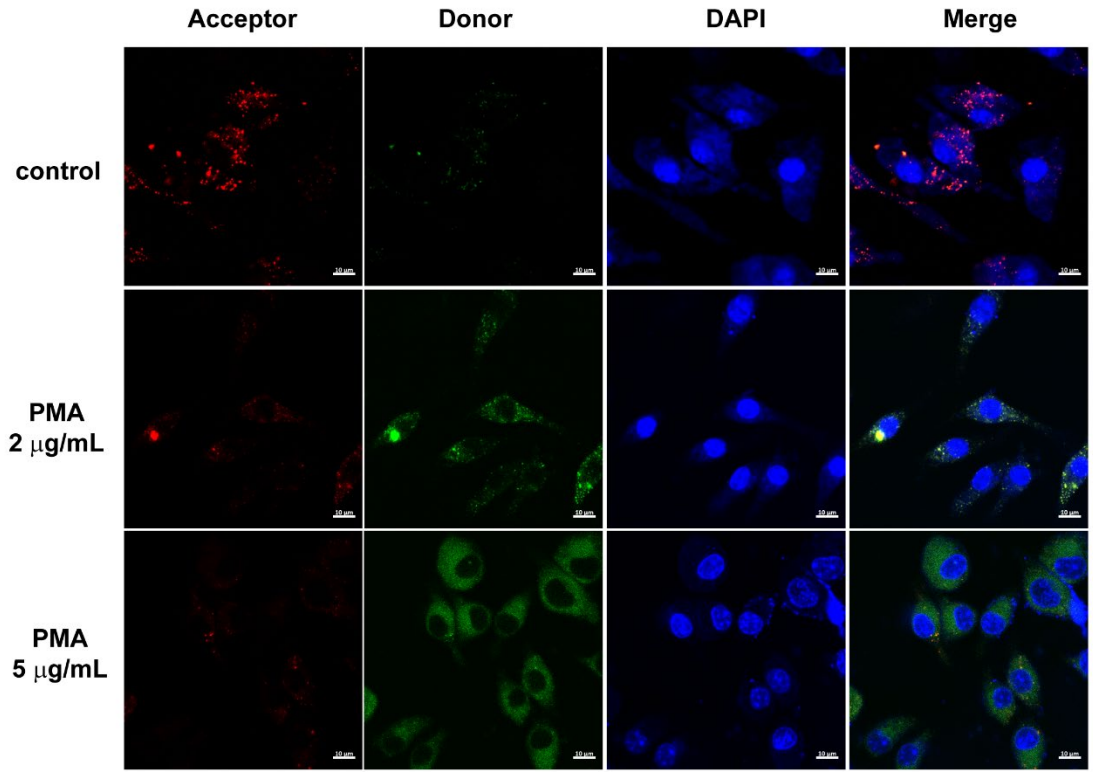


Figure S14. CLSM images of J774A.1 macrophage cells after treatment with empty MF micelles and 4 h incubation with PMA at two concentrations (2 and 5 μg/mL). For images, cells were stained with DAPI (Blue, Ex: 405 nm, Em: 410−538 nm); MF micelles were excited at 543 nm; the green channel corresponding to Cy3.5 (Em: 565−662 nm) and the red channel corresponding to CyOH (Em: 671−758 nm) were monitored.

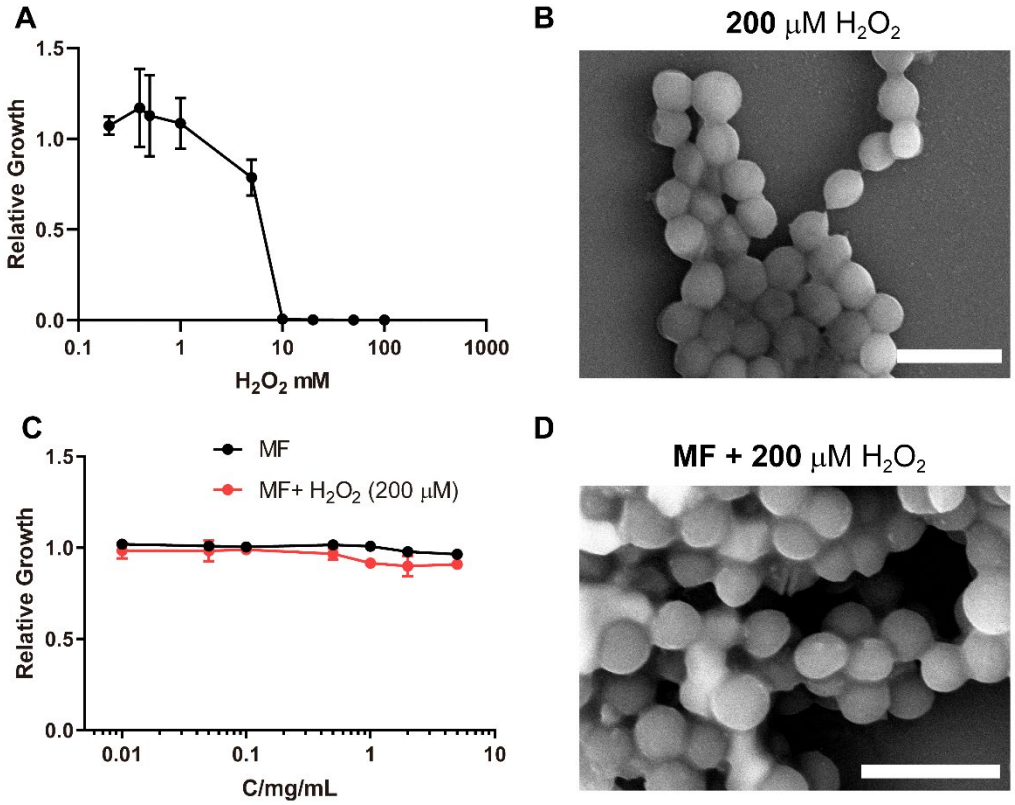


Figure S15. (A) Antibacterial effect of H_2O_2 at various concentrations (B) SEM images of *S. aureus* after treatment for 12 h with 200 μM H_2O_2; (C) antibacterial activity of MF and MF plus 200 μM H_2O_2; (D) SEM images of *S. aureus* after treatment for 12 h with MF plus 200 μM H_2O_2. Scale bar: 5 μm.

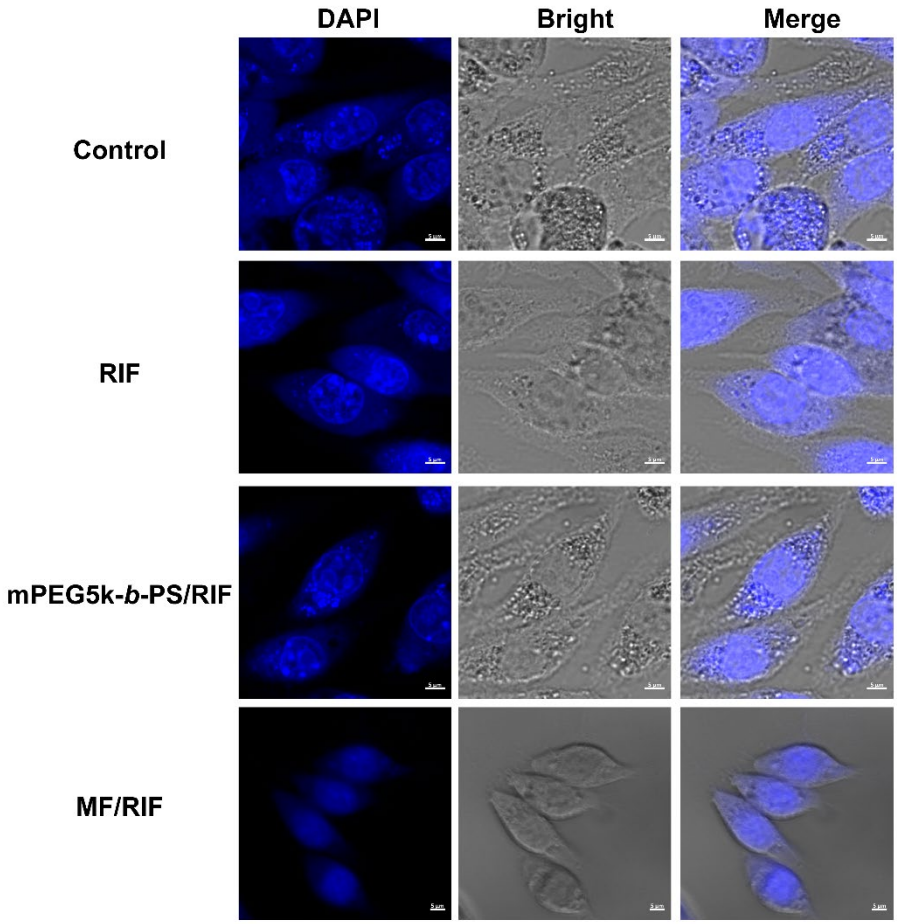


Figure S16. Confocal fluorescence images of *S. aureus*-infected J774A.1 cells treated with control (PBS), free RIF (8 μg/mL), equivalent control RIF-loaded micelles (mPEG5k-b-PS/RIF) and equivalent MF/RIF for 24 h. Blue channel corresponds to cells stained with DAPI (scale bar = 5 μm).

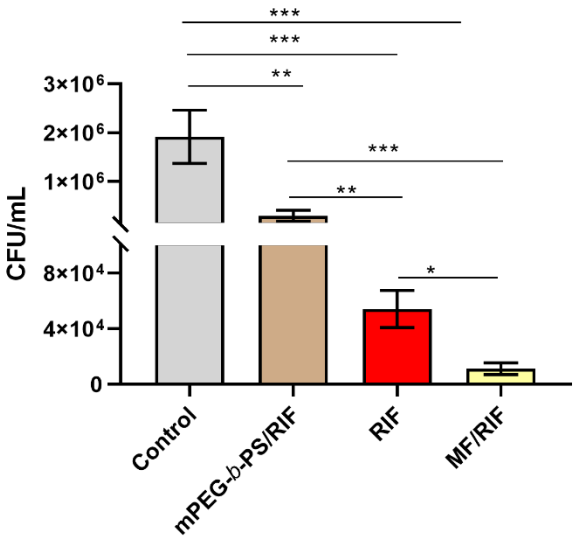


Figure S17. CFU counts of *S. aureus* following infected and treatment of J774A.1 macrophages with various formulations. The data are presented as the means ± SD. * indicates $p < 0.5$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.