

SUPPORTING INFORMATION

Self-adaptive antibacterial surface with bacterium-triggered antifouling-bactericidal switching properties

Yidan Zhang, Xiang Zhang, Yu-Qing Zhao, Xin-Yang Zhang, Xiaokang Ding,
Xuejia Ding, Bingran Yu, Shun Duan,* Fu-Jian Xu*

State Key Laboratory of Chemical Resource Engineering, Key Lab of Biomedical Materials of Natural Macromolecules (Beijing University of Chemical Technology), Ministry of Education, Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, Beijing 100029, China

*To whom all correspondence should be addressed:

E-mail: duanshun@mail.buct.edu.cn (S Duan); xufj@mail.buct.edu.cn (F J Xu).

1. Materials and methods

1.1 Materials.

Polyurethane film was purchased from Shanghai Qinggen Industrial Co., Ltd(China). *N,N,N',N'',N'''*-pentamethyl diethylenetriamine (PMDETA) was purchased from Sigma-Aldrich (USA). Methanol, ethanol, acetonitrile, isopropyl ether, tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) and sodium chloride (NaCl) were purchased from Beijing Chemical Works (China). Triethylamine (TEA), (3-aminopropyl)triethoxysilane (APTES), copper (I) bromide (CuBr), 1-bromodecane, hydroxyethyl methacrylate (HEMA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), pyridinium chlorochromate (PCC), and copper (II) bromide (CuBr₂) were purchased from Energy Chemical (China). 2-bromoisobutyryl bromide (BIBB) was purchased from TCI Chemical Industry Co. (China). Methoxypolyethylene glycol amine (PEG-NH₂, $M_w=20,000$ Da) was purchased from Shanghai Macklin Biochemical Co., Ltd (China). Tryptone and yeast extract were purchased from Oxoid (UK).

1.2 Preparation of surface-initiated atom transfer radical polymerization (SI-ATRP) initiator-modified PU (PU-Br).

In a 100 mL flask, 4.68 mL of APTES and 3.696 mL of TEA were added into 30 mL of anhydrous THF. With ice water cooling and strong stirring, 2.728 mL of BIBB was added dropwise, and the solution was stirred for 3 h at room temperature. The precipitate was removed by vacuum filtration, and the product (APTES-Br) was purified by evaporation. PU films were cut into pieces of 0.4 cm × 0.4 cm, and washed alternatively in deionized water and ethanol for three times (10 min for each time) by

ultrasonic cleaning, followed by drying in oven. In a 50 mL flask, 0.3 mL of APTES-Br was dissolved into 10 mL of 90% ethanol solution, stirred for 2 h at room temperature. PU films pretreated by oxygen plasma (90 W, 5 min) were immersed into APTES-Br solution, and stirred for 2 h at 50 °C to prepare PU-Br. To remove the unreacted APTES-Br, the samples were oscillated alternatively in deionized water and ethanol for three times, then dried in oven and cured for 15 min at 100 °C in vacuum.

1.3 Preparation of block polymer brushes-grafted PU (PU-PQH).

The typical SI-ATRP process was performed based on our previous work.^{S1} Poly [2-(dimethyl decyl ammonium) ethyl methacrylate] (PQDMAEMA) brushes was prepared by SI-ATRP of 2-(dimethyl decyl ammonium) ethyl methacrylate (QDMAEMA) from PU-Br. To prepare QDMAEMA, 2.68 mL of DMAEMA and 3.9 mL of 1-bromodecane were added into 10 mL of acetonitrile in a 50 mL flask and reacted for 24 h at 40 °C. After cooling to room temperature, the solution was slowly dripped into 200 mL of isopropyl ether, and then the precipitate was separated by centrifugation. The precipitate was dissolved the in acetonitrile and then carried on the precipitation-centrifugation process for another two times. For SI-ATRP, 711.4 mg of QDMAEMA and 200 μ L of PMDETA were added into 10 mL of deionized water/methanol mixture solution (volume ratio=1:1) in a 50 mL flask. Under protection of nitrogen, 10 mg of CuBr₂ and two pieces of PU-Br were added into the mixture. After nitrogen bubbling for 20 min, 32.4 mg of CuBr was added into the flask, and the flask was tightly sealed. After stirring for 4 h at 30 °C, PQDMAEMA-modified PU was prepared (denoted as PU-PQ). The samples were taken out from the flask, placed in a clean 50 mL centrifuge

tube, and alternately oscillated in deionized water and methanol for three times. The PU-PQ samples were naturally dried. To prepare P(HEMA-*b*-QDMAEMA) brushes by SI-ATRP, two pieces of PU-PQ, 250 μ L of HEMA, 200 μ L of PMDETA, 26 mg of CuBr₂, and 84 mg of CuBr were successively added into 10 mL of deionized water/methanol solution (1:1) in a 50 mL flask under protection of nitrogen, and then the mixture was stirred for 1 h at 30 °C to prepare P(HEMA-*b*-QDMAEMA)-modified PU (denoted as PU-PQH). The subsequent cleaning process of the samples was as described above.

1.4 Preparation of PEG-grafted PU-PQH by Schiff base reaction (PU-PQ-PEG).

In a 15 mL centrifuge tube, 30 mg of PCC and one piece of PU-PQH were added into 3 mL of deionized water, and the mixture was stirred at 30 °C for 6 h to prepare aldehyde-modified PU-PQH (denoted as PU-PQ-CHO). Then, the PU-PQ-CHO samples were removed, and alternately oscillated in deionized water and methanol for three times. After natural drying, PU-PQ-CHO was immersed into phosphate buffer saline (PBS) containing 25 mg/mL PEG-NH₂ (M_w = 20,000 Da), and stirred for 24 h at 30 °C. After the reaction, the samples were alternately washed in deionized water and methanol for three times, and naturally dried. The PU-PQ-PEG samples were immersed in 3 mL HAc-NaAc buffer (molar ratio of HAc:NaAc = 37:63, pH = 5.0) shaking (150 r/min) at 37°C for 24 h to remove PEG from PU-PQ-PEG samples (denoted as PU-PQ-RE).

1.5 Physical and chemical characterization.

The chemical structures of APTES-Br and QDMAEMA were characterized by 400

MHz ^1H NMR spectroscopy (Bruker ARX, USA). The morphologies of samples were observed by scanning electron microscopy (SEM, JSM-7500F, JEOL, Japan) and atomic force microscope (AFM, Bruker Dimension Icon, Bruker, Santa Barbara, CA, USA). Surface chemical element valences of the samples were investigated by X-ray photoelectron spectroscopy (XPS, Kratos AXIS-His, Shimadzu, Japan) equipped with Al K α X-ray source. Surface wettability of the samples was studied by water contact angle meter (WCA, Dataphysics, Germany). Surface zeta potential of samples was measured by solid surface zeta potential analyzer (SurpassTM 3, Anton Paar, Austria).

1.6 Evaluation of antifouling properties.

The samples were divided into four groups, including PU, PU-PQ-CHO, PU-PQ-PEG and PU-PQ-RE. All the samples were cut into pieces with the size of 0.4 cm \times 0.4 cm, and placed into 48-well plates. The samples were soaked in PBS for 2 h at 37 $^\circ\text{C}$, and then moved to the wells with 1 mL of PBS containing 0.25 mg/mL fluorescein isothiocyanate-labelled bovine serum albumin (FITC-BSA, synthesized following the procedure in the work of Yiu et al.^{S2}). The samples were incubated with FITC-BSA solution for 2 h at 37 $^\circ\text{C}$ in the dark. After rinse with deionized water gently, the samples were transferred into PBS and oscillated by a shaker (37 $^\circ\text{C}$, 150 r/min) for 3 min. The samples were washed as described above for three time, and dried under nitrogen. The whole cleaning operations were carried out in the dark. The samples were observed by confocal laser scanning microscope (CLSM, Leica SP8, Germany), and the amount of absorbed BSA was determined by Image J software based on the fluorescent images.

Staphylococcus aureus (*S. aureus*) was chosen as the representative bacteria. *S.*

aureus was incubated for 8 h (37°C, 150 r/min) in Luria broth (LB) media to the density of 10⁸ CFU/mL. The bacterial suspension was centrifuged to remove LB media and diluted with PBS to reach the density of 10⁸ CFU/mL. The samples of six groups (PU, PU-PQ, PU-PQH, PU-PQ-CHO, PU-PQ-PEG and PU-PQ-RE) were cut into pieces with the size of 0.3 cm × 0.3 cm, and put into 96-well plates. In each well, 200 μL of bacterial suspension at the density of 10⁸ CFU/mL was added and incubated for 10 h. Then the samples were washed gently to remove loosely attached bacteria, and stained for 10 min with a mixed solution containing SYTO 9 (6 μmol/L) and propidium iodide (PI, 30 μmol/L) from a live/dead staining kit (L7012, Thermo Fisher Scientific, USA) in the dark. The stained samples were observed by CLSM.

1.7 Characterization of antifouling-bactericidal switching behavior.

S. aureus suspension was diluted to the density of 10⁵ CFU/mL with LB media. The samples of PU, PU-PQ-CHO and PU-PQ-PEG were placed into 96-well plates, and 200 μL bacterial suspension was added into each well. After being incubated for 10 h or 24 h, the samples were washed gently and stained by SYTO 9 and PI. The stained samples were observed by CLSM. The samples of PU, PU-PQ-CHO and PU-PQ-PEG were incubated in PBS at 37 °C for two weeks and repeat the above experiments of characterization of antifouling-bactericidal switching behavior.

1.8 Antibacterial performances under *in vitro* circulating flow condition.

Peristaltic pump (BT301L-YT15, Baoding Lead Fluid Technology Co., Ltd., China), silicone tubes (with the inner diameter of 0.6 cm) and 500 mL beaker were used to build the circulating flow system. The samples (PU and PU-PQ-PEG) was cut into pieces

with the size of 0.6 cm × 0.4 cm and placed in each end of the silicone tube. In the beaker, 200 mL of bacterial suspension (10^7 CFU/mL, PBS) was added, and incubated at 37 °C with stirring at 250 r/min. Both ends of the silicone tube were kept under the liquid level. At first, the pump speed was set as 200 r/min for 10 min to completely fill the tube with bacteria suspension, and then turned to 10 r/min for 48 h. After circulation test, the samples of PU and PU-DMH were removed from the tube and gently washed by PBS. The samples were stained by SYTO 9 and PI and observed by CLSM. The bacterial suspension at density of 10^5 CFU/mL in LB media was used to repeat the above experiment for 10 h and 24 h, respectively.

1.9 Biocompatibility evaluation.

Red blood cells were isolated from rat blood. In a 50 mL centrifuge tube, 1 mL of rat blood was added into 20 mL normal saline and centrifuged at 2000 r/min for 15 min. The supernatant was discarded, and the operation was repeated for another two times. Red blood cells were removed and diluted to 4% suspension with normal saline. In a 96-well plate, samples of PU, PU-PQ-CHO and PU-PQ-PEG were placed into the wells. In each well, 100 μL of red blood cell suspension and 100 μL normal saline were added to immerse the samples. In addition, positive and negative control groups were set. The positive control group was containing 100 μL red blood cell suspension and 100 μL cell lysis solution (RIPA R0020, Solarbio, China), and the negative control group was treated with normal saline. The 96-well plate was kept standing for 1 h at 37 °C. The suspension in each well was mixed and transferred into a 1.5 mL centrifuge tube. After centrifugation (2000 r/min, 15 min), 100 μL of the supernatant was added into a new 96-well plate to measure the absorbance at 545 nm by a microplate reader (Cytation 3, Biotek, USA). Hemolysis ratio was calculated according to the following

formula:

$$\text{Hemolysis ratio (\%)} = \frac{\text{OD}_{\text{test}} - \text{OD}_{\text{neg}}}{\text{OD}_{\text{pos}} - \text{OD}_{\text{neg}}} \times 100\% \quad (1)$$

where OD_{test} , OD_{pos} and OD_{neg} represented the absorbance values of the tested sample, the positive control (cell lysis solution) and the negative control (normal saline), respectively.

Mouse fibroblast cells (L929) were used for cytotoxicity test. To prepare the extract, the samples of PU, PU-PQ-CHO and PU-PQ-PEG were sterilized by 75% ethanol, rinsed by PBS for three times and incubated with RPMI 1640 media (3 cm²/mL) at 37 °C for 24 h. The L929 cells were cultured in RPMI 1640 media containing 10% fetal bovine serum (FBS), 100 U/mL of penicillin, and 100 mg/mL of streptomycin at 37 °C in 5% CO₂ humidified atmosphere. When proliferating to 80% confluence, the cells were digested by 0.25% trypsin (Sigma, USA), seeded into 96-well plate at a density of 10⁴ cells per well with 100 μL culture media, and cultured at 37 °C for 24 h. Then the media were removed, and a mixed solution was added into each well was added comprising of 50 μL of extract and 50 μL of RPMI 1640 media containing 20% FBS, 200 U/mL penicillin, and 200 mg/mL streptomycin. After being cultured for another 24 h at 37 °C, the mixed media were removed and 100 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) solution (0.5 mg/mL) was added into each well. After incubation at 37 °C for 4 h, MTT solution was take out and 100 μL of DMSO was added into each well. The absorbance at 560 nm of each well was measured by a microplate reader (Cytation 3, Biotek, USA). The absorbance of PU group was

defined as 100%.

1.10 *In vivo* experiments.

Eight-week-aged female BALB/C mice were divided into two groups (three mice in each group) for different densities of inoculated bacteria. The mice were anesthetized by isoflurane and the hair was removed from the back of the mice. Incisions with the length of 0.5 cm were made on both sides of the spine, and skin and muscle were separated. Before implantation, 10 μ L of *S. aureus* suspension with PBS (10^9 CFU/mL for low bacteria density group and 10^{11} CFU/mL for high bacteria density group) was dripped on each sample and dried in the clean bench. The bacterium-contaminated samples were implanted into the incisions, while the side with bacteria was contacted with muscle, and the incisions were sutured. PU samples were implanted on the left side and PU-PQ-PEG on the right side. After one day of implantation, the samples were taken out and investigated by CLSM with SYTO 9/PI staining. The muscle tissues closed to the samples were harvested and added into normal saline (100 mg of tissue with 900 μ L of saline). After being homogenized, the mixture was diluted with normal saline (10 times for low bacteria density group and 1000 times for high bacteria density group), and 10 μ L was take out to spread on solid LB media to check the counts of colonies.

1.11 Statistic analysis.

At least three samples were tested in each group. The results were presented as mean \pm standard deviation. When two groups were compared, the differences were assessed by *t*-test. When more than two groups were compared, ANOVA was performed. A

significance level of $p = 0.05$ were applied using Origin 7.1 software.

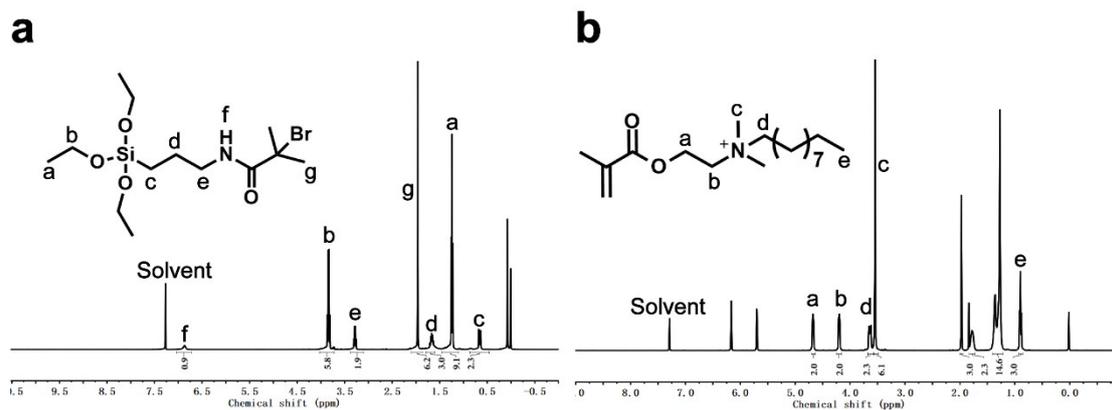


Figure S2. ^1H NMR spectra of (a) APTES-Br in CDCl_3 , and (b) the QDMAEMA in CDCl_3 .

As shown in Figure S2a, the chemical structure of APTES-Br was confirmed by ^1H NMR. The chemical shift at 1.24 ppm (peak a) and 3.83 ppm (peak b) were attributed to methyl protons ($\text{CH}_3\text{-CH}_2$) and methylene protons ($\text{CH}_3\text{-CH}_2\text{-O}$) of ethoxyl group, respectively. The chemical shift at 0.66 ppm (peak c) was assigned to methylene protons adjacent to silicon atom ($\text{Si-CH}_2\text{-CH}_2$). The chemical shift at 1.67 ppm (peak d) was attributed to methylene protons of aminopropyl groups ($\text{CH}_2\text{-CH}_2\text{-CH}_2$). The chemical shift at 3.28 ppm (peak e) was attributed to methylene protons adjacent to imino group ($\text{CH}_2\text{-CH}_2\text{-NH}$). The chemical shift at 6.87 ppm (peak f) was attributed to protons of imino groups ($\text{CH}_2\text{-NH-C}$). The chemical shift at 1.96 ppm (peak g) was attributed to methyl protons adjacent bromine atom ($\text{CH}_3\text{-C-Br}$). The purity of product was determined as $\sim 90\%$ based on the integral ratio of peak d and peak f as 3:0.9.

The chemical structure of QDMAEMA was also determined by ^1H NMR (Figure S2b). The chemical shift at 4.68 ppm (peak a) was attributed to methylene protons adjacent to ester group ($\text{O-CH}_2\text{-CH}_2$). The chemical shift at 4.20 ppm (peak b) was attributed to methylene protons adjacent to quaternary ammonium group ($\text{CH}_2\text{-CH}_2\text{-N}$). The chemical shift at 3.55 ppm (peak c) was attributed to methyl protons of quaternary ammonium group ($\text{CH}_3\text{-N}$). The chemical shift at 3.64 ppm (peak d) was attributed to methylene protons of decyl adjacent to quaternary ammonium group ($\text{CH}_2\text{-CH}_2\text{-N}$). The chemical shift at 0.90 (ppm) was attributed to methyl protons of decyl groups ($\text{CH}_3\text{-CH}_2$). The purity of product was determined as $\sim 98\%$ based on the integral ratio of peak a and peak c as 2:6.1.

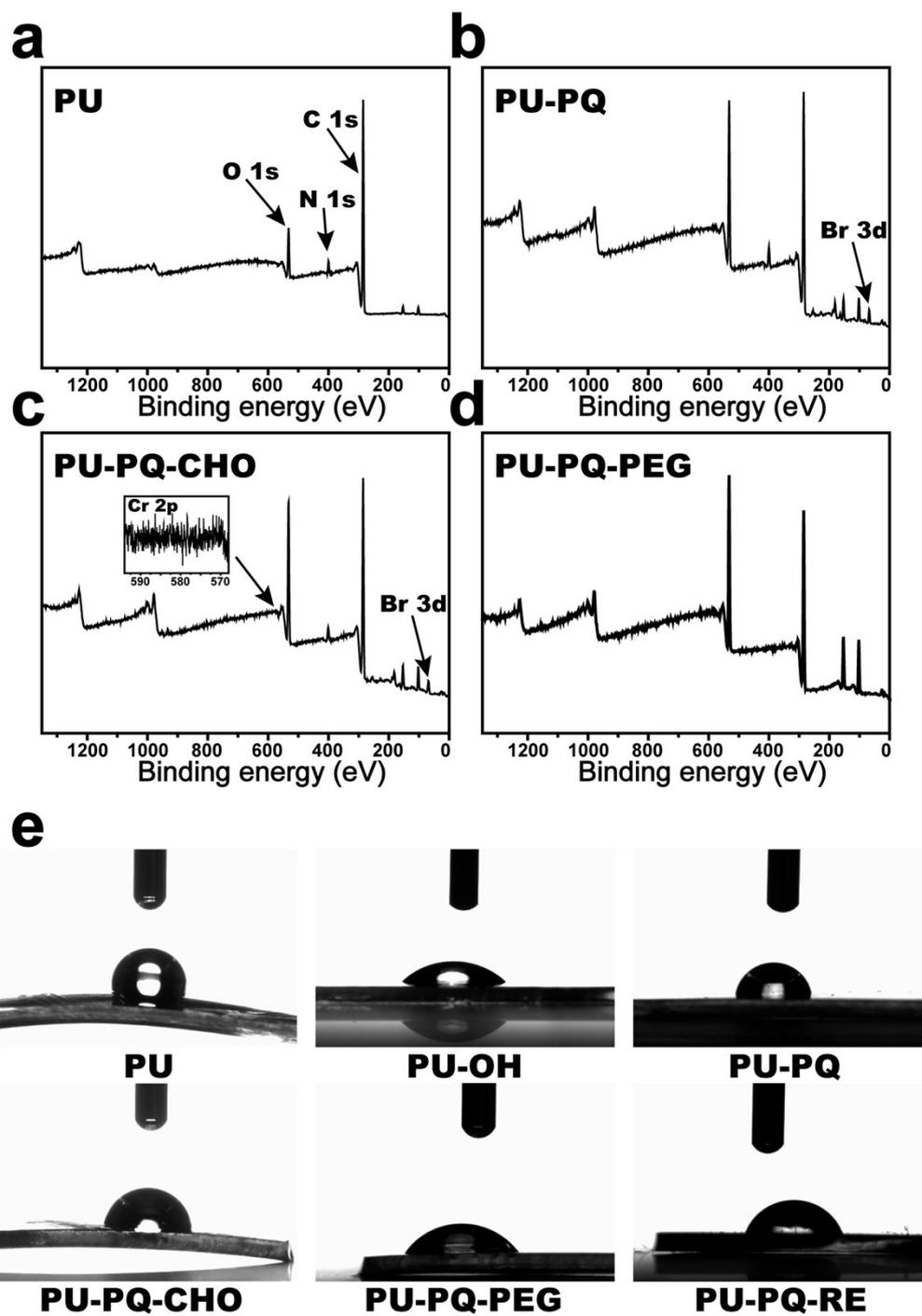


Figure S3. XPS wide-scan spectra (a, b, c and d, the inset is the Cr 2p spectrum of PU-PQ-CHO) and representative images of water contact angles (e).

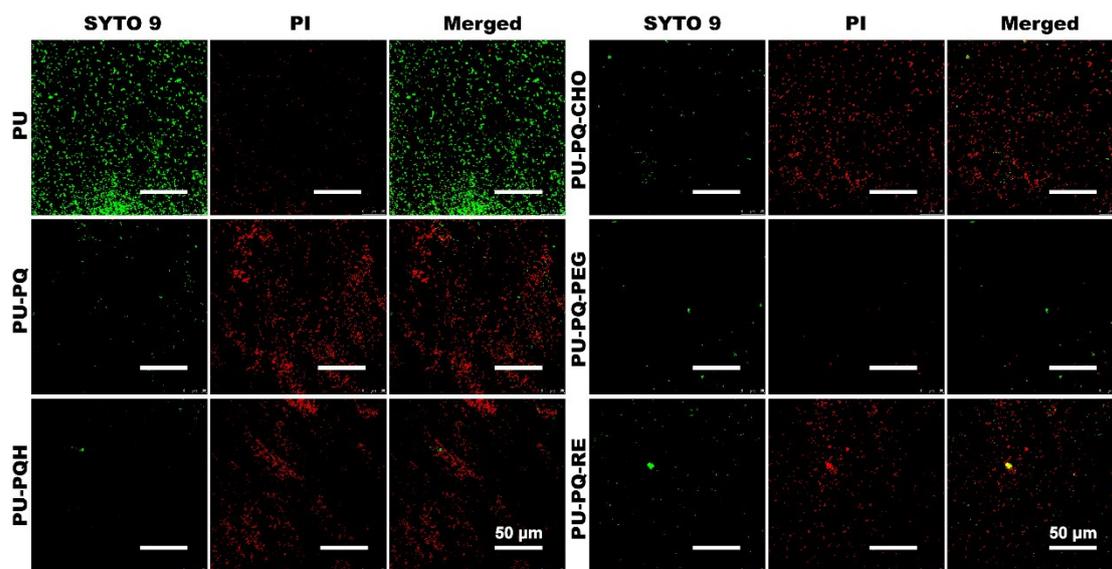


Figure S4. Representative CLSM images of anti-fouling test against *S. aureus*.

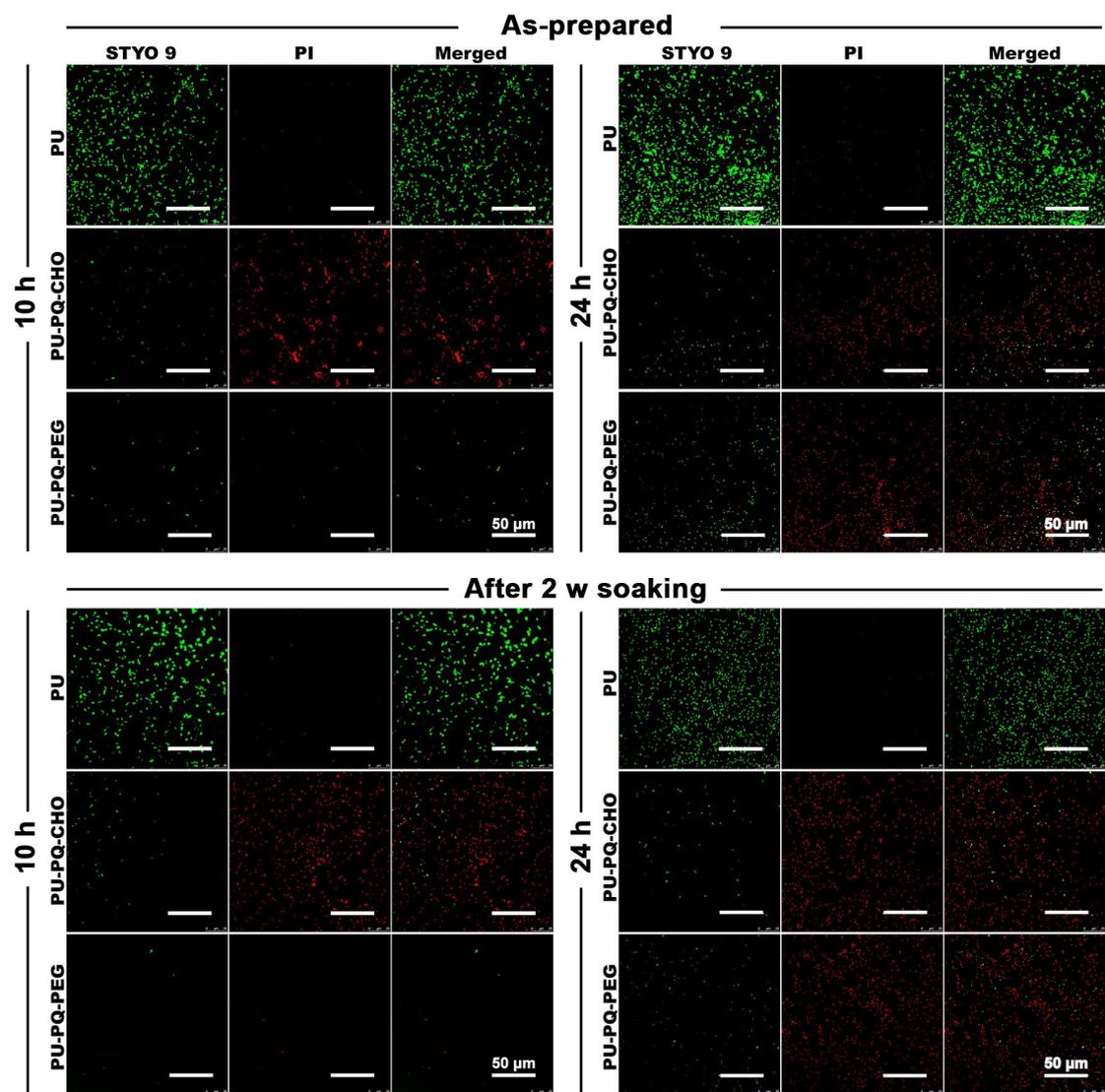


Figure S5. Representative CLSM images of antibacterial test against *S. aureus* of as-prepared samples and soaked samples in PBS for 2 w.

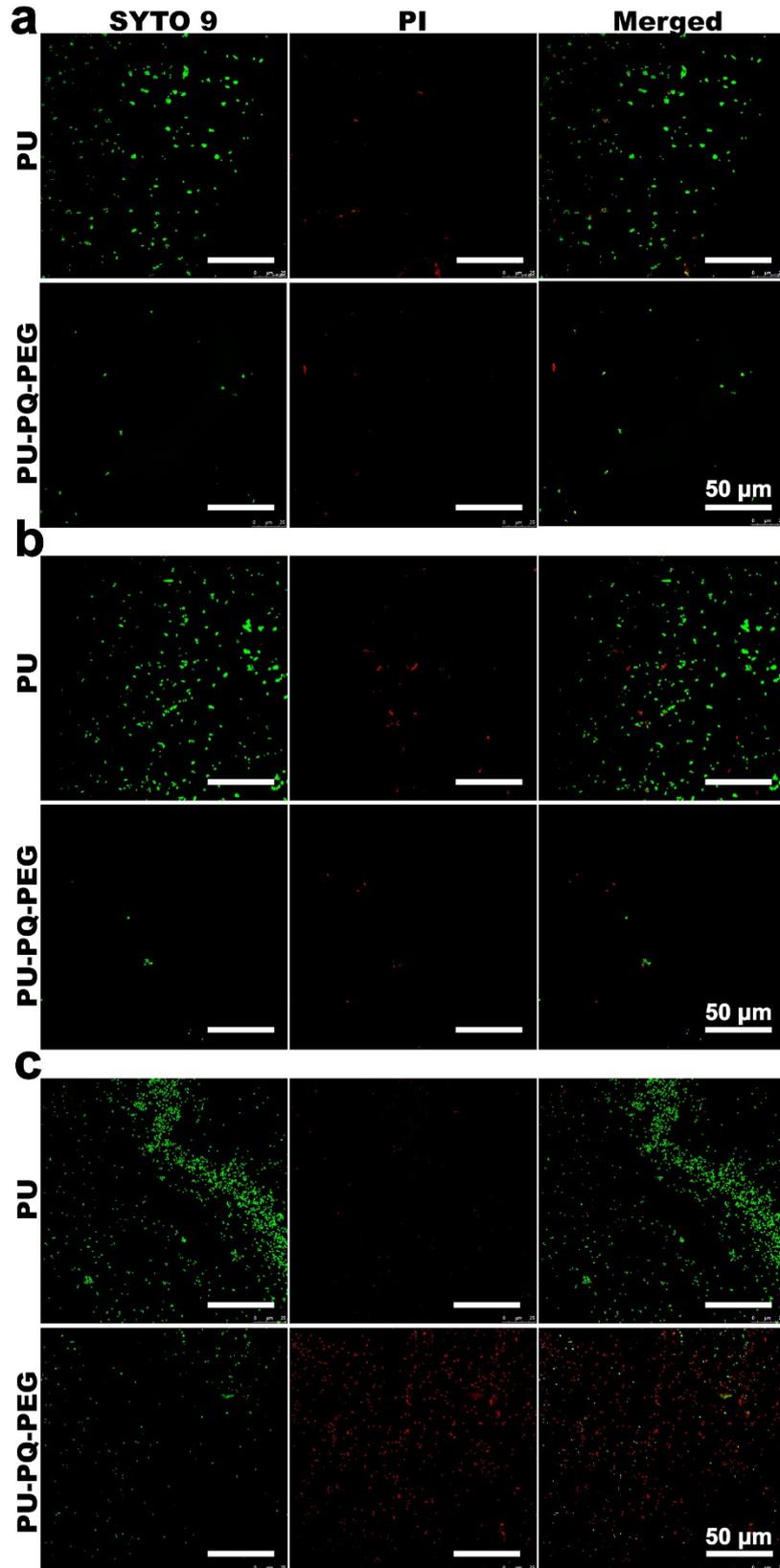


Figure S6. Representative CLSM images of dynamic anti-fouling test for *S. aureus* (a); CLSM images of dynamic antibacterial test against *S. aureus* for 10 h (b) and 24 h (c).

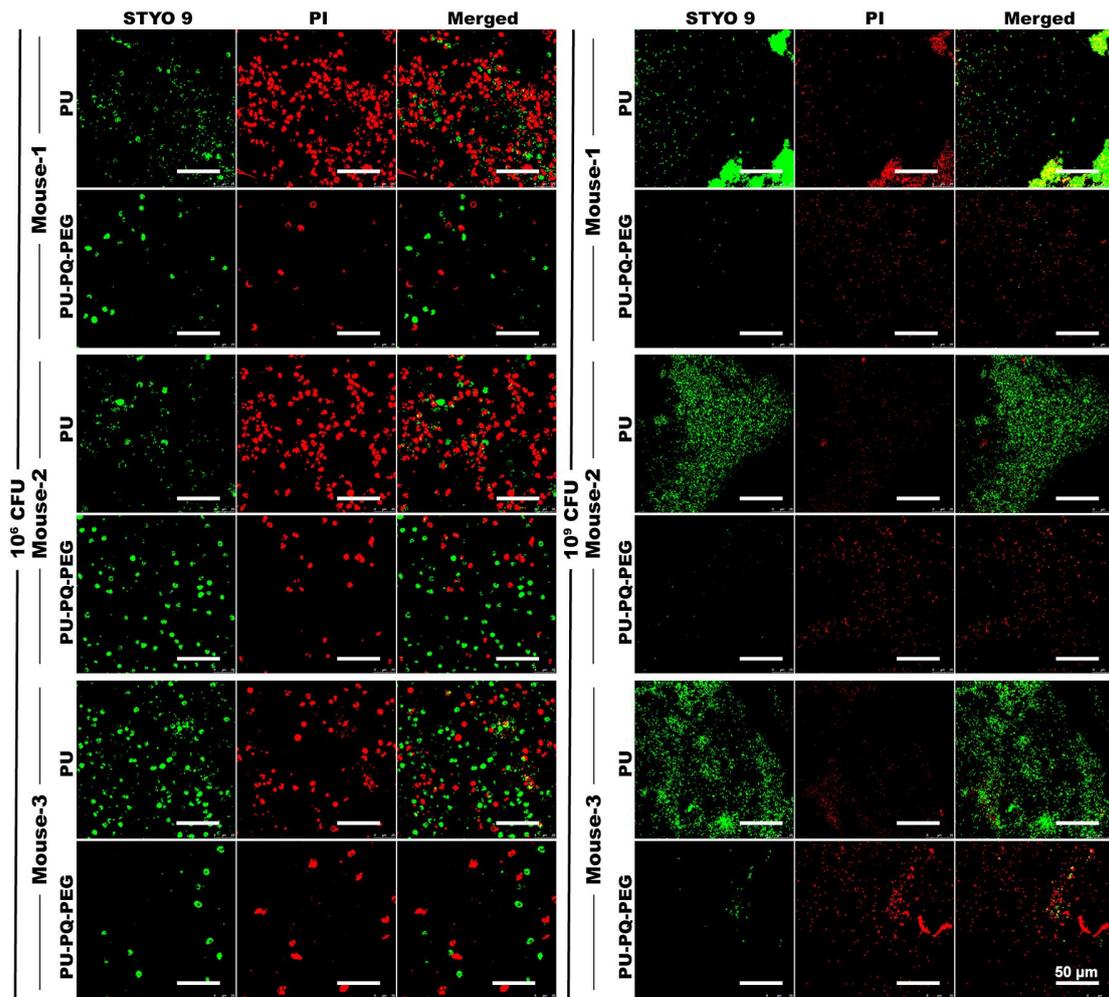


Figure S7. Representative CLSM images of *S. aureus* on PU and PU-PQ-PEG surfaces under different *in vivo* infected conditions.

3. References

- S1. X. Jin, Y.-H. Xiong, X.-Y. Zhang, R. Wang, Y. Xing, S. Duan, D. Chen, W. Tian and F.-J. Xu, *Adv. Funct. Mater.*, 2019, **29**, 1807915.
- S2. H. H. P. Yiu, H.-j. Niu, E. Biermans, G. van Tendeloo and M. J. Rosseinsky, *Adv. Funct. Mater.*, 2010, **20**, 1599-1609.