Supporting Information

A Versatile pH-responsive Peptide Based Dynamic Biointerface for Tracked Bacteria killing and Infection Resistance

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1. Experimental Section

Materials

Hydrophilic monomer 2-Hydroxyethyl acrylamide (HEAAm, 99%, Sigma), silane reagent 3-(trimethoxysilyl)propyl methacrylate (MPS, 98%, Sigma), photo initiator 2-hydroxy-4′-(2-hydroxyethoxy) -2-methylpropiophenone (HHMP, 98%, sigma), 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, 99%, Tokyo Kachina), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride (EDAC, ≥98%, Sigma), N-hydroxysuccinimide (NHS, 98%, Sigma), 3-aminopropyl methacrylamide hydrochloride (APMA, 95%, Lanzhou Porsechem, China), 2-Chlorotrityl Chloride Resin (1.03 mmol/g, Tianjin Nankai, China), the Fmoc-protected amino acids (≥95%, Chengdu Chengnuo, China), Fmoc-NH-PEG_8-CH_2CH_2COOH (≥95%, Chengdu Pukang, China), Tris (hydroxymethyl) aminomethane (Tris base, ≥99.0%, Aladdin), 2-Morpholinoethanesulfonic Acid (MES-Na, ≥99.0%, Aladdin) and *N*-methyl-2-pyrrolidone (NMP, J&K Scientific), FITC-PEG2000-SH (≥95.0%, Shanghai Ziqibio, China) were used as received. Acrylic acid (AA, ≥99.0%), Acetic acid (Ac, ≥99.5%), Trifluoroacetic acid (TFA, ≥99.5%), Acetonitrile (ACN, ≥99.0%), Piperidine (≥99.0%), Diisopropylethylamine (DIPEA, 99%), Methanol (MEOH, ≥99.5%), Formic acid (FA, ≥98.0%) and all the other AR-grade solvents in this work were purchased from Shanghai Reagent General Factory and used as received. Dichloromethane (DCM), Triethylamine (TEA), Tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were dried with CaH_2 and distilled by a general method before use. The HPLC-grade solvent was purchased from Sigma-Aldrich (Shanghai, China). The peptide synthesis was performed at Qiangyao Biotech (Shanghai, China). Quartz substrates (10 mm in diameter) (Haoneng, China) were used as received. LIVE/DEAD™ BacLight™ Bacterial Viability kit was purchased from Thermo Fisher. Peptone, yeast extract and Cell Counting Kit (CCK8) were purchased from Solarbio (Beijing, China). 0.25% Trypsin/EDTA solution, streptomycin and penicillin were purchased from Gibco BRL (USA). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from HyClone (USA). The mouse fibroblast (L929) cells were kindly provided by Cell Bank/Stem Cell Bank, Chinese Academy of Sciences. *Escherichia Coli* (*E. coli*, ATCC25922) was kindly provided by School of Pharmacy, Jiangsu University.

Synthesis of pH-responsive peptides P1 (Ac-X) and moiety of P2 (X-APMA)

The pH-responsive peptides Ac-$WH_9(QL)_6K_2$ (P1, Ac-X) and $WH_9(QL)_6K_2$-(PEG8)-[N-(3-Aminopropyl) -methacrylamide] (X-APMA) were synthesized on 2-chlorotrityl chloride polymer resin using a standard Fmoc-based solid phase synthesis strategy, and the specific synthetic route of P1 was synthesized according to a previously reported method[1]. After removal of the Fmoc group of the final amino acid, the amino group was capped with an acetyl group. Then, the protected peptide acid was cleaved from the resin using 2% TFA in DCM solution and purified by HPLC. X-APMA was synthesized by firstly grafted Fmoc-NH-PEG8-CH_2CH_2COOH to the 2-chlorotrityl chloride resin, and then the following amino acids were synthesized according to the preparation method of P1. To introduce APMA to the C-terminal of P1-PEG8, excessive N-(3-aminopropyl) methacrylate hydrochloride was used and catalyzed by PyBOP. Then the two pH-responsive peptides were characterized with ESI-MS and HPLC to confirm the peptide sequence and purity.

Synthesis of bacteria binding peptide Y (p937)

In our previous research, the bacteria binding peptide Y (p937, WGLHTSATNLYLHGGGC) was used to fabricate a dual-functional peptide coupling gold nanoconjugate (Au@p937 NRs) for the detection and photothermal ablation of bacteria, in which p937 had the capacity for specific binding to bacteria, along with a residue of cysteine that was able to conjugate with the nanorods (Au NRs)[2]. p937 was synthesized on 2-chlorotrityl chloride polymer resin using a standard Fmoc-based solid phase synthesis strategy and characterized with ESI-MS and HPLC to confirm the peptide sequence and purity.

Ac-$WH_9(QL)_6K_2$ (P1, Ac-X)

ESI-MS (3184.62): 1062.6 $[M+3H]^{3+}$,797.1 $[M+4H]^{4+}$, 638.3 $[M+5H]^{5+}$; **HPLC**: 97.43 % in purity.

$WH_9(QL)_6K_2$-(PEG8)-[N-(3-Aminopropyl)-methacrylamide] (X-APMA)

ESI-MS (3690.21): 1231.61 $[M+3H]^{3+}$, 923.92 $[M+4H]^{4+}$, 739.28 $[M+5H]^{5+}$, 616.22 $[M+6H]^{6+}$; 528.29 $[M+7H]^{7+}$; **HPLC**: 95.39 % in purity.

WGLHTSATNLYLHGGGC (Y, p937)

ESI-MS (1786.99): 894.53 $[M+2H]^{2+}$, 596.62 $[M+3H]^{3+}$; **HPLC**: 96.86 % in purity.

High performance liquid chromatography (HPLC) analysis

Reserved-phase HPLC was performed on an Agilent HPLC system by using a Kromasil 100-5C18 column (5 μm, 4.6 × 250 mm, column temperature 30 °C). Buffer A (0.1% TFA in ACN) and Buffer B (0.1% TFA in water) were used as mobile phase. The flow rate was 1.0 mL/min with a gradient elute. Inject volume was 10 μL and run time was 20 min. For the purification of P1, the gradient elute was 20%-100% buffer A (linear) in 9 min, and purity was 97.43%. For X-APMA, the gradient elute was 20%-100% buffer A (linear) in 7.872 min with purity of 95.39%. For p937, the gradient elute was 20%-100% buffer A (linear) in 9.583 min, and purity was 96.86%.

Electrospray ionization mass spectrometry (ESI-MS)

Electrospray ionization mass spectrometry spectra were recorded on AB SCIEX API-150EX. Buffer: 45% ACN/45% MeOH/10% H_2O/0.1% FA; Flow rate: 0.25 mL/min; Run time: 0.5-1 min; NEB: 12.00; CUR: 6.00; Ion Source: ESI; IS: +4500.

MD Simulation details

All simulations were performed using the GROMACS 2020 software package, and the CHARMM36 force field in combination with TIP3P water molecules. An integration time step of 2 fs was used, with H-bond bonds constrained using the LINCS algorithm. Electrostatic interactions were treated with the Particle-Mesh-Ewald (PME) method applying a real-space cutoff of 1.0 nm. In this system, the initial structure of peptide X was constructed by Gaussian 09, and obtained by relaxation of 5 ns in an aqueous solution under constant temperature and pressure. In the initial structure of the system, the centroid distance between the two peptides is 2 nm. The box size of the neutral system is 9 × 9 × 9 nm^3 and contains 23,602 water molecules. The box of the acid system is 12 × 12 × 12 nm^3, contains 56,260 water molecules and 22 Cl molecules, to neutralize the charge of the system. The main reason for the large boxes in acidic conditions is to avoid the interaction between the periodic images.

Self-assembly of pH-responsive peptides

(1) Sample preparation: The powders of peptides P1 and X-APMA (2 mg, each) were dissolved in HFIP (1 mL). An aliquot of each peptide with 125 μL HFIP was transferred into tube and dried in a vacuum oven (Jinghong Co., Ltd., China) at 25°C for about 3 hours. Samples were prepared by dilution from each dried peptide sample to a concentration of 250 μg/mL in either pH 7.4 solution (Tris buffer, 20 mM) or pH 5.7 solution (MES buffer, 20 mM). The samples were incubated at 4°C for 12 hours.

(2) Characterization:

i) Atomic Force Microscopy (AFM): 10 μL of prepared samples were deposited onto the freshly cleaved mica surface and dried in air, and the samples were rinsed twice. All of the images were mapped by AFM (multimode 8, Bruker, Germany) under ambient conditions in air. Ultrasharp silicon cantilevers (OMCL-AC160TS-R3, Olympus Co.) were used for AFM imaging. AFM imaging was performed at Tapping-mode with a scan frequency of 1 Hz and optimized feedback parameters. The resolution of the images was 512 × 512 pixels. All the images were firstly flattened and analyzed using the NanoScope Analysis software.

ii) Thioflavin T (ThT) assay: ThT fluorescence assay was employed to monitor the peptide aggregation and fibrillation. The fluorescence intensity was recorded by using a Hitachi F-4500 fluorescence spectrophotometer (Japan) with excitation at 450 nm and emission at 470 - 600 nm. A total of 200 μL sample solution composed of solvent, peptide solution and 1 mM ThT solution was added into a 0.1 cm quartz cell for the final measurement. The volume ratio of solvent, peptide solution and ThT was 2:1:1. All the measurements were repeated in triplicate and averaged the intensity value of every sample.

iii) Circular Dichroism (CD) spectra: The data were obtained to evaluate the secondary structure of peptides at room temperature by circular dichroism spectrometry (PTC-348W1, Japan JASCO Co.). Data were collected from 250 nm to 190 nm at room temperature (RT) using a 1 mm cuvette, a bandwidth at 1 nm, scan rate at 100 nm/min and a response time of 0.5 sec. A quartz cell was used with a sample volume of 300 μL and a path length of 1 mm. Each spectrum was averaged from three scans. The background signal of Tris buffer and MES buffer has been subtracted in advance.

Preparation of peptide P1 grafted biointerface

The preparation of the peptide P1 containing substrate can refer to the previous synthesis method[3], can be simply summarized as follows: the quartz glass pieces were firstly hydroxylated by the piranha solution (mixture of 2:1 concentrated H_2SO_4 to 30 % H_2O_2 solutions), at 90^{o}C for 1h. Then the slides were washed with ultrapure water and dried with N_2 3 times respectively. The clean slides were immersed in a silanization solution for 24 hours prepared by diluting MPS (1 mL) in the mixture of ethanol (10 mL) and diluted Ac (0.1 mL, 10% v/v) for surface methacrylate-functionalization, and washed with ethanol and dried with N_2. Then polymer brush was grafted on the slides surface through photo-initiated polymerization, HEAAm (172.5 mg), acrylic acid (34 μL), photo initiator HHMP (5 mg) were dissolved in 2 mL of DMF solution. After filtration with a 0.22 μm membrane, the solution was purged with N_2 for 30 min. Then, 200 μL of the solution was immediately placed between a cover glass slide and the methacrylate-functionalized quartz. Polymerization was immediately initiated under UV light (365 nm) and lasted for 12 min at 25^{o}C. After the reaction, the cover glasses were removed with forceps and the resultant polymer brush-grafted quartz slides were washed with ethanol and ultrapure water for three times and dried with N_2 for further use.

Subsequently, 1 mg peptide P1 powder and 3.8 mg/4.6 mg EDC/NHS were dissolved in 2 mL DMF, placing the modified polymer brush into the reaction vessel with the surface of the quartz plate facing up, and then added 250 μL/slide (P1-125 μg/slide), 500 μL/slide (P1-250 μg/slide), 1mL/slide (P1-500 μg/slide) of the reaction solution of the peptide P1 at room temperature for 24-hour reaction, and different P1-grafted biointerface (P1-125 μg, P1-250 μg, P1-500 μg) obtained.

Characterization of the quartz slides

The German Data physics OCAH 200 contact angle meter was used to measure the static water contact angle of the quartz glass slides in different modification processes at room temperature. Each sample was measured three times with 4 μL deionized water droplets, and the average value was used for analysis. The surface chemical composition of the quartz glass slide was analyzed and determined by Thermo Science ESCALAB 250Xi (UK) X-ray photoelectron spectrometer. Atomic force microscope (Bruker, Multimode 8) in tapping mode was used to

measure the change of the surface roughness of the quartz wafer during polymer grafting.

pH responsiveness of peptide based biointerface

i) QCM online monitoring of peptide assembling and disassembling: QCM-D (QSense, Sweden) *in-situ* on-line was used to monitor peptide assembling and disassembling on the dynamic biointerface. The surface of the gold chip was washed at 70 °C for 5 min with the solution of H_2O : $NH_3 \cdot H_2O$: H_2O_2 (5:1:1, V:V:V). Subsequently, the chip was cleaned with distilled water and ethanol solution, dried with N_2, and then immersed in a methanol solution of 5 mM N, N'-Bis(acryloyl)cystamine for 3 hours. After three times of washing with methanol and distilled water, the gold chip was dried with N_2, and then the anti-adhesion polymer brush poly-(HEAAm-*co*-AAm) and peptide P1 (P1-250 μg) were grafted on the gold chip successively (refer to subsequent preparation methods). The on-line monitoring of peptide X-APMA (200 μg/mL) self-assembling and disassembling in Tris buffer (pH 7.4) and MES buffer (pH 5.7) respectively by QCM-D was performed successively.

ii) pH responsiveness of fluorescent molecules (Peptide-FITC)

(1) Synthesis of Peptide-FITC (X-APMA-PEG-FITC): 100 μg dried peptide X-APMA and 0.5 mg SH-PEG2000-FITC were dissolved in 100 μL alkaline DMSO (pH 8.0) and reacted for 12 h in the dark. 0.5 mg of SH-PEG2000-FITC dissolved in 100 μL of alkaline DMSO solution was used as the negative control.

(2) pH responsiveness of fluorescent molecules: We washed the peptide P1 modified quartz slides obtained above with DMF and ethanol for 3 times, and dried it with N_2 and laid in the 24-well plate, with a addition of 400 μL Tris buffer (pH 7.4). The solution obtained from the experimental and the negative group above were added to the quartz surface with amount of 100 μL/well respectively for 6-hour reaction at 4 ºC. Subsequently the obtained surface was washed with Tris buffer solution for 5 times, and then it was observed under the Olympus inverted fluorescence microscope (IX73). Finally, we placed all the reacted quartz slides with MES buffer for 1 h and 1.5 h incubation, and washed them with MES buffer for 5 times, and then they were observed by fluorescence microscope for the release of fluorophores.

Bacteria capture and release relied on pH-responsive peptide based biointerface

(1) Synthesis of peptide conjugate P2 (X-APMA-Y)*:* 2 mg/mL X-APMA and Y peptide（$m_{X\text{-}APMA}$: m_Y=1:1）were dissolved in alkaline DMSO for 12-hour reaction. The P1 grafted biointerface was used as negative control.

(2) E. coli capture and release for different P1-grafted biointerfaces: The inoculated liquid culture medium of LB was incubated in a shaking bed at 37 °C, and the colony forming units (CFUs) were determined by measuring the optical density (OD) at 600 nm (OD600=1.0 is approximately 1.0×10^8 CFU/mL) and diluted to 10^7 CFU/mL after SYTO 9 prestained. The excess reaction solution was removed from the different amount of peptide grafted quartz surface (P1-125 μg, P1-250 μg and P1-500 μg) and rinsed with DMF and ethanol for 3 times, after that they were dried with N_2, and then the quartz plates were placed into a 24-well plate with addition of 400 μL of Tris buffer, and P2 conjugate with a mount of 100 μL/well subsequently for 6-hour reaction at 4℃. After that, the solution covering the quartz were removed and then the quartz plates were further rinsed with Tris buffer (pH 7.4) for 3 times, then 500 μL of prestained bacteria solution was added for 1 h incubation at room temperature in the dark. Finally, we washed the obtained slides surface with Tris buffer solution for 5 times, and observe it under the Olympus inverted fluorescence microscope (IX73). The interface of the captured bacteria was further incubated in a 24-well plate with MES buffer for 1.5 h, and the residual bacteria was observed under a fluorescence microscope after the plate was rinsed by MES buffer solution for 5 times.

(2) E. coli capture and release for different P2-introduced biointerfaces: As described above, the excess reaction solution was removed from the peptide P1 grafted quartz surface (P1-250 μg) and rinsed and dried with N_2, and then the quartz plates were placed into a 24-well plate with addition of Tris buffer, and P2 conjugate with different mount of 50 μL/well (P2-100 μg), 100 μL/well (P2-200 μg) and 200 μL/well (P2-400 μg) to a total of 500

μL per well, and subsequently for 6-hour reaction at 4℃. The P1-grafted interface was used as control. The subsequent operations were the same as the above ***(2) E. coli capture and release for different P1-grafted biointerfaces.***

Cell culture

The resuscitated mouse fibroblast (L929) cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin at 37 °C and 5% CO_2. L929 cell culture medium was used to confirm the cell adhesion, cytotoxicity of peptides and peptide based biointerface.

Anti-cell adhesion of biointerface

The quartz slides grafted polymer brush poly-(HEAAm-*co*-AAm) and peptide P1 were prepared according to the method above, with different amount of P1 (125, 250, 500μg/slide). The P2 containing dynamic biointerfaces based peptide self-assembling were prepared. The above three kinds of quartz plates were put into 24 well plate. 1 mL L929 cell culture medium (10^4 cells/mL) was added to the quartz modified surface and cultured in RPMI 1640 medium with 5% CO_2 at 37 °C. Subsequently, cell morphology was recorded under a microscope equipped with a digital camera at 3 h and 14 h, respectively. The proportion of nonadherent cells in the microscope field were calculated. Three parallel experiments were conducted in each group, and the average value was taken from three separate experiments.

Cytotoxicity measurement

L929 cells were seeded onto a 96-well plate at a density of 10^4 cells/well and incubated for 24 hours at 37 °C in an incubator with 5% of CO_2. After that, the culture medium was removed. 10 μL of peptide solution at various concentrations (5000, 2500, 1250, 625., 312.5, 156.25, 78.12, 39.06, 19.53 μg/mL) was mixed with 90 μL fresh culture medium in a 96-well plate. After another 24-hour incubation, the Cell Counting Kit (CCK8) was performed to quantify the cell viability by monitoring the UV absorbance at 450 nm. Cell culture without peptides was used as a negative control. All the experiments were performed in six replicates.

Antibacterial/bactericidal properties of constructed dynamic biointerface

E. coli was cultured overnight, and resuspended in Tris buffer (pH 7.4) and MES buffer (pH 5.7) and then diluted to OD=0.1 (~10^7 CFU/mL). The constructed dynamic bionterface containing P1/P2 assembly was placed in a 24-well plate with addition of 0.5 mL of bacterial solution (Tris buffer, pH 7.4) for 1 hour incubation at room temperature and then washed the unbinding bacteria with Tris buffer for 5 times, added 0.5 mL of MES buffer (pH 5.7) for further 1.5 h incubation, and then the plate was rinsed with MES buffer for 5 times. The washed solution was collected and centrifuged for bacteria enrichment, and resuspend in 0.5 mL MES buffer, and incubated at room temperature for 18 hours and then it was ready for test of tracked disinfection. For bacteria defense test of biointerface, we covered the remaining quartz surface with 0.5 mL of OD=0.1 bacterial solution (MES buffer) and it was incubated at room temperature for another 18 hours. Meanwhile, Tris and MES bacteria solution (OD=0.1) were incubated for 18 hours at room temperature as control. LIVE/DEAD (SYTO-9/PI) staining was performed on the obtained bacterial solution, and it was observed under the Olympus inverted fluorescence microscope (IX73). The number of dead bacteria in each field of view was counted and its mortality was calculated for all the samples prepared above.

Statistical analysis

All quantitative data were presented as mean ± standard deviation (S.D.) with no less than three replicates for each experimental condition. Statistical analyses were mainly performed using one-way analysis of variance (ANOVA) followed by Tukey's test. Two-way analysis of variance was only used when comparisons were made with two or more interconnected variables. Differences between two groups are considered significant when the p-value is less than 0.05.

2. Supplementary Figures and Tables

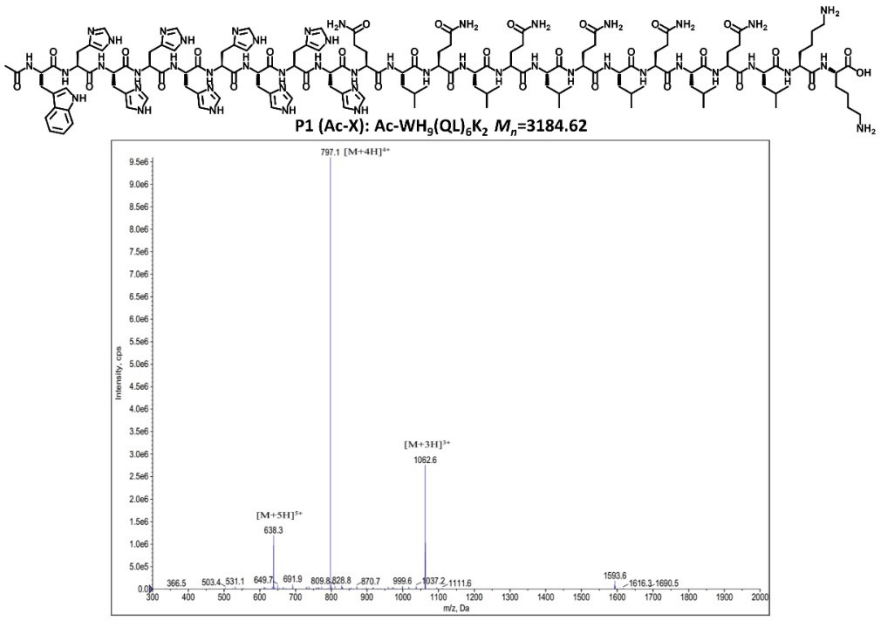

Figure S1. Chemical structure, molecular weight and ESI mass spectrum of the peptide P1.

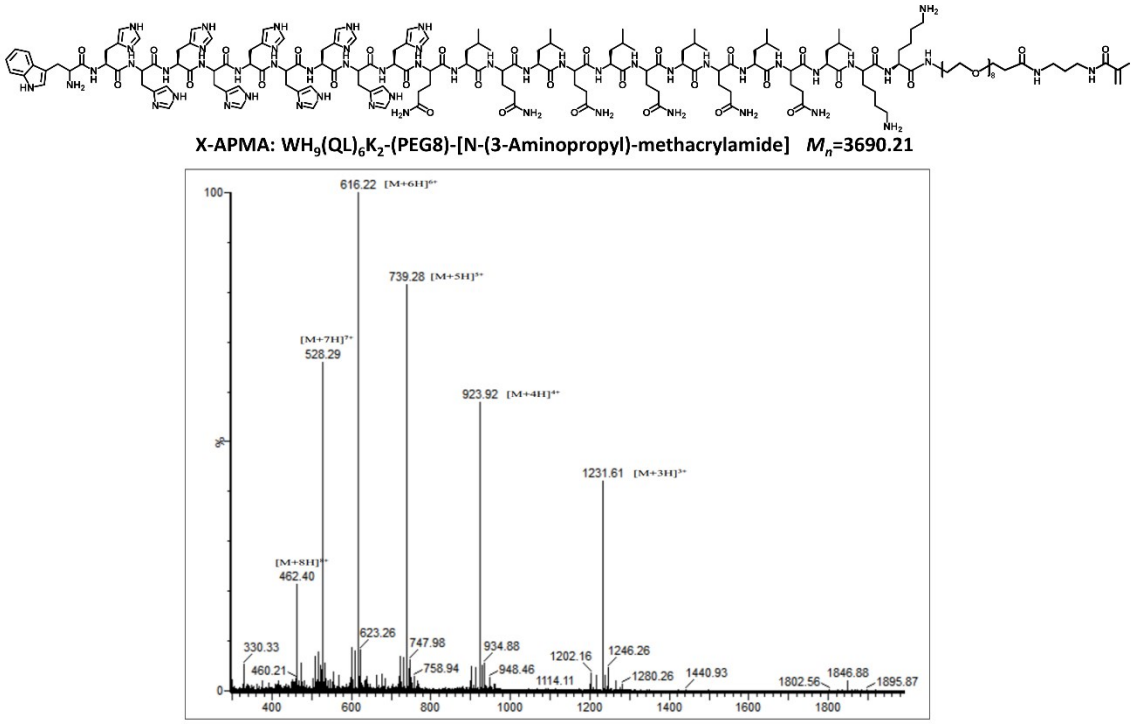

Figure S2. Chemical structure, molecular weight and ESI mass spectrum of the peptide X-APMA.

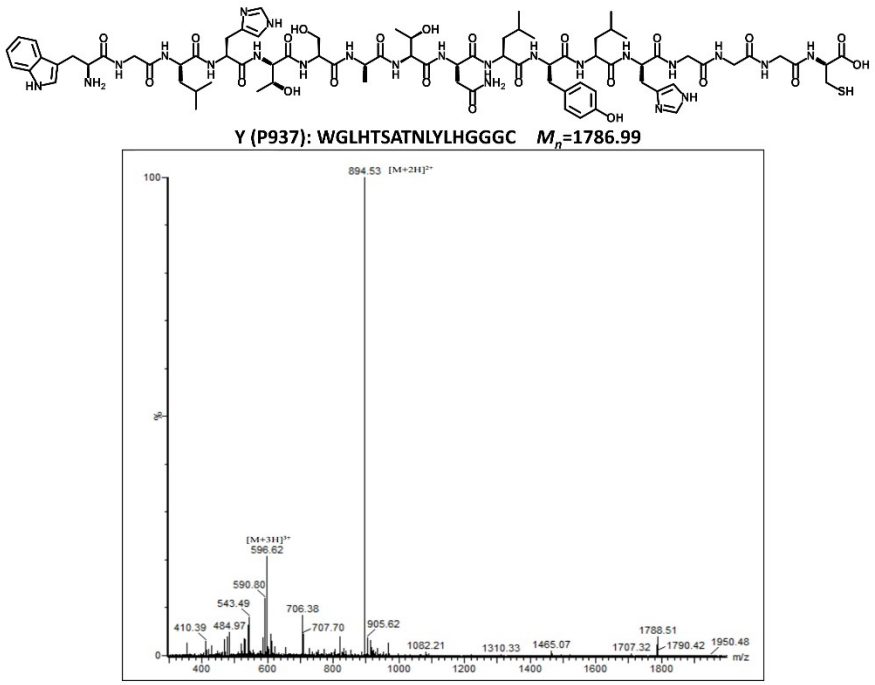

Figure S3. Chemical structure, molecular weight and ESI mass spectrum of the peptide Y (p937).

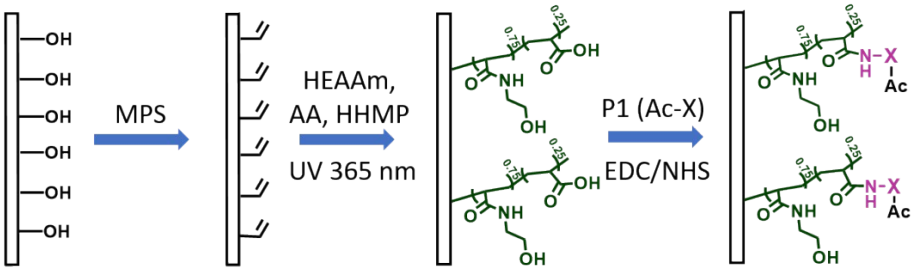

Figure S4. Schematic procedure for preparing the P1-containing polymer-brush-grafted quartz slide. The molar ratio of HEAAm and AA was 3:1.

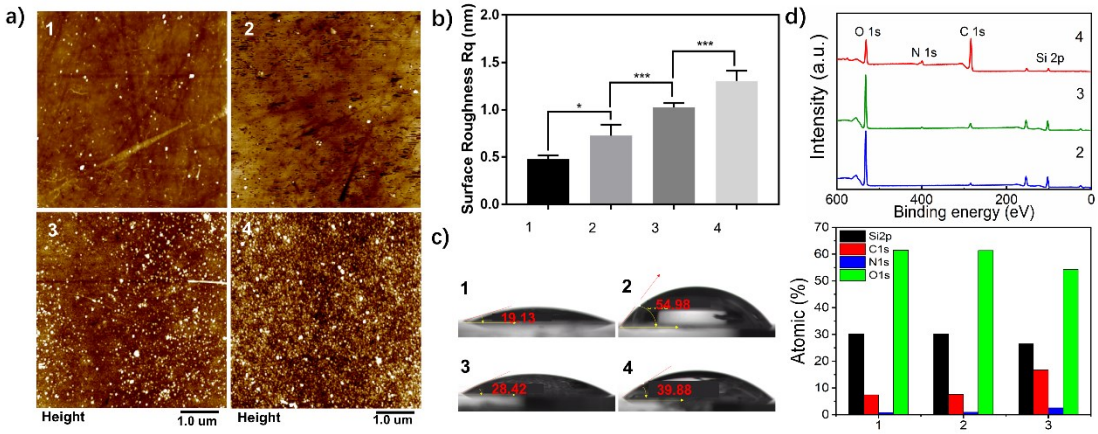

Figure S5. Modification and characterization of peptide based biointerface. a-b) AFM characterization and roughness data statistics (t-test 1 vs. 2 ($p<0.05$), 2 vs. 3 and 3 vs. 4 ($p<0.001$)). c) Static water contact angles of the biointerface in each modified process. d) The photoelectron energy spectrum (XPS) and relative element contents of surface at each modification. 1-4 represent hydroxylation, alkylation, polymer brush grafting and peptide modification on the quartz surface, respectively.

Table S1. Quantification of chemical element composition on the surface of quartz slide, and 2-4 represent alkylation, brush grafting and peptide modification of the quartz surface, respectively.

Atomic Name	Atomic (%)		
	2	3	4
Si2p	30.13	26.46	5.5
C1s	7.56	16.7	67.14
N1s	0.95	2.55	7.96
O1s	61.35	54.29	19.4

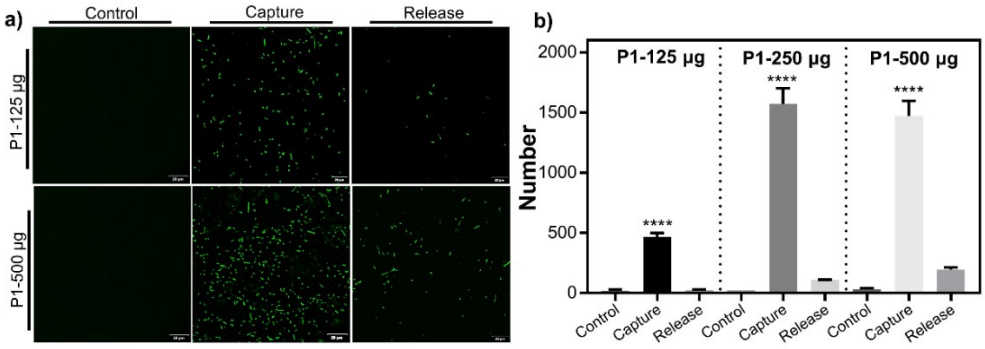

Figure S6. a) The bacteria capturing and releasing on the peptide based dynamic biointerface modified by different amounts of peptides (Polymer (P1-0), P1-125 and P1-500) and the constant amounts of peptides P2-200 µg under different conditions i.e. in Tris buffer at pH 7.4 and MES buffer at pH 5.7, respectively. b) The statistics of the number of captured and released bacteria on the substrate in different cases (one-way ANOVA, $p<0.0001$).

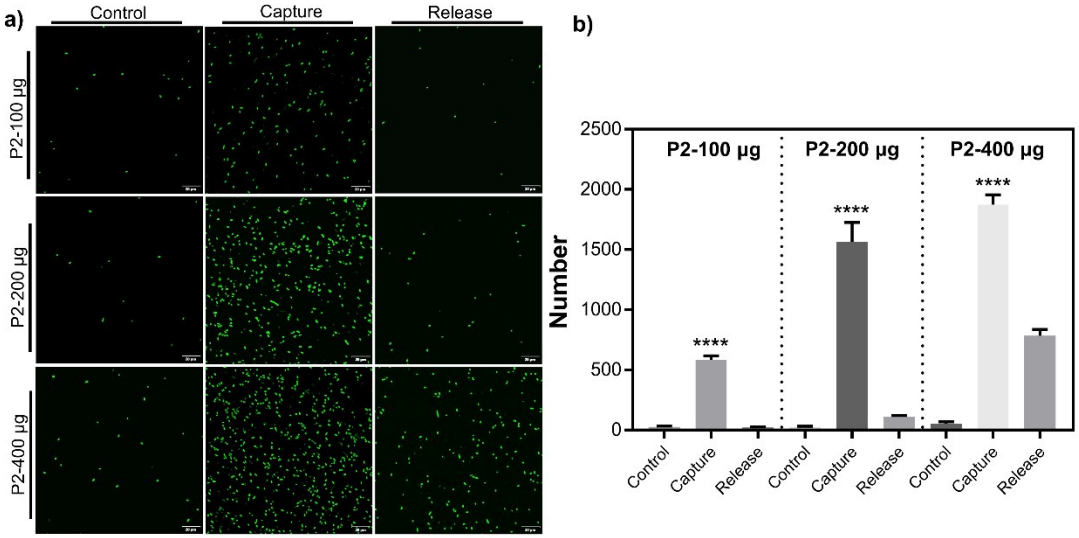

Figure S7. a) The bacteria capturing and releasing on the peptide based dynamic biointerface modified by the constant amounts of peptides P1-200 µg and different amounts of peptides (P2-0 µg (Control), P2-100 µg, P2-200 µg and P2-400 µg (Capture and Release)) under different conditions i.e. in Tris buffer at pH 7.4 and MES buffer at pH 5.7, respectively. b) The statistics of the number of captured and released bacteria on the substrate in different cases (one-way ANOVA, $p<0.0001$).

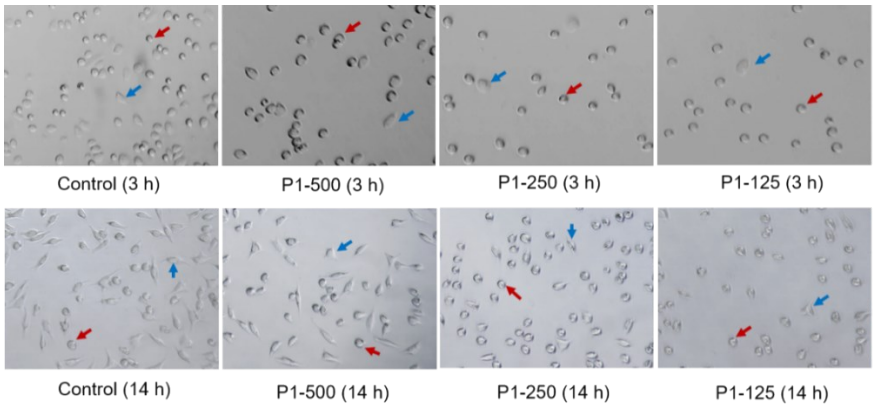

Figure S8. Anti-cell adhesion of biointerface (unadhered cells are indicated by the red arrows and adhered cells are indicated by the blue arrows).

3. References

1. W. K. Chen, S. X. Li, P. Renick, S. Yang, N. Pandy, C. Boutte, K. T. Nguyen, L. P. Tang and H. Dong, *J Mater Chem B*, 2019, **7**, 2915-2919.
2. Q. Y. Chen, L. W. Zhang, Y. H. Feng, F. Shi, Y. B. Wang, P. Wang and L. Liu, *J Mater Chem B*, 2018, **6**, 7643-7651.
3. L. Liu, X. H. Tian, Y. Ma, Y. Q. Duan, X. Zhao and G. Q. Pan, *Angew Chem Int Edit*, 2018, **57**, 7878-7882.