Supporting Information

Towards Infection Resistant Surfaces: Achieving High Antimicrobial Peptide Potency by Modulating the Functionality of Polymer Brush and Peptide

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Synthesis of PDMA-co-APMA brushes on Ti-surface

The Ti surface was firstly deposited with surface initiator ((11-(2-bromo-2-methyl)-propionyloxy)-undecyl-trichlorosilane. Copper (II) chloride ($CuCl_2$, 1.5 mg), copper (I) chloride (CuCl, 10 mg) and Me_6TEN (60 μL) were added successively into a glass tube followed by adding 20 mL Milli-Q water. The solution was degassed with three freeze-pump-thaw cycles. The solution was then transferred into the glove box. The catalyst solution (10 mL) was mixed very well before adding 160 μL DMA and 56 mg APMA. The modified quartz slides were immersed in the polymerization mixture. Soluble methyl 2-chloropropionate (25 μL from a stock solution of 40 μL in 5 mL methanol) was added immediately to the reaction mixture. The polymerization was allowed to proceed at RT (22°C) for 2 h. The substrate was then rinsed thoroughly with Milli-Q water and sonicated in water for 10 min. The soluble polymer formed along with the surface grafted polymer was collected by dialysis (molecular weight cut off: 1000) against water for 1 week.

PMPC-*co*-APMA brush was grafted on quartz slide using similar procedures by adding 500 mg MPC and 94 mg APMA into the catalyst solution (10 mL). Soluble methyl 2-chloropropionate (7 μL from a stock solution of 40 μL in 5 mL methanol) was added immediately to the reaction mixture and the reaction was left at room temperature for 24 h.

PMPDSAH-*co*-APMA brush was grafted on quartz slide using similar procedures with adding 1060 mg MPDSAH and 123 mg APMA into the catalyst solution (10 mL). Soluble methyl 2-chloropropionate (8 μL from a stock solution of 40 μL in 5 mL methanol) was added immediately to the reaction mixture and the reaction proceeded for 2 h.

ITC: ITC experiments on the interaction of peptide E6 with PMPDSAH were carried out at 25.0 °C using a MicroCal iTC200 calorimeter (MicroCal, Northampton, USA). A solution of 22 μM PMPDSAH was loaded into the sample cell and a solution of 440 μM peptide E6 was placed in the injection syringe. A titration experiment consisted of 14 consecutive injections of 2-μl volume and 5-s duration each, with a 3-min interval between injections. Dilution heat of peptide E6 was measured by injecting peptide E6 solution into PBS buffer alone and was subtracted from the experimental curves prior to data analysis. The resulting data (injections 2–14) were fitted to a single set of identical sites model using the MicroCal ORIGIN software supplied with

the instrument. Raw heat data of peptide E6 titration into PMPDSAH solution shows very weak endothermic peaks.

Confocal Microscopy: A solution of the SYTO 9 (0.4 uL) and propidium iodide (PI) (2 μL) dyes in PBS buffer (2 mL) was prepared. After incubation of the substrate with dye solution at room temperature in dark environment for 15 min, the substrates were washed with sterilized water and dried. The samples were then examined using a laser confocal microscope. Samples were imaged using a Nikon C2+ Eclipse Ti-E inversion confocal microscope with a Plan Aprochromat VC 60x/1.4 Oil DIC N2 OFN25 objective. All images were acquired under identical exposure conditions and processed using NIS-Elements C software (Nikon Instruments Inc.)

X-ray photoelectron spectroscopy: X-ray photoelectron spectroscopy (XPS) was performed using a Leybold LH Max 200 surface analysis system (Leybold, Cologne, Germany) equipped with a Mg Kα source at a power of 200 W. Elements were identified from survey spectra.

AFM Morphology: Atomic force microscopy measurements were performed on a commercially available multimode system with an atomic head of 130 × 130 μm2 scan range which used a NanoScope IIIa controller (Digital Instruments, Santa Barbara, CA). Surface morphology was examined under ambient condition in contact mode using a commercially manufactured V-shaped silicon nitride (Si3N4) cantilever with gold on the back for laser beam reflection (Veeco, NP-S20).

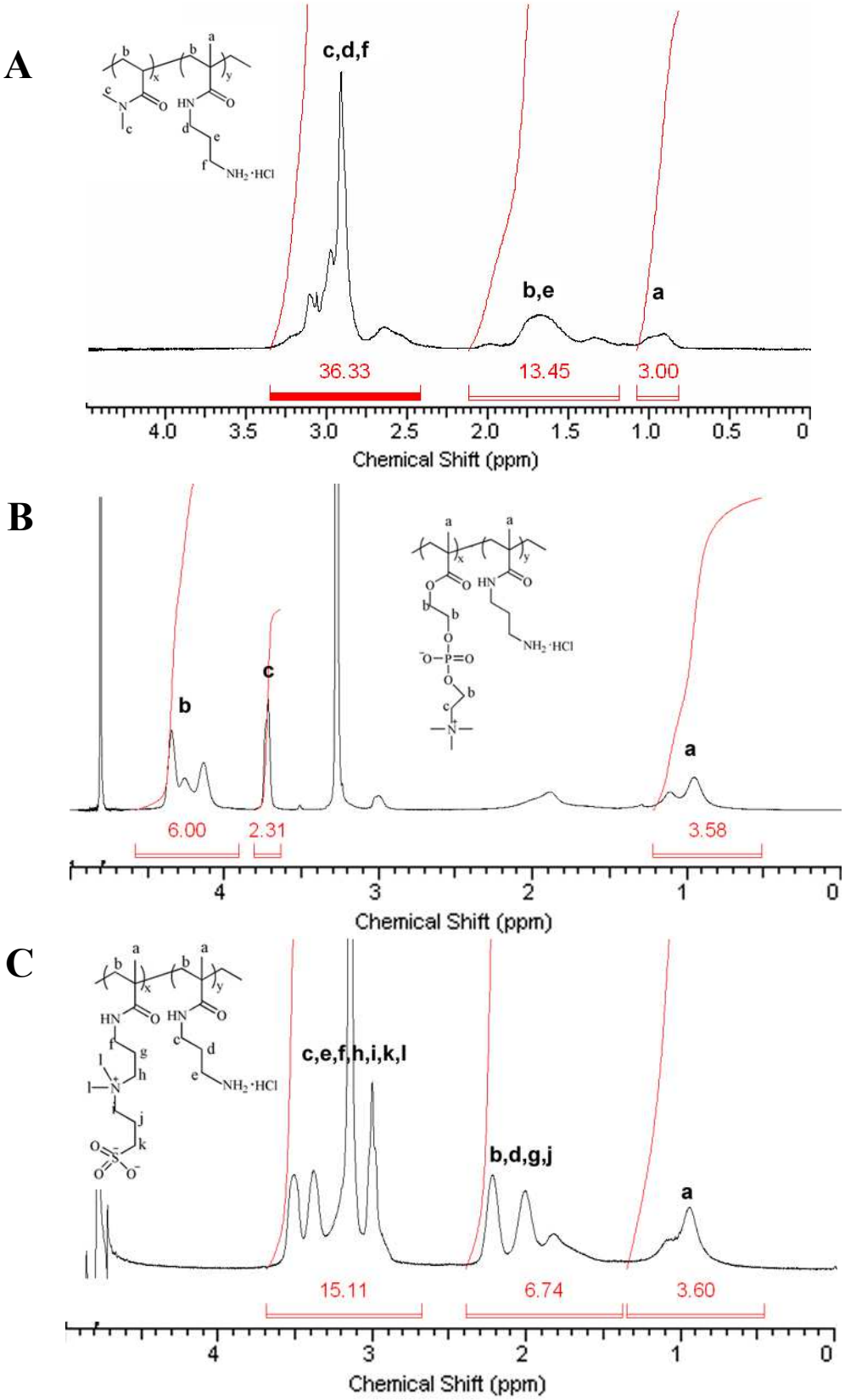

Figure S1. ^{1}H NMR spectra of PDMA-*co*-APMA (A), PMPC-*co*-APMA (B) and PMPDSAH-*co*-APMA (C) formed in the solution. The calculated molar ratio of DMA to APMA, MPC to APMA and MPDSAH to APMA are 4.7:1, 5.2:1 and 4.2:1 respectively.

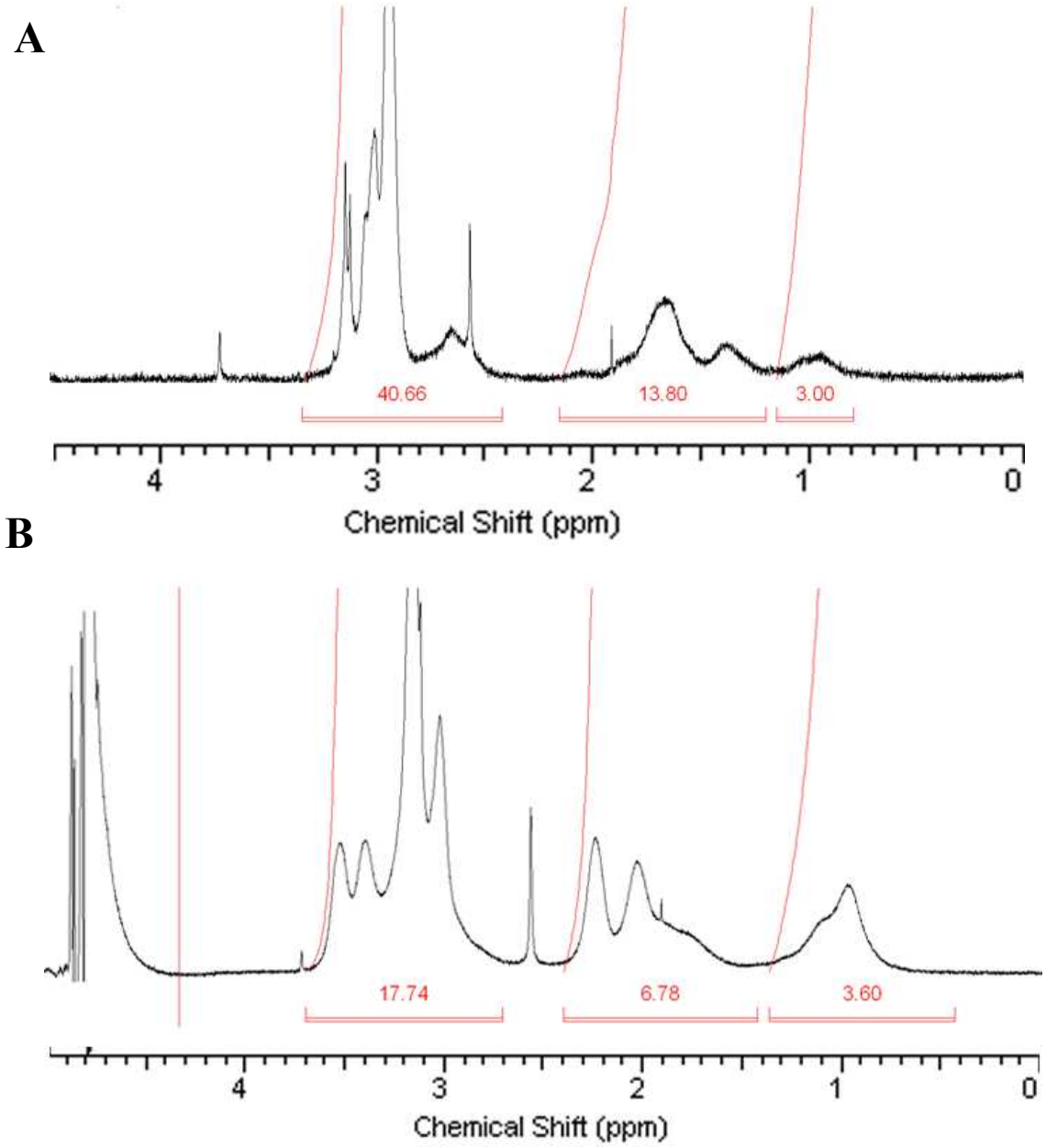

Figure S2. ^{1}H NMR spectra of PDMA-*co*-APMA (A) and PMPDSAH-*co*-APMA (B) cleaved from the NPs surface. The calculated molar ratios of DMA to PMA, MPDSAH to APMA are 4.9:1 and 4.7:1, respectively. The polymer was cleaved from the NPs by using 0.1M NaOH for 7 days.

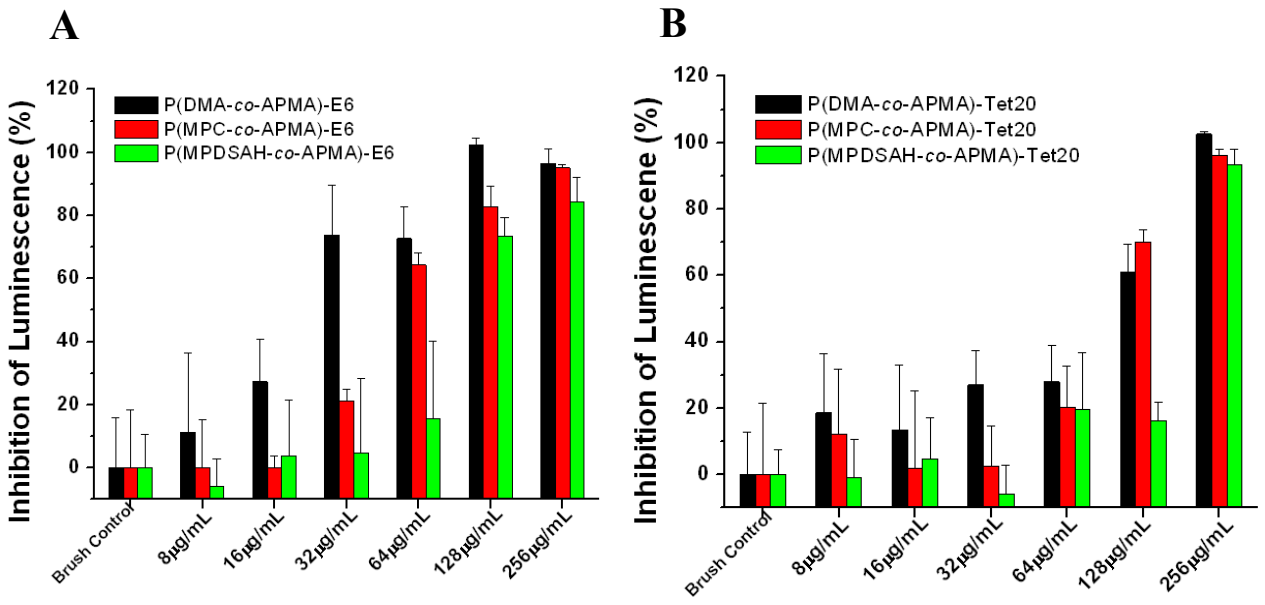

Figure S3. Inhibition of luminescence of *E. coli* upon incubation with nanoparticles modified with peptide E6 (A) and Tet20 (B) immobilized copolymer brush for 4h.

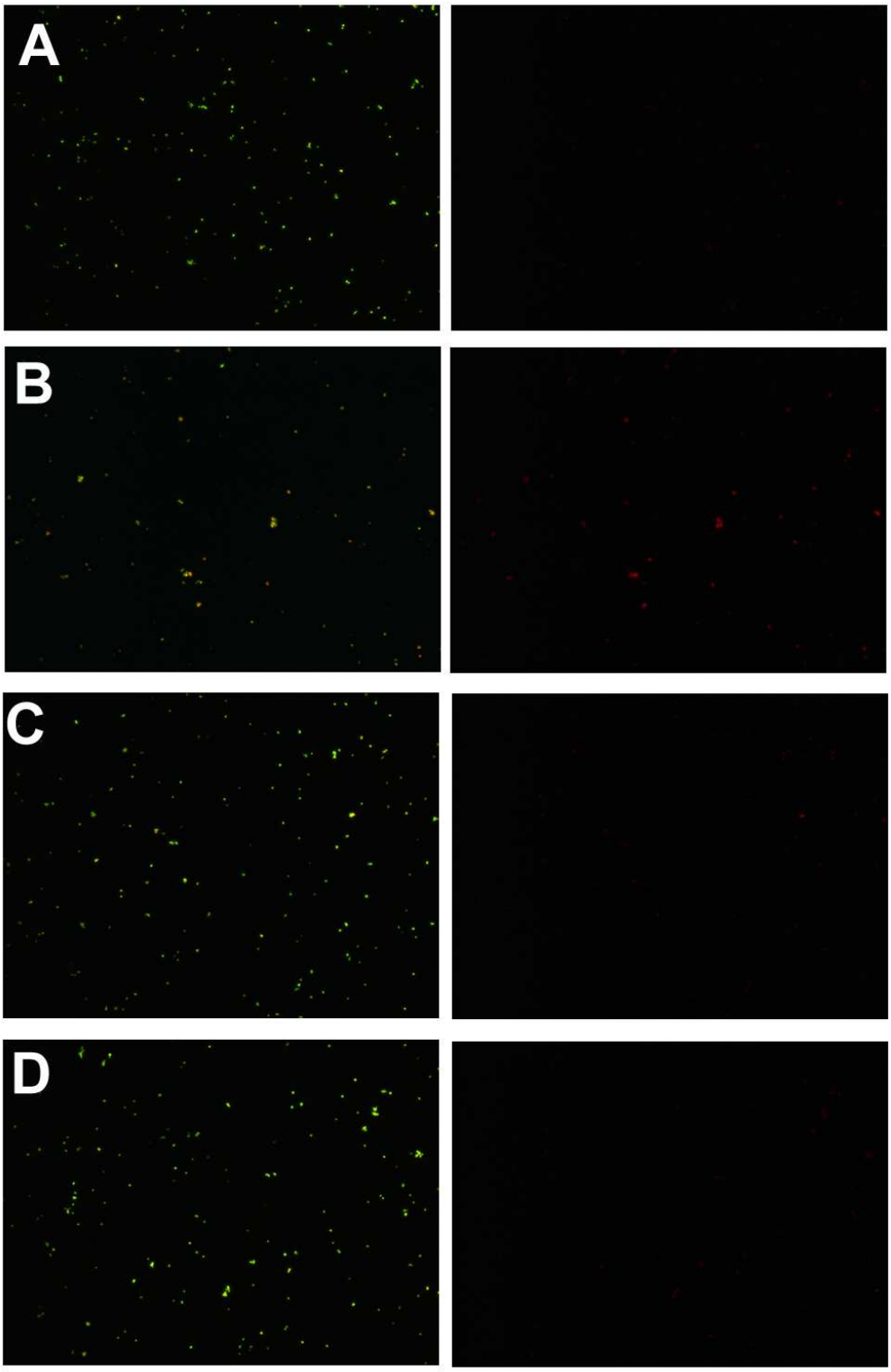

Figure S4. Representative fluorescence microscopy images (left: FITC channel; right: rhodamine channel) of bare Ti (**A**), P(DMA-*co*-APMA)-E6 (**B**), P(MPC-*co*-APMA)-E6 (**C**) and P(MPDSAH-*co*-APMA)-E6 (**D**) coated Ti surface by live/dead bacteria staining after a 4h incubation with *S. aureus*.

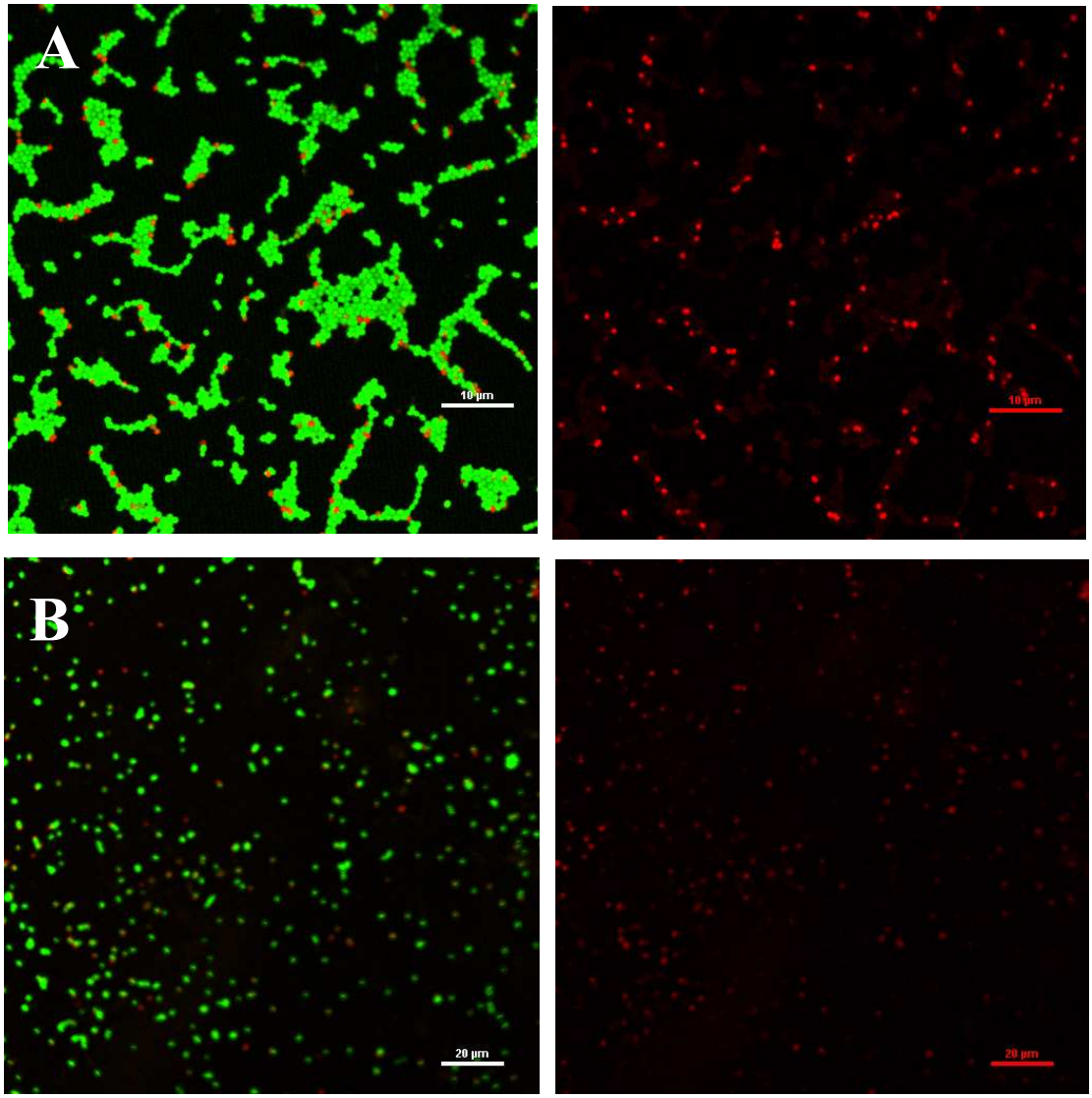

Figure S5. SYTO9/PI staining analyzed with confocal microscopy. *S. aureus* adhered onto the substrate was stained with SYTO9 and PI and examined with confocal microscopy. Merged confocal images (left) and red channel (Ex: 633 nm) (right) of the bare Ti (A), PDMA-*co*-APMA-E6 (B) coated Ti surface are shown. The dead percentage of bacteria on the bare Ti surface is about 12 ± 3.4%. While on the Ti surface grafted with P(DMA-*co*-APMA)-E6, the dead percentage is about 52 ± 9.7%.

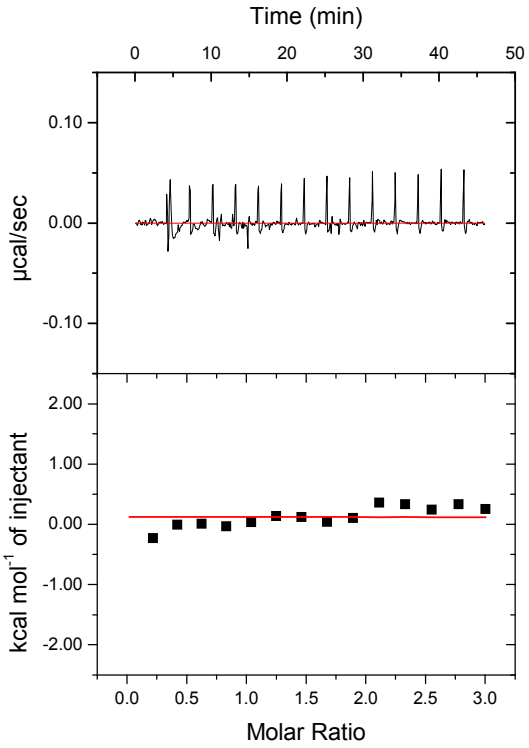

Figure S6. Titration curves (upper panels) and binding isotherms (lower panels) for the titration of peptide E6 (440 µM) into 22 µM PMPDSAH (Mn 320000, PDI 1.5) in 150 mM PBS at pH 7.4.

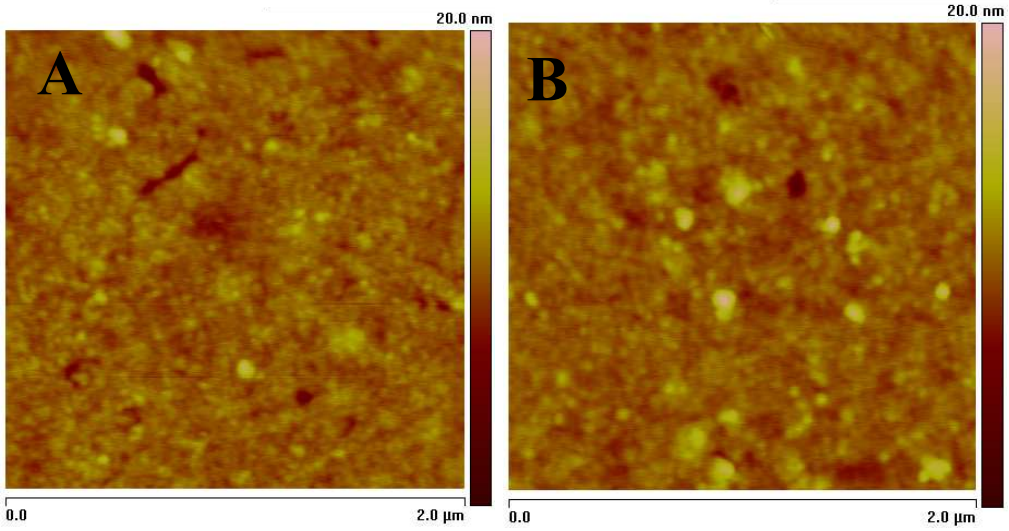

Figure S7. Surface morphology of Ti-PDMA-*co*-APMA-E6 and Ti-PDMA-*co*-APMA-Tet20. The surface roughness was 1.7 nm and 1.5 nm, respectively.

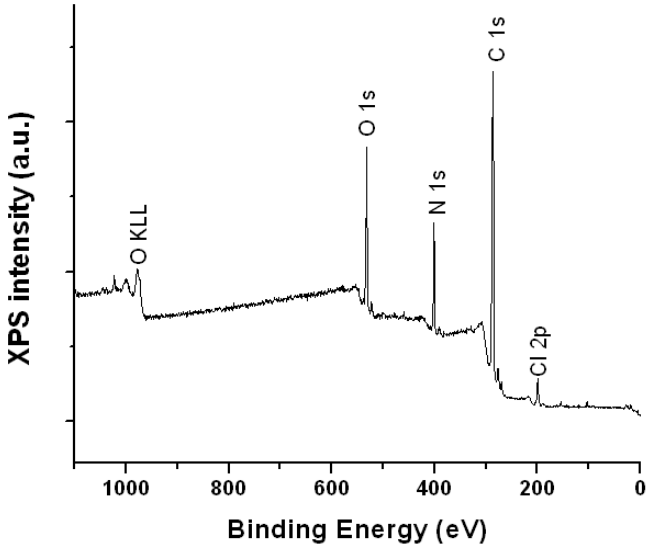

Figure S8. XPS survey scan of Ti-PDMA-co-APMA.

Table S1. Characterization of AMPs tethered polymer brushes on silicon substrate

Samples	Thickness (nm)		Swelling ratio		Molecular weight and PDI	Grafting density (chains/nm^2)	Water contact angle after peptide conjugation
Si-PDMA-*co*-APMA (E6)	24.1±1.2 (d) 48.6 ± 3.2 (w)	49.3 ± 1.1 (d) 79.1 ± 3.4 (w)	2.02	1.6	2.4E5, 1.5	0.06	46.3±4.2
Si-PDMA-*co*-APMA (Tet20)		39±1.1 (d) 68 ± 4.7 (w)		1.74			66.3±2.3

d: dry state; w: wet state.