

Supporting information

Acidity triggered charge-reversible multilayer for the construction of adaptive surfaces with switchable bactericidal and bacterial repelling functions

Jindan Wu,^{*ab} Sufang Zhao,^b Shuting Xu,^b Xiaoyu Pang,^b Guoqiang Cai,^{ab} Jiping
Wang^{*ab}

^aMOE Key Laboratory of Advanced Textile Materials & Manufacturing Technology,
Zhejiang Sci-Tech University, Hangzhou 310018, P. R. China;

^bMOE Engineering Research Center for Eco-Dyeing & Finishing of Textiles, Zhejiang
Sci-Tech University, Hangzhou 310018, P. R. China.

*Please address correspondences to

Dr. Jindan Wu (wujindan@zstu.edu.cn), Fax: +86-571-86843666.

1. The synthesis and characterization of PAA-dopa

PAA-dopa was synthesized via the carbodiimide chemistry between carboxylic acid species of PAA and amino groups in dopamine. The ^1H NMR and IR measurement were used to characterize the structure of synthesized PAA-dopa (Figure S1). The peaks at δ 6.80-6.60 attributed to H in the aromatic rings of catechol moieties, and peaks at δ 2.5-1.4 attributed to H in the polymeric backbone. In the FTIR spectrum, the new peaks at 1528 cm^{-1} , 1587 cm^{-1} and 1625 cm^{-1} were attributed to the stretch vibration of C=C bonds in the aromatic rings of catechol moieties, C-N and C=O bonds in the amide groups formed between PAA and dopamine. These results suggested that catechol moieties have been successfully introduced to PAA backbone.

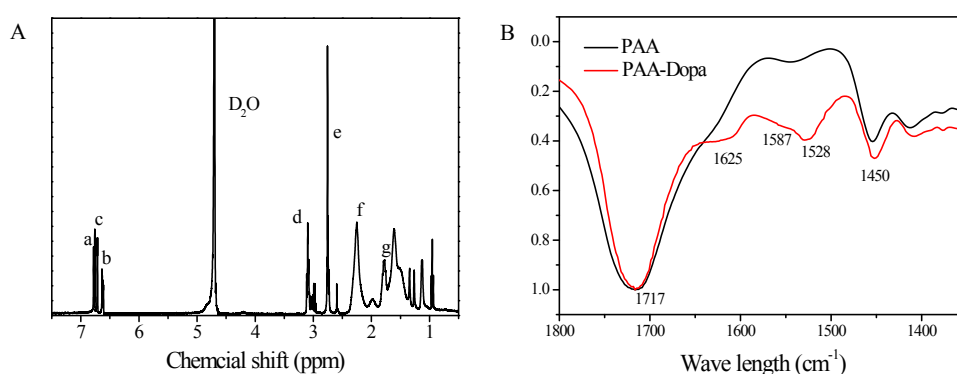


Figure S1 The (A) ^1H NMR and (B) IR spectra of PAA-dopa.

In order to get high crosslinking density, the catechol content (mol%) in PAA molecules was optimized. It was determined by dividing integral area of peaks at δ 6.80-6.60 (H in the aromatic rings) by that of peaks at δ 2.5-1.4 (H in PAA backbone). As the amount of fed dopamine increased, the catechol content also increased (Table S1). The catechol content reached above 9% when the feed ratio of PAA and dopamine was

3:5. In the following investigation, PAA grafted with 9% catechol units was used.

Table S1 The catechol content (mol%) in PAA molecules at different feed ratio of PAA and dopamine.

PAA (mol)	Dopamine (mol)	Catechol content (%)
3	1	0.79
3	2	1.47
3	3	4.55
3	4	5.00
3	5	9.09

2. The LBL assembly of (Q-CS/PAA-dopa)_n multilayer

The LBL assembly was monitored by water contact angle measurement. Surface deposited with Q-CS was relatively hydrophobic with WCA above 60°, while surface with PAA-dopa as the outermost layer turn to more hydrophilic (WCA~50°) (Figure S2). The surface WCA changed alternately during the deposition layer by layer, indicating that the assembly was successful.

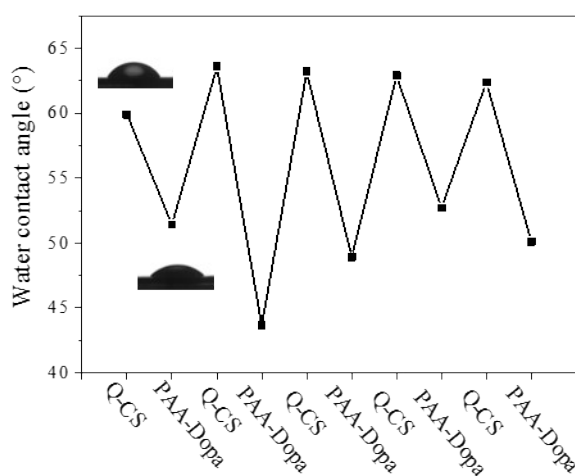


Figure S2 The variation of water contact angle of multilayer during the LBL assembly.

To find an optimal bilayer number, the thickness and roughness of multilayers with 3, 5, 7 bilayers were also measured (Figure S3). Surface deposited with (Q-CS/PAA-dopa)₃ was inhomogeneous since the multilayer was too thin to cover the substrate. When the bilayer number increased, the thickness of multilayer grew from tens to hundreds of nanometers. The thickness of (Q-CS/PAA-dopa)₇ reached 450 nm, and its roughness was about 200 nm. Combining appropriate thickness and low roughness of multilayer, surface assembled with (Q-CS/PAA-dopa)₅ was used in the following investigation.

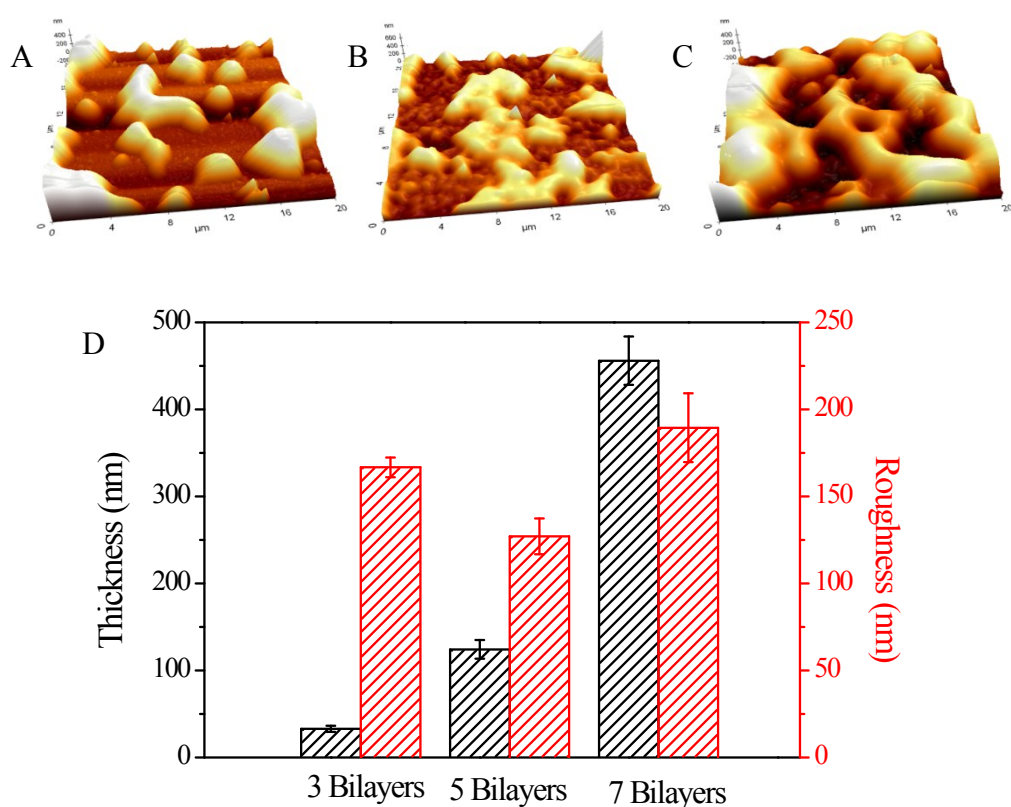


Figure S3 (A-C) The surface morphologies of (A) (Q-CS/PAA-dopa)₃, (B) (Q-CS/PAA-dopa)₅ and (Q-CS/PAA-dopa)₇ multilayer; (D) the thickness and roughness of multilayers measured by AFM.

3. The XPS analysis of (Q-CS/PAA-dopa)_n multilayer

The peaks of N1s and O1s were analyzed and the results were shown in figure S4. In N1s spectrum, the peaks at 399 eV and 402.4 eV were attributed to -NH₂ and -NH₃⁺ groups in Q-CS, and those at 400.9 eV were attributed to amide groups in PAA-dopa. On the crosslinked multilayer, the peaks of -NH₂ and -NH₃⁺ groups strengthened, indicating that more Q-CS was detected by XPS. Similarly, in the O1s spectrum, the relative intensity of peaks attributing to ether (530.4 eV) and hydroxyl groups (531.8 eV) in Q-CS increased on the surface of crosslinked multilayer. Since the detection range of XPS technology is about 10 nm, the results confirmed that more chitosan penetrating to the top layer on the crosslinked multilayer. A more compact structure was formed after crosslinking.

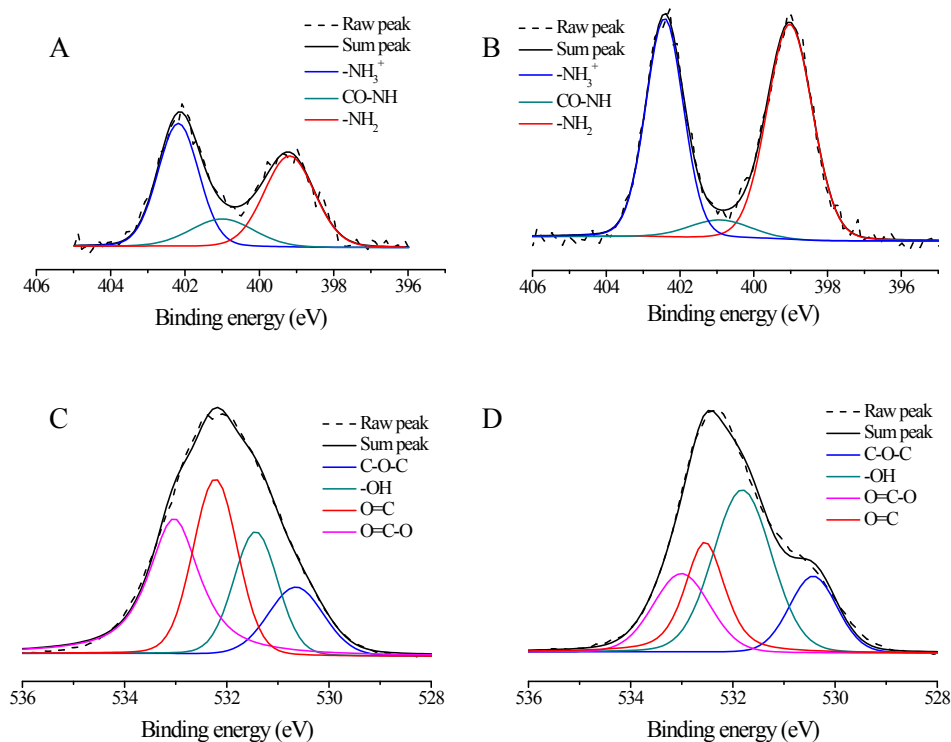


Figure S4 The (A, B) N1s spectrum and (C, D) O1s spectrum of (A, C) uncrosslinked and (B, D) crosslinked multilayer.

4. The stability of (Q-CS/PAA-dopa)_n multilayer

In order to test the stability of the multilayer, the maximum reusable cycle number was measured. The thickness of multilayer after being treated with pH 5.0 and 7.4 sequentially for different cycles was shown in figure S5. It was found that the thickness kept above 100 nm and the multilayer was stable in the first 5 cycles. Then it decreased to 80 and 70 nm at cycle 8 and 10, respectively. It was suggested that the multilayer might start to dissociated after reused for 5 cycles. The reason may be that the polydopamine (the crosslinking of the multilayers) was not so stable at acidic conditions during long-term application¹.

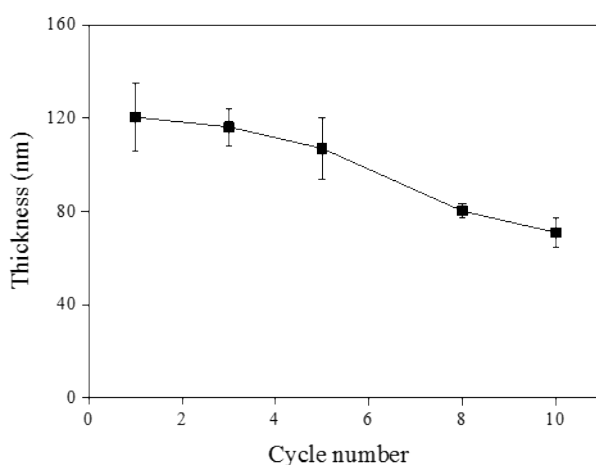


Figure S5: The thickness of multilayer measured by AFM. The multilayers were treated with buffers at pH 5.0 and 7.4 for different cycles.

The antibacterial performance of multilayer in different cycles was also investigated. The bactericidal efficiency was indicated by the percentage of dead bacteria (Figure S6). The experiment follows the same procedure in section 2.7. For *E. coli*., the percentage of dead bacteria maintained in the range of 76% to 81%, indicating that the killing effect kept high after 10 cycles although the integrity of multilayer might be lost. However, the percentage of dead *S. aureus* decreased significantly in cycle 8. The value reduced from 91% to 61%. As shown in CLSM images, the number of live

S. aureus, indicated by green fluorescence, increased when the multilayer was recycled for more than 5 cycles (Figure S7). In summary, the multilayer was stable and the bactericidal performance for both *E. coli*. and *S. aureus* kept high in the first 5 cycles.

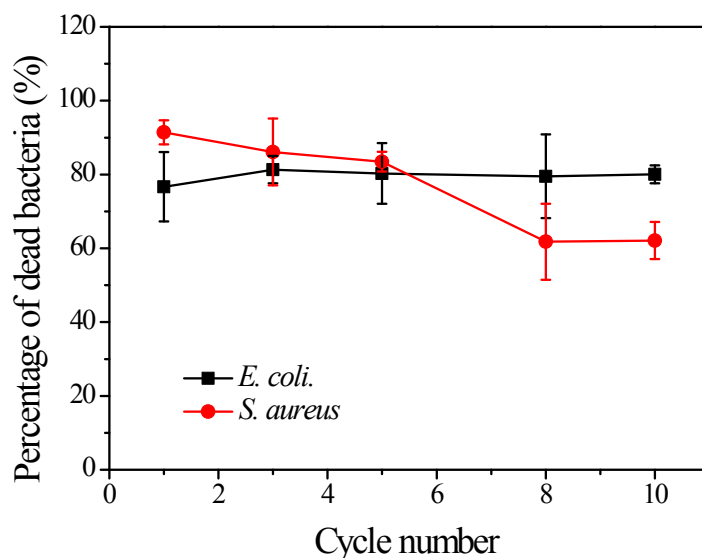


Figure S6 The percentage of dead bacteria on the surface at pH 5.0 in different cycle.

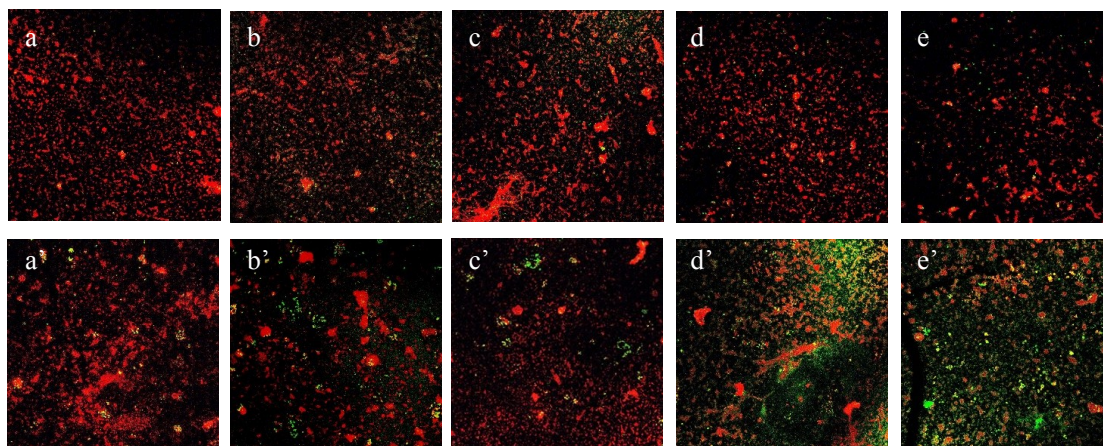


Figure S7 The merged CLSM images of (a-e) *E. coli*. and (a'-e') *S. aureus* on surfaces at pH 5.0 in (a, a') cycle 1, (b, b') cycle 3, (c, c') cycle 5, (d, d') cycle 8 and (e, e') cycle 10. The live bacteria were indicated by green fluorescence and the dead bacteria were indicated by red fluorescence.

5. The bacterial viability in acidic condition

The viability of bacteria at pH 5.0 and 7.4 was tested. A certain volume of bacterial

suspension was diluted with PBS buffer (pH 5.0 or 7.4), followed by incubated at 37 °C for 4 h with shaking at 150 r/min. Then, the bacteria suspension was coated onto nutrient agar plates and cultured for 24 h at 37 °C. The number of bacteria decreased 10% and 30% at pH 5.0 for *E. coli*. and *S. aureus*, respectively, comparing to that at pH 7.4 (Table S2). On the other hand, the percentage of live bacteria on the multilayer was 95% (*E. coli*.) and 65% (*S. aureus*) at pH 7.4, and it decreased to 20% (*E. coli*.) and 6% (*S. aureus*) at pH 5.0 (Figure 7C). So, the percentage of live bacteria decreased 79% and 91% for *E. coli*. and *S. aureus*, respectively. Therefore, it was believed that the death of bacteria on the multilayer was mainly caused by contacting to the positively charged surface at pH 5.0.

Table S2 The number of *E. coli*. and *S. aureus* incubated in PBS at pH 5.0 and pH 7.4 for 4 h.

	pH 5.0	pH 7.4
<i>E. coli</i> .	1.8×10^6 cfu/mL	2.0×10^6 cfu/mL
<i>S. aureus</i>	1.6×10^7 cfu/mL	2.3×10^7 cfu/mL

References:

1. H. Wei, J. Ren, B. Han, L. Xu, L. Han and L. J, *Colloid Surface B*, 2013, 10, 22-28.