

## Supporting Information

### **Mussel-inspired chimeric protein as a novel facile antifouling coating**

Haishan Qi <sup>a,b,c,#</sup>, Weiwei Zheng <sup>a,b,c,#</sup>, Xiao Zhou <sup>a,b,c</sup>, Chen Zhang <sup>a,b,c</sup> and Lei Zhang <sup>a,b,c\*</sup>

a. Department of Biochemical Engineering, School of Chemical Engineering and Technology,

Tianjin University, Tianjin 300072, China

b. Key Laboratory of Systems Bioengineering (Ministry of Education), Tianjin University, Tianjin

300072, P. R. China

c. Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin

University, Tianjin, 300072, China

#. Qi and Zheng contributed equally to this work

\*Correspondence to: Lei Zhang

E-mail: lei\_zhang@tju.edu.cn

## **Materials and Methods**

### **1. pET28a-MP-KE plasmid construction**

The DNA sequence encoding MP-KE was designed as shown in Fig. 1a. The gene was synthesized based on the codon preference of *Escherichia coli* and inserted in pET28a vector (Novagen, Madison, WI) between *NdeI* and *HindIII* restriction sites by Genewiz (China). The resulting recombinant plasmid was named as pET28a-MP-KE which was further introduced into the *E. coli* strain BL21(DE3)pLysS expression host.

### **2. Protein expression**

A single colony from a freshly streaked plate was cultured overnight at 37°C in 5 mL Luria-Bertani (LB) medium containing 50 µg/mL chloramphenicol and kanamycin. 2.5 mL of the culture was inoculated into 250 mL of the above medium in a 1 L baffled flask and cultivated at 37°C, 250 rpm. Then the culture was induced by adding of Isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 1.0 mM until OD<sub>600</sub> reached 0.6-0.8. The culture was further incubated at 37°C and 250 rpm for another 10 h, after which the cells were harvested by centrifugation at 6000 rpm for 20 min at 4°C.

### **3. Protein purification**

After centrifugation, the cell pellets were washed three times with 10 mM phosphate buffer. The precipitates were then resuspended with phosphate buffer and lysed by ultrasonication with ice-water bath and lysates were centrifuged at 4 °C, 12000 rpm for 20 min. The collected cell debris were washed twice with wash buffer (2 M urea in 50 mM phosphate containing 4 mM EDTA and 0.6% Trison X-100) for 30 min at 25 °C and inclusion body was harvested through centrifugation. Then 50% acetic acid was used to resuspend inclusion body and extract fusion protein at 250 rpm for 2h. After centrifugation, supernatant was collected and dialyzed in 5% acetic acid buffer with stirring overnight at 4°C using porous membrane (MWCO = 10,000 Da). The protein solution was further concentrated by ultrafiltration and lyophilized to get purified fusion protein. In addition, the purified protein was analyzed by a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) with a 4700 Proteomics Analyzer (Applied Biosystems) in the positive ion linear mode.

### **4. Hydroxylation of tyrosine residues**

The conversion of tyrosine residues in the recombined protein MP-KE (2 mg/mL) to Dopa

was performed using 200 U/mL tyrosinase (Sigma,  $\geq 2,000$  U/mg) in 0.1 M PBS buffer containing 20 mM sodium borate and 25 mM ascorbic acid at 25°C with stirring and bubbling for 5 h.<sup>1</sup> Enzyme activity was halted with 0.2 mL of 6 M HCl per mL of reaction. After modification, the mixture was dialyzed (MWCO = 10,000 Da) in 5% acetic acid at 4°C and concentrated by ultrafiltration, after which, samples were lyophilized and collected. The proportion of tyrosine residues modified to Dopa was measured by UV Spectroscopy. Borate-Dopa complexes showed higher  $\lambda_{\max}$  than uncomplexed Dopa in ultraviolet region.<sup>2</sup> A microplate spectrophotometer reader (Multiskan GO microplate Spectrophotometer, Thermo Scientific, Vantaa, Finland) was used to measure absorbance of 1 mM Dopa standard and modified MP-KE in 0.1 M HCl and 0.1 M sodium borate (pH 8.5) from 285 nm to 296 nm, respectively. The spectra differences were measured by subtracting the spectra of the Dopa standard or modified MP-KE in 0.1 M HCl from that obtained in 0.1 M sodium borate. The Dopa standard exhibited a subtraction difference  $\lambda_{\max}$  of 292 nm with a  $\epsilon$  value of 3200/M/cm. Using the  $\lambda_{\max}$  and  $\epsilon$  of 1 mM Dopa standard, the Dopa concentration can be calculated according to Beer's law:<sup>2</sup>

$$A = \epsilon bc$$

Where A is absorbance at 292 nm,  $\epsilon$  is the molar absorptivity with 3200/M/cm, b is the path length of the sample which is 0.051 cm in this work, c is the molar concentration of Dopa in solution.

## 5. NBT/glycinate staining assay

NBT/glycinate assay was used to detect Dopa in MP-KE after hydroxylation with tyrosinase.<sup>3</sup> 200  $\mu$ L of 2 mg/mL modified MP-KE, unmodified MP-KE and water were added to 1 mL 0.5 mg/mL nitroblue tetrazolium (NBT) in 2 M potassium glycinate (pH 10). After 1 h incubation in darkness at 25°C with shaking, the absorbance of samples were determined using a multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland) from 350 nm to 1000 nm. Besides, a visual detection of Dopa was also performed with PVDF membrane. In brief, 10  $\mu$ L of 2 mg/mL modified MP-KE, unmodified MP-KE and water were dropped on PVDF membrane, respectively. After blotting, the membrane was immersed in 2 M potassium glycinate (pH 10) containing 0.5 mg/mL NBT and incubated in darkness at 25°C and 50 rpm for 1 h. The membrane was washed twice with 0.2 M sodium borate (pH 8.5) and then counterstained with

0.1% Ponceau S in 5% acetic acid.

## **6. Surface coating and AFM analysis**

Coverslips, glass beads ( $d = 250 \mu\text{m}$ ), mica sheets ( $d = 9.9 \text{ mm}$ ) and polytetrafluoroethylene (PTFE) were washed with acetone, alcohol and MilliQ water successively. After dried at  $40^\circ\text{C}$  for 1 h, the coverslips were immersed in 2 mg/mL modified MP-KE, unmodified MP-KE and water respectively and incubated at  $25^\circ\text{C}$  for 12 h. The morphology and structure of glass surface coated with chimeric protein was characterized by atomic force microscopy (AFM) under ambient conditions. The commercial silicon AFM probes with resonant frequency of 300 kHz and force constant of 40 N/m were used with a tapping mode.

## **7. Water contact measurement**

Water contact measurement was conducted to characterize the hydrophilicity of substrate materials coated with different chimeric proteins. The measurement was performed at  $25^\circ\text{C}$  with 5  $\mu\text{L}$  droplets.

## **8. Protein adsorption assay**

The evaluation of nonfouling behavior was performed on glass beads and PTFE plates coated by different samples with bovine serum albumin (BSA) labeled with fluorescein isothiocyanate (FITC-BSA). Glass beads and PTFE plates after modification were exposed to 0.1 mg/mL FITC-BSA and then incubated at  $25^\circ\text{C}$  and 60 rpm for 30 min covered with aluminum foil, followed by rinsing with PBS for three times. Fluorescence microscopy images were obtained with inverted microscope (Nikon Eclipse Ti-S) excited by blue light. The amount of FITC-BSA adsorbed on plates were determined by bicinchoninic acid (BCA) colorimetric assay at 562 nm with a subtraction difference before and after FITC-BSA adsorption.<sup>4</sup>

## **9. Bacteria adhesion assay**

*E. coli* carrying green fluorescent protein (GFP) gene and *S. aureus* were chosen as model biological pollutant for Gram-negative and Gram-positive bacteria, respectively. Both the bacteria strains were cultured in LB medium. The culture was harvested by centrifugation at  $4^\circ\text{C}$  and 2000 rpm for 15 min at when the  $\text{OD}_{600}$  reached 1.0. The pellets were washed by sterile PBS for three times and then resuspended to an  $\text{OD}_{600}$  value of 1.0. Coverslips coated with different protein samples were immersed in bacterial solution and incubated at  $37^\circ\text{C}$  and 150 rpm for 2 h. Then coverslips were washed three times with sterile 0.9% NaCl. For visible observation of *S.*

*aureus*, the coverslips were stained with syto 9 solution in darkness for 30 min. Bacterias were imaged with inverted fluorescence microscope (Nikon Eclipse Ti-S) excited by blue light. The number of bacteria were counted and calculated according to the microscopy image results.

#### **10. Hemocompatibility test**

To evaluate the hemocompatibility, hemolysis assay was conducted by co-incubation different glass plates with diluted blood (20% fresh blood and 80% 0.9% NaCl) at 37°C for 1 h.<sup>5</sup> Equally diluted blood with 0.9% NaCl and MilliQ water were used as negative and positive control, respectively. Samples were centrifuged at 1000 rpm for 10 min and the absorbance of supernatants was measured at 541 nm. Hemolysis ratio (HR) was calculated according to the following equation:

$$HR = (A_S - A_N) / (A_P - A_N)$$

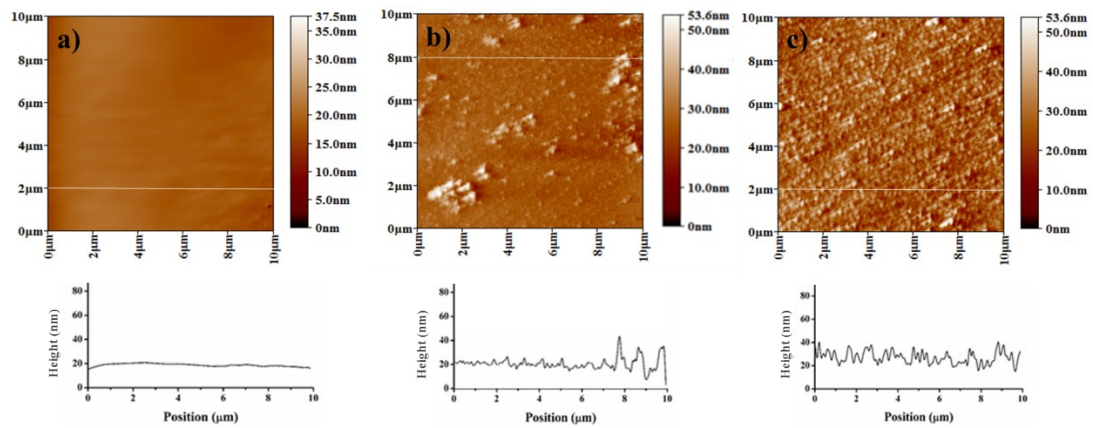
$A_S$ ,  $A_N$  and  $A_P$  are the absorbance at 541 nm of samples, negative control and positive control, respectively.

#### **11. Cytotoxicity assay**

The NIH/3T3 fibroblasts were cultured in DMEM supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum at 37°C and 5% CO<sub>2</sub>.  $7.5 \times 10^4$  cells/well were seeded in 24-well plate and incubated for 12 h. The modified mica sheets were rinsed three times with sterilized PBS and then soaked in sterilized PBS with ultraviolet irradiation for 30 min. The mica sheets were added into 24-well plate with cells and co-incubated. A LIVE/DEAD viability/cytotoxicity kit (Invitrogen, CA, USA) was used to evaluate the viability of cells.<sup>4</sup> After 24 h of incubation, the culture medium and mica sheets were gently removed. Cells were incubated in staining solution (2 μM calcium-AM and 4 μM ethidium homodimer) at 37°C for 30 min in darkness. Cells were observed with inverted fluorescence microscope and the viability was calculated according to the number of live and dead cells.

MAKPSYPPTYKAKPSYPPTYKAKPSYPPTYKAKPSYPPTYKAKPSYPPTYKAKPSYPPTYKAKPSYPPTYKSSEEYKGGYYPGNTY  
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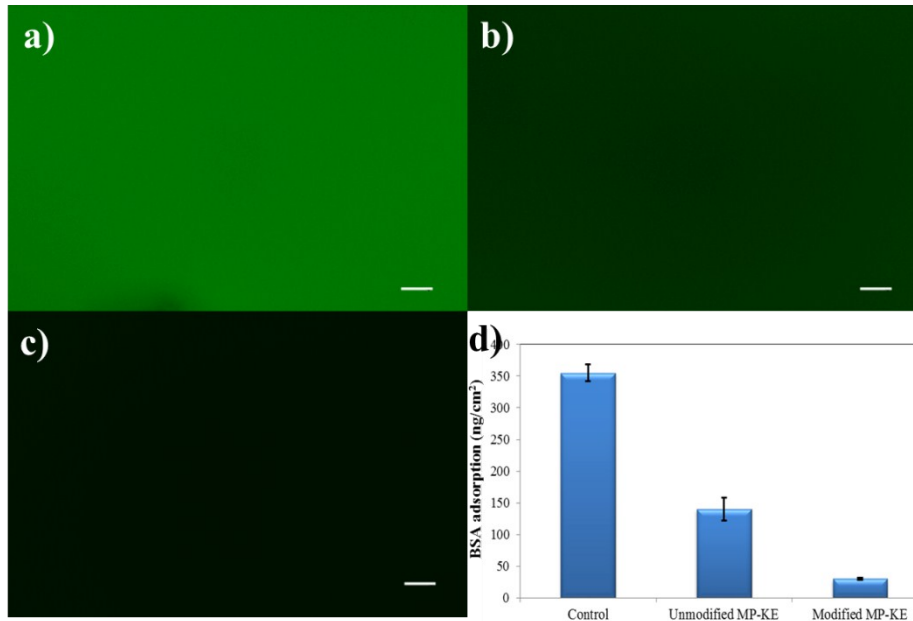
**Fig. S1.** The primary sequences of chimeric protein MP-KE.



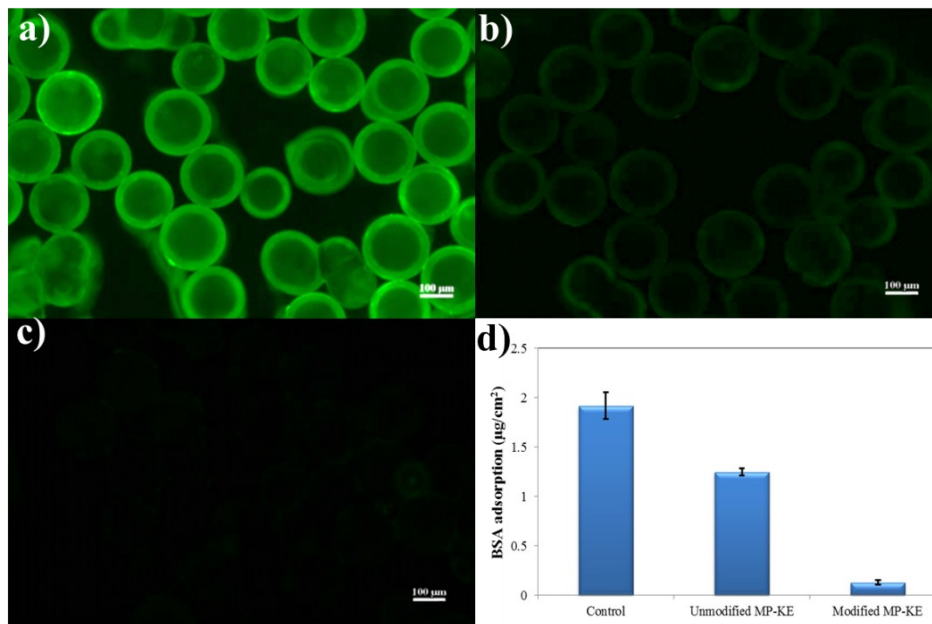
**Fig. S2.** AFM height images of different glass samples, uncoated surface (a), unmodified MP-KE coated surface (b), and modified MP-KE coated surface (c). The bottom curves show the representative height differences for each sample.



**Fig. S3.** Water contact angles of different titanium samples, uncoated surface (a), unmodified MP-KE coated surface (b), and modified MP-KE coated surface (c).



**Fig. S4.** Fluorescence microscopy results of BSA-FITC incubated with different PTFE samples. a) uncoated PTFE, b) unmodified MP-KE coated PTFE, and c) modified MP-KE coated PTFE. (scale bar = 100  $\mu\text{m}$ ); d) the BSA adsorption amount of corresponding surfaces.



**Fig. S5.** Fluorescence microscopy results of BSA-FITC incubated with different glass beads after been kept 7 weeks. a) uncoated beads, b) unmodified MP-KE coated beads, and c) modified MP-KE coated beads (scale bar = 100  $\mu\text{m}$ ); d) the BSA adsorption amount of corresponding surfaces.

**Table S1. Dopa conversion yield of modified MP-KE (4 mg/L) according to UV Sepctroscopy method.**

	A <sub>292</sub> in 0.1 M HCl	A <sub>292</sub> in 0.1 M sodium borate	$\Delta A$	Dopa concentration	Protein concentration	Dopa number per molecule	Tyrosine number per molecule	Tyrosine modification efficiency
Modified MP-KE	0.302	0.191	0.111	0.68 mM	0.129 mM	5	44	11%



## References

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