Supporting information

Self-assembled peptide nanofibrils designed to release membrane lysing antimicrobial peptide

Xiangyu Sha¹, Ping Li², Yonghai Feng¹, Dan Xia³, Xiaohua Tian¹, Zengkai Wang¹, Yanlian Yang², Xiaobo Mao^{*4,5}, Lei Liu^{*1}

¹Institute for Advanced Materials, School of Material Science and Engineering, Jiangsu University, Zhenjiang 212013, China.

²National Center for Nanoscience and Technology, China.

³Research Institute for Energy Equipment Materials, Tianjin Key Laboratory of Materials, Laminating Fabrication and Interface Control Technology, College of Materials Science and Engineering Hebei University of Technology, Tianjin, China.

⁴Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA

⁵Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, 21205, USA

*Corresponding author.

E-mail address: liul@ujs.edu.cn (Prof. Lei Liu) xmao4@jhmi.edu (Prof. Xiaobo Mao)

List of Supporting information

TableS1: MIC of the designed peptides

Figure S1: Colonies formed of *E. coli* after treated with peptide

Figure S2: Antimicrobial activity of designed peptides for E. coli and Salmonella

Figure S3: The height of bacteria E. coli. by in-situ AFM analysis

Figure S4: SEM images of bacterial morphology treated by designed peptides.

Figure S5: The calcium imaging indicated by Fluo3-AM probe inside of the E. coli.

Figure S6: TEM images for Self-assembly and disassembly structure of the peptide K₃(FA)₄K₃

Figure S7: AFM images for Self-assembly and disassembly structure of the peptide K₃(FA)₄K₃

Figure S8: TEM images for Self-assembly and disassembly structure of the peptide K₆(FA)₄

Figure S9: AFM images for Self-assembly and disassembly structure of the peptide K₆(FA)₄

Figure S10: Surface coverage of K₃(FA)₄K₃ peptide fibrils in self-assembling and disassembling

Figure S11: The evaluation of cell viability of bacteria Salmonella for long term antimicrobial activity test

	Gram	MIC (µg mL ⁻¹)					
Strains	stain	K ₃ (FA	K ₆ (FA	Boc-F	KRLF	DLP-	cHABPI-AMP ⁴
) ₄ K ₃)4	- γ	KEFL	PH/	
				4 - FV-	FSLR	DLP-	
				OMe ¹	KY ²	PHc ³	
Concentration							
CFU mL ⁻¹		2~7	2~7 ×	2~7 ×	2~7 ×	2~7 ×	$2 \sim 7 \times 10^{6}$
		$\times 10^{6}$	106	106	106	106	
E.coli	-	63	125	250	125	32/12	32
						8	
Salmonella	-	32	63				

Table S1 MIC of the designed peptides



Figure S1 Colonies formed of *E. coli* after treated with peptide K₃(FA)₄K₃ (63 µg mL⁻¹)



Figure S2 Antimicrobial activity of designed peptide $K_3(FA)_4K_3$ (1 × MBC) and $K_6(FA)_4$ (1 × MBC) against pathogens cells: (a) The cells viability of *E. coli*, (b) Salmonella in case of peptide $K_3(FA)_4K_3$ (1 × MBC); the untreated bacteria cells are shown as control



Figure S3. The height of bacteria *E. coli*. by *in-situ* AFM analysis treated by designed peptides (up panel) and without treatment as control (down panel).



Figure S4. SEM images of bacterial morphology treated by designed peptides. (A) *E. coli* treated by PBS (B) *E. coli* treated by $K_3(FA)_4K_3$ (C) *E. coli* treated by $K_6(FA)_4K_3$



Figure S5 The calcium imaging indicated by Fluo3-AM probe inside of the E. coli. with and without the treatment of peptides. The depleted intensity of fluorescence implied the calcium efflux.



Figure S6 Self-assembly and disassembly structure of the peptide $K_3(FA)_4K_3$ (500 µg mL⁻¹) by TEM, (a) incubation for 1 Day; (b) incubation for 3 Days; (c) incubation for 5 Days at pH=12; (d) continue to incubate for 12 h; (e) incubate for 24 h; (f) incubate for 48 h at pH=7



Figure S7 Self-assembly and disassembly structure of the peptide $K_3(FA)_4K_3$ (500 µg mL⁻¹) by AFM, (a) incubation for 1 Day; (b) incubation for 5 Days; (c) continue to incubate for 48 h at pH=7



Figure S8 Self-assembly and disassembly structure of the peptide $K_6(FA)_4$ (500 µg mL⁻¹) by TEM, (a) incubation for 1 Day; (b) incubation for 3 Days; (c) incubation for 5 Days at pH=12; (d) continue to incubate for 12 h; (e) incubate for 24 h; (f) incubate for 48 h at pH=7



Figure S9 Self-assembly and disassembly structure of the peptide $K_6(FA)_4$ (500 µg mL⁻¹) by AFM, (a) incubation for 1 day; (b) incubation for 5 days; (c) continue to incubate for 48 h, at pH=7



Figure S10 Surface coverage of K₃(FA)₄K₃ peptide fibrils in self-assembling and disassembling.



Figure S11. The evaluation of cell viability of bacteria Salmonella treated by two peptide based nanofibrils with twice more injection of bacteria for the exploration of long term bacteriocidal action.

References

1. Shankar, S.; Singh, G.; Rahim, J. U.; Qayum, A.; Sharma, P. R.; Katoch, M.; Rai, R., Investigation of α / γ hybrid peptide self - assembled structures with antimicrobial and antibiofilm properties. Journal of Peptide Science 2020, 26, e3243, 4-5.

2. Schnaider, L.; Rosenberg, A.; Kreiser, T.; Kolusheva, S.; Gazit, E.; Berman, J., Peptide Self-Assembly Is Linked to Antibacterial, but Not Antifungal, Activity of Histatin 5 Derivatives. Msphere 2020, 5, e00021-20.

3. Wu, D.; Gao, Y.; Tan, Y.; Liu, Y.; Wang, L.; Zhou, M.; Xi, X.; Ma, C.; Bininda-Emonds, O. R.; Chen, T., Discovery of distinctin-like-peptide-ph (dlp-ph) from the skin secretion of phyllomedusa hypochondrialis, a prototype of a novel family of antimicrobial peptide. Frontiers in microbiology 2018, 9, 541.

4. Yazici, H.; Habib, G.; Boone, K.; Urgen, M.; Utku, F. S.; Tamerler, C., Self-assembling antimicrobial peptides on nanotubular titanium surfaces coated with calcium phosphate for local therapy. Materials Science and Engineering: C 2019, 94, 333-343.