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The Purpurin-Peptide Derivative for Selective Killing of Gram-Positive Bacteria *via* Insertion of Cell Membrane

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1. **Synthesis and characterization**

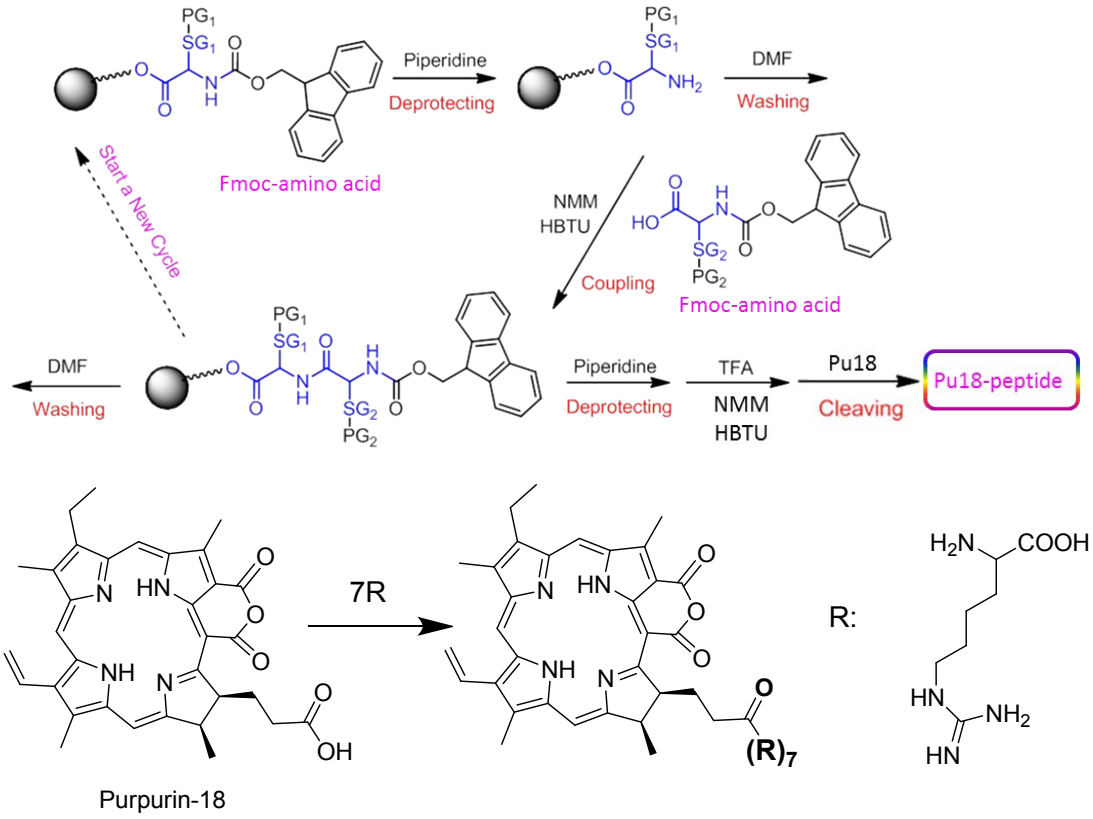

Figure S1. The solid-phase peptide synthesis way and the synthesis route of PA7.

The synthesis of Arginine-Chlorophyll derivatives was based on a standard solid-phase peptide synthesis techniques using Fmoc-coupling chemistry. Fmoc-Arg(Pbf)-Wang resin (loading: 0.321 mmol/g) was used as the solid phase support. The deprotection of Fmoc group on N-terminal was used piperidine (20%, v/v) in anhydrous DMF for 10 min. The qualitative of Fmoc deprotecion was tested by the Kaiser reagent (ninhydrin, phenol, VC 1:1:1, v/v). And the carboxy group of amino acid was activated by NMM (0.4 M) and HBTU (the same mole of amino acid) in anhydrous DMF. The amino acid coupling was reacted at RT for 1h. Repeat the above operation and the reaction time was prolonged as the increase of the number of amino acid. After seven Arg(Pbf) amino acid have linked, Pu-18 was added into the reaction solution with the same synthesis method as amino acid coupling. The final compound cleavage from the resin and deprotection of the amino acid side chains was performed by using the mixture solution of TFA (95%, v/v), H_2O (2.5%, v/v) and TIPS (2.5%, v/v), the solution react in ice bath for 30 min and then further at room temperature for 4 h. After extraction filtration of the mix solution, the filtered fluid was collected and the residual TFA was removed by a vacuum rotary evaporator. The final products were precipitated by cold anhydrous diethyl ether. The precipitate was collected by centrifuge and dried under vacuum drying oven. Finally, the raw products were purified by using a Hitachi HPLC system (L-7100, Japan), with the mobile phase: acetonitrile (5-65%) and ultrapure water containing 0.1% TFA.

MS (MALDI-TOF) calculated for $C_{75}H_{116}N_{32}O_{12}$, 1657.93 m/z, found 1659.20.

PA7: ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 9.86 (s, 1H), 9.58 (s, 1H), 8.95 (s, 1H), 8.20 (t, 1H), 8.00 (s, 2H), 6.47 (d, 1H), 6.28 (d, 1H), 5.12 (d, 1H), 4.58 (t, 1H), 4.19-4.29 (m, 10H), 3.79 (s, 3H), 3.00-3.19 (m, 22H), 2.57-2.91 (m, 14H), 2.37 (t, 2H), 1.25-1.78 (m, 45H).

Anal. Calcd for C75H116N32O12 (%): C, 54.33; H, 7.05; N, 27.03. Found: 54.61, 7.14, 27.08.

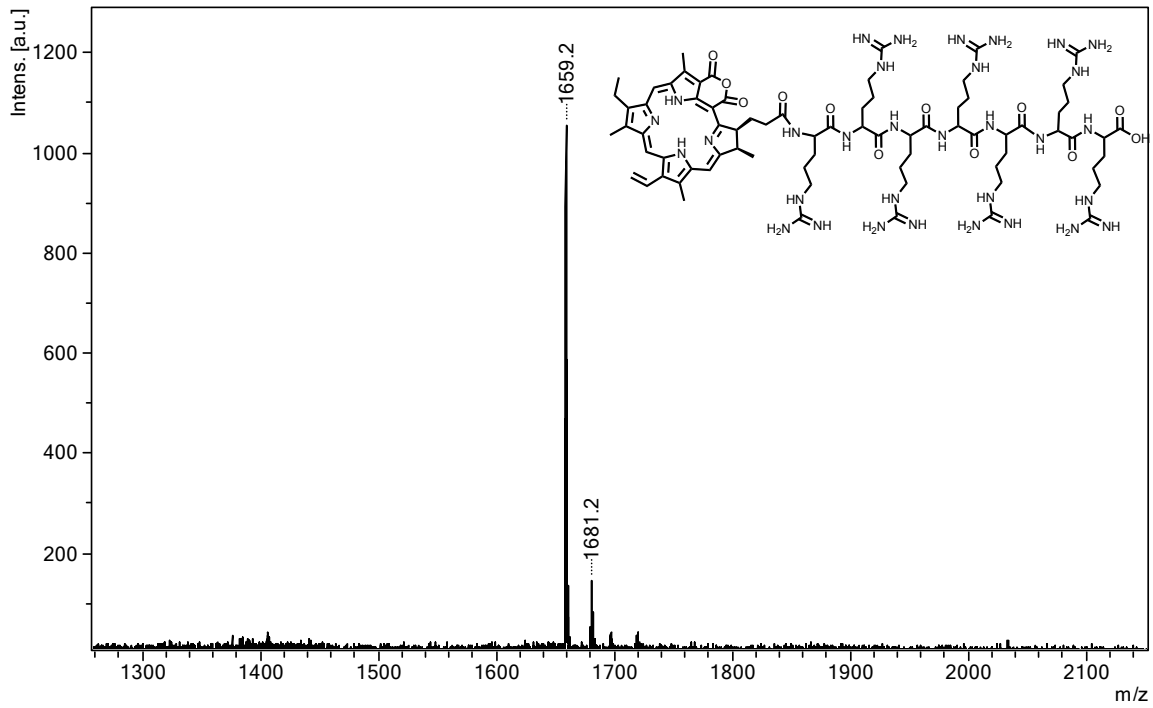

Figure S2. The MALDI-TOF spectrum of PA7.

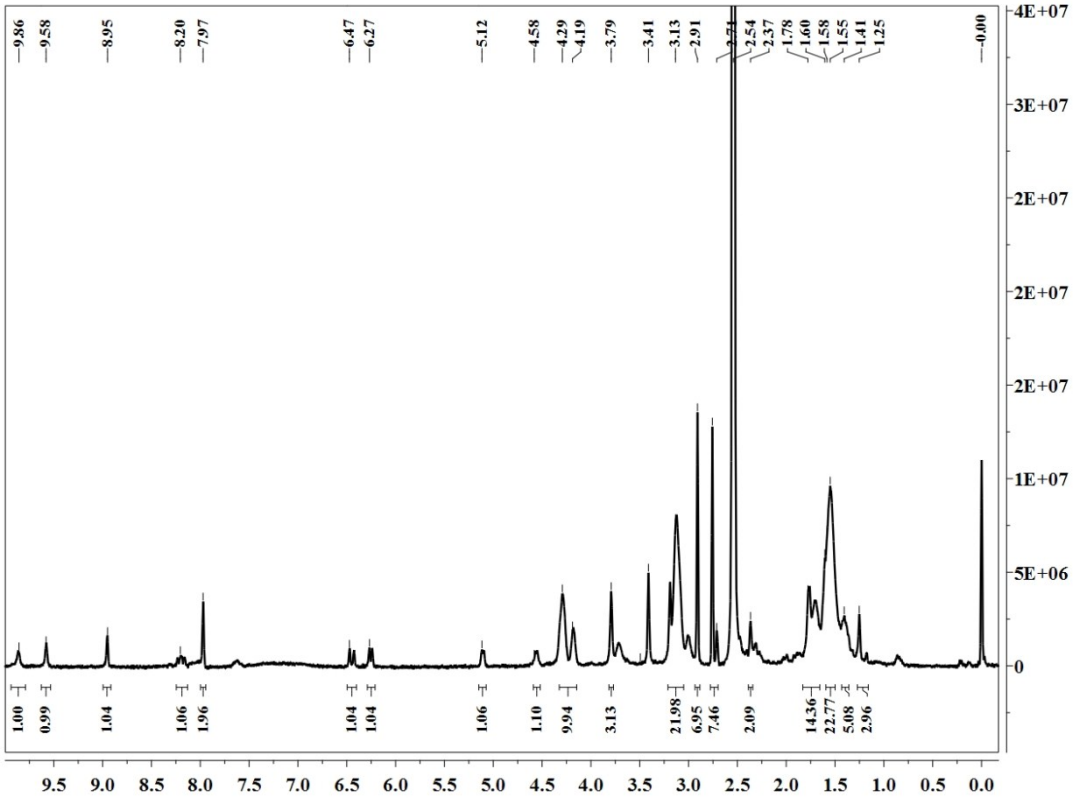

Figure S3.[1]H NMR spectrum of PA7 in DMSO.

2. Optical properties of PA7

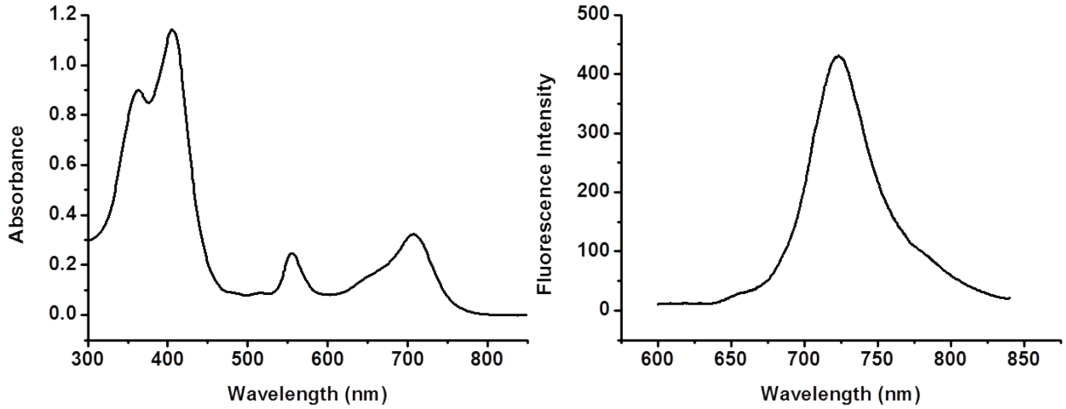

Figure S4. The absorption (20 μM) and fluorescence spectrum (5 μM) of PA7 in PBS buffer.

3. CLSM images of *S. epidermidis* and *P. aeruginosa* treated by PA7

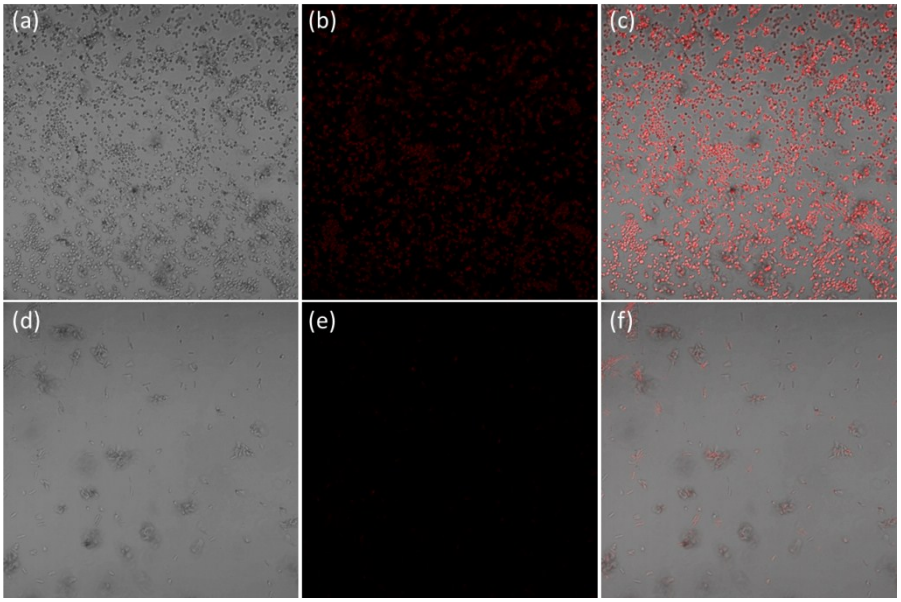

Figure S5. CLSM images of a, d) bright field, b, e) fluorescent images, and c, f) merged images; a, b, c) Gram-positive *S. epidermidis* and d, e, f) Gram-negative *P. aeruginosa* incubated with PA7 (5 μM) in pH 7.4 PBS buffer (10 mM) for 0.5 h. Confocal laser scanning microscopy (CLSM: 405 nm excitation; 620–720 nm emission channel).

4. The fluorescence intensity of PA7 on different bacteria

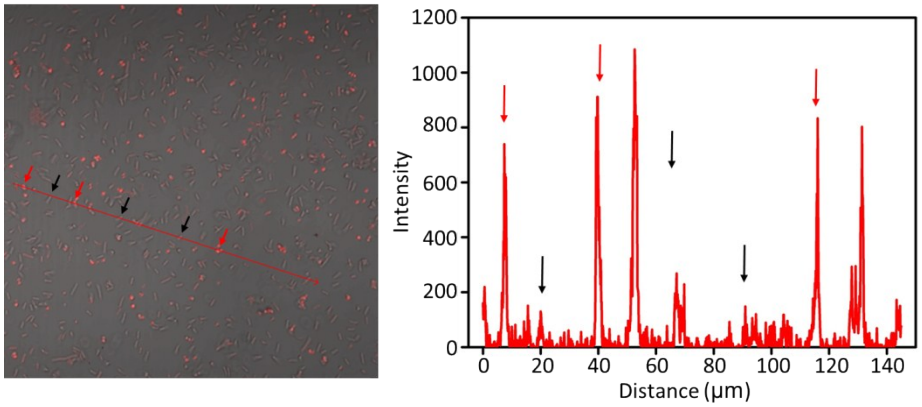

Figure S6. Overlay CLSM image (left) and quantification (right) of mixture of *S. aureus* and *E. coli* treated by PA7, The red arrows and black arrows indicated *S. aureus* cells and *E. coli* cells, respectively.

5. Photodynamic activity of PA7 under the dark

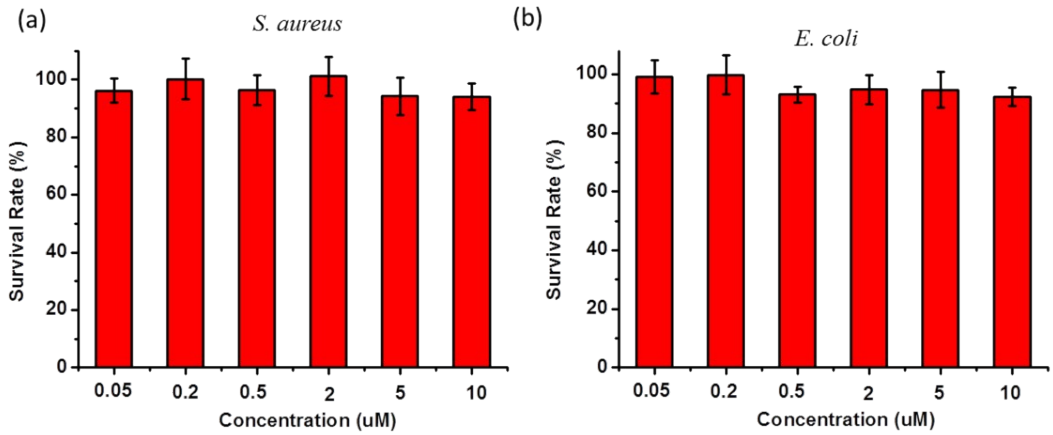

Figure S7. Photodynamic activity of *S. aureus* (a) and *E. coli* (b). The bacteria were incubated with PA7 at concentrations of 0.05, 0.2, 0.5, 2, 5, and 10 μM without light.

6. ROS detection

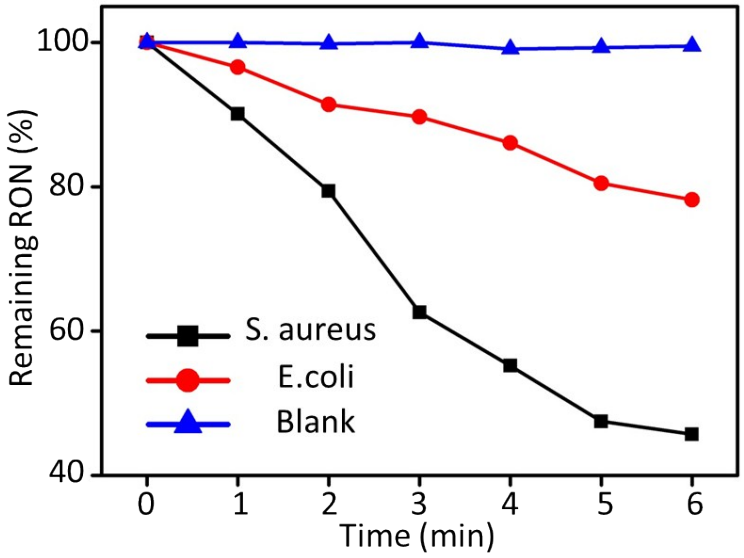

Figure S8. Singlet oxygen generation was measured using RNO as a sensor according to irradiation time, using RNO alone with light irradiation as the blank control.

7. Selectivity binding to bacteria

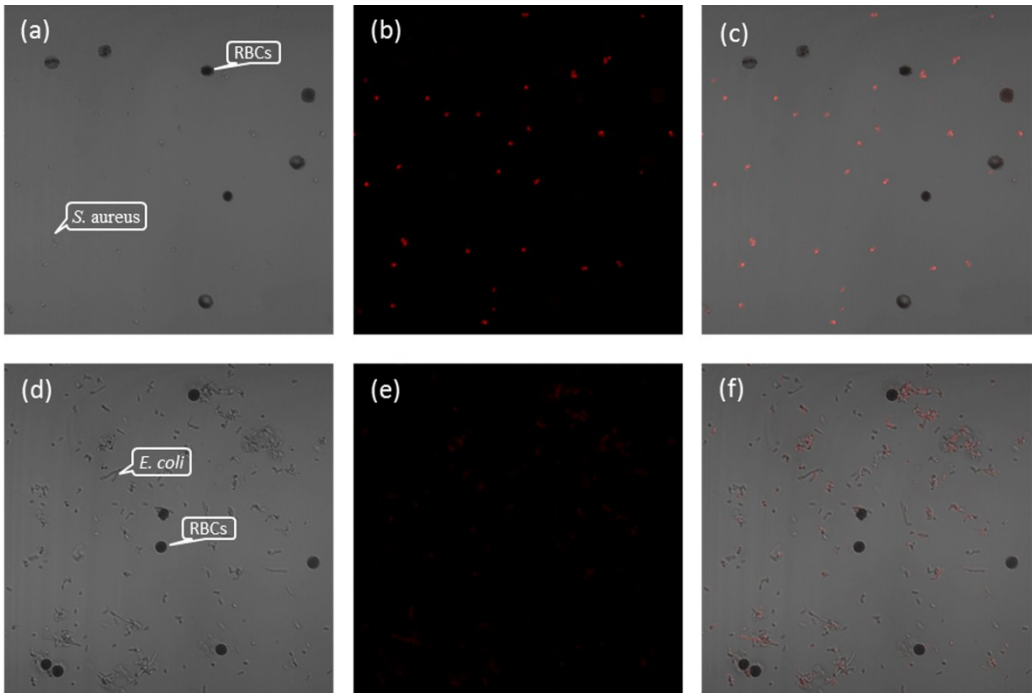

Figure S9. PA7 selectively binds to bacteria in the mixed solution of bacteria (10^8 CFU/mL) and RBCs (0.05% by volume). a, d) bright field, b, e) fluorescent images, and c, f) merged images; a, b, c) RBCs mixed with Gram-positive *S. aureus*; d, e, f) RBCs mixed with Gram-negative *E. coli.* Images were acquired after 0.5 h incubation with PA7 (5 μM) in pH 7.4 PBS buffer.