Electronic Supplementary Information for

Bacteria-mediated in-situ polymerization of peptide-modified acrylamide for enhancing antimicrobial activity

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General procedures

The acrylamide-functionalized tetraethylene glycol-Trp-Arg-Lys (**Am-WRK**, China Peptides, Suzhou, China), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, TCI), LIVE/DEAD™ BacLight™ bacterial viability kit (Thermo Fisher), and other commercially available chemicals (Innochem) were purchased and used as received. The water-soluble chain transfer agent CTA was synthesized according to the previous work.[1] Luria-Bertani (LB) medium for bacteria culture was prepared by dissolving 8 g tryptone, 4 g yeast extract, and 8 g NaCl in 800 mL sterile water, followed by sterilization in an autoclave at 120 °C for 20 min. *Escherichia coli* TOP 10 (*E. coli*) was obtained through transfecting *E. coli* (Beijing Bio-Med Technology Development). *Pseudomonas aeruginosa* (*P. aeruginosa*), *Bacillus subtilis* (*B. subtilis*), *Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*) were obtained by China General Microbiological Culture Collection Center.

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Bruker Avance III 400 MHz HD spectrometer at room temperature. Mass spectrum results were obtained by a Bruker Autoflex III-MALDI-TOF-MS. Gel permeation chromatography (GPC) results were obtained on a Waters 1515 system and calibrated with polystyrene standards in DMF (with 0.05 M LiBr). The values of OD_{600} were obtained on a microplate reader (BIO-TEK Synergy HT) using black 96-well plates (Thermo Scientific, lighttight, flat bottom, non-sterile). Infrared (IR) spectra were obtained on a Bruker VERTEX 70V Fourier-transform IR spectrometer. The scanning electron microscope (SEM) images were captured on a Hitachi S-4800 SEM with an accelerating voltage of 10.0 kV. Confocal laser scanning microscope (CLSM) images were captured on an Olympus FV 1200 microscope.

Photoinitiated polymerization of Am-WRK in solution

The monomer Am-WRK, water-soluble chain transfer agent CTA and photoinitiator LAP were prepared as stock solutions (D_2O) of 100 mM, 100 mM, and 4% w/v, respectively. A mixture of 10 μL CTA, 12.5 μL LAP, and 1 mL Am-WRK (molar ratio of [Am-WRK]:[CTA]:[LAP] =

100:1:1) was irradiated for 30 min (λ = 365 nm, 20 mW/cm^2). The reaction mixture was subjected to ^{1}H-NMR characterization and the monomer conversion was calculated based on the integrations of acrylic protons (5.80 ppm and 6.25 ppm, set as 3 H in total) and aromatic protons of tryptophan (7.01-7.72 ppm, [5+*n*] H), where monomer conversion = *n* / (*n*+1). The unreacted Am-WRK monomer was removed by dialysis (molecular weight cut-off 1 kDa) for three days. After freeze-drying, the PAm-WRK was obtained as a white solid and redissolved in DMF for GPC measurements. The number-average molecular weight (M_n) of PAm-WRK was 88.4 kg/mol with a polydispersity index of 1.46.

Antibacterial performance test of Am-WRK and PAm-WRK

Stock solutions of Am-WRK were prepared in a concentration gradient of 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 mM (2× concentrations listed in Fig. 1d). Stock solutions of PAm-WRK were prepared with mass fractions equal to the corresponding Am-WRK solutions. The bacteria suspension (*E. coli* or *P. aeruginosa*) was adjusted to OD_{600} = 1 and diluted 500 times by LB medium. In each well of a 96-well plate, 50 μL of Am-WRK (or PAm-WRK) and 50 μL of the diluted bacterial suspension were mixed and the OD_{600} was immediately recorded as $OD_{600}(0)$. The 96-well plate was placed at 37°C for 18 h for incubation before the OD_{600} was measured again and recorded as $OD_{600}(t)$. The antibacterial activity was presented by the bacterial viability = $OD_{600}(t)$ / $OD_{600}(0)$.

Bacteria-mediated polymerization of Am-WRK

Bacteria suspensions (*E. coli*, *S. aureus,* or *P. aeruginosa*) in the LB medium were adjusted to OD_{600} = 1. Then, 100 μL of the bacterial solution was transferred into a transparent centrifuge tube containing 100 μL of Am-WRK stock solution (50 mM in LB medium). After thoroughly mixed, bacterial-mediated polymerization was carried out under white light irradiation (λ > 420 nm, 60 mW/cm^2) for 30 min. The bacteria suspension in the dark without Am-WRK was used as the blank group. The bacteria suspension under irradiation without Am-WRK was labeled as the control group "(+) Light", while the bacteria suspension in the dark with an equal concentration of Am-WRK was

labeled as the control group "(+) Am-WRK".

Measurement of the antibacterial activity related to the polymerization

After the bacteria-mediated polymerization, each bacteria suspension was diluted 10^4 times by PBS, and 100 μL of this diluted suspension was coated onto the solid LB medium in a Petri dish (90 mm in diameter). After incubation at 37°C for 18 h, colony-forming units (CFU) were counted on each dish. The antibacterial activity was calculated as Inhibition Rate (IR) = ($C_0 - C$) / C_0 × 100%, where C was the number of CFU in the polymerization group or control group, and C_0 was the number of CFU in the blank group of untreated bacteria. At least 3 independent replicates were carried out under each reaction condition.

SEM images

The bacteria were fixed in 2.5% glutaraldehyde (PBS) solution for 12 h and resuspended in PBS after centrifugation (7200 rpm, 3 min). The bacteria were resuspended in a series of ethanol-water mixtures (20%, 40%, 60%, 80%, and 100% EtOH, v/v) and dropped on the silicon wafer. After evaporation under ambient conditions, the sample was sputtered with gold for SEM analysis.

Procedures for bacterial viability staining

1.5 μL SYTO 9 and 1.5 μL propidium iodide (PI) were dissolved in 1 mL PBS to a final concentration of 5 μM and 30 μM, respectively. 1 mL bacterial suspension was centrifuged to remove the supernatant and mixed with 1 mL of the above dye mixture. The bacteria were resuspended and incubated for 15 min at room temperature in the dark. The dye mixture was replaced by PBS before CLSM imaging.

Resazurin indicator experiments

In a 2 mL glass vial, 50 μL resazurin aqueous stock solution (0.01% w/w) was mixed with 450

μL bacterial suspension (*E. coli* or *P. aeruginosa*, OD_{600} = 1). A screw cap was replaced, and the vial was kept in the dark except for taking digital photos. "Light" samples were irradiated under white light for 30 min (λ > 420 nm, 60 mW/cm^2).

References

1. H. Lu, Y. Huang, E. Zhang, Y. Liu, F. Lv, L. Liu, Y. Ma, S. Wang, *ACS Macro Lett.* 2021, 10, 996-1001.

Supplementary Figures

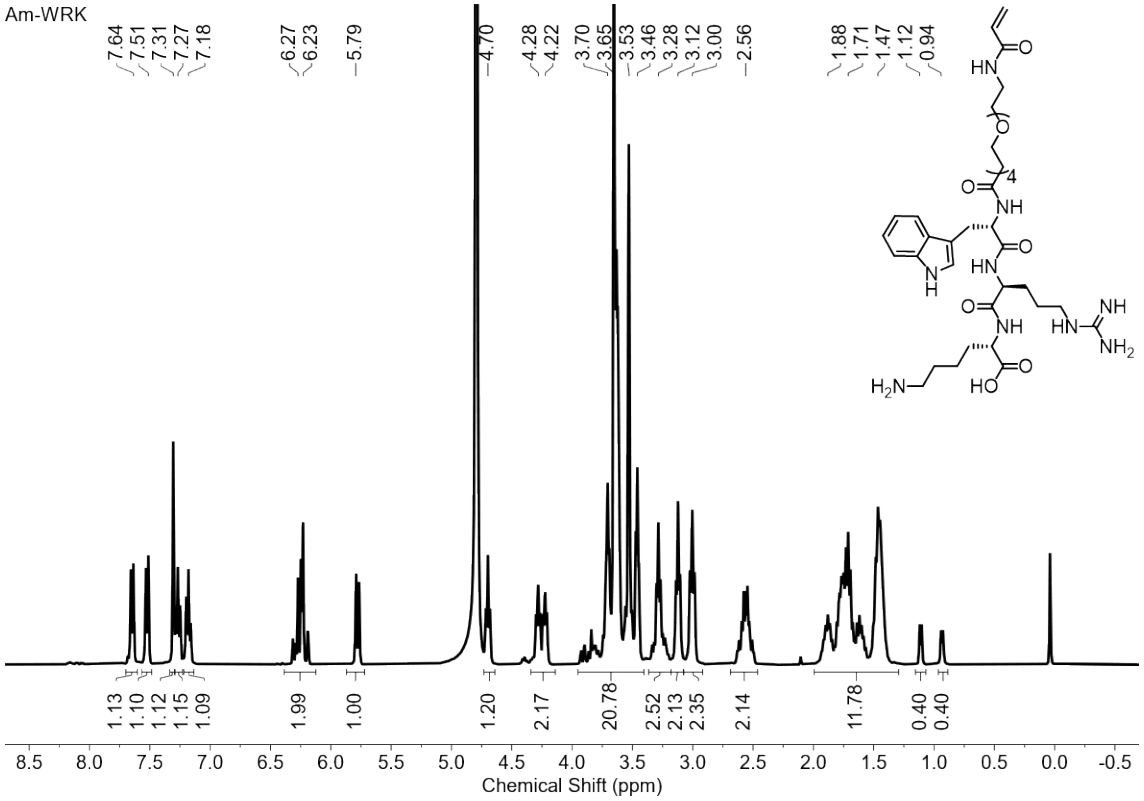

Figure S1. ^{1}H-NMR spectrum of the Am-WRK monomer in D_2O.

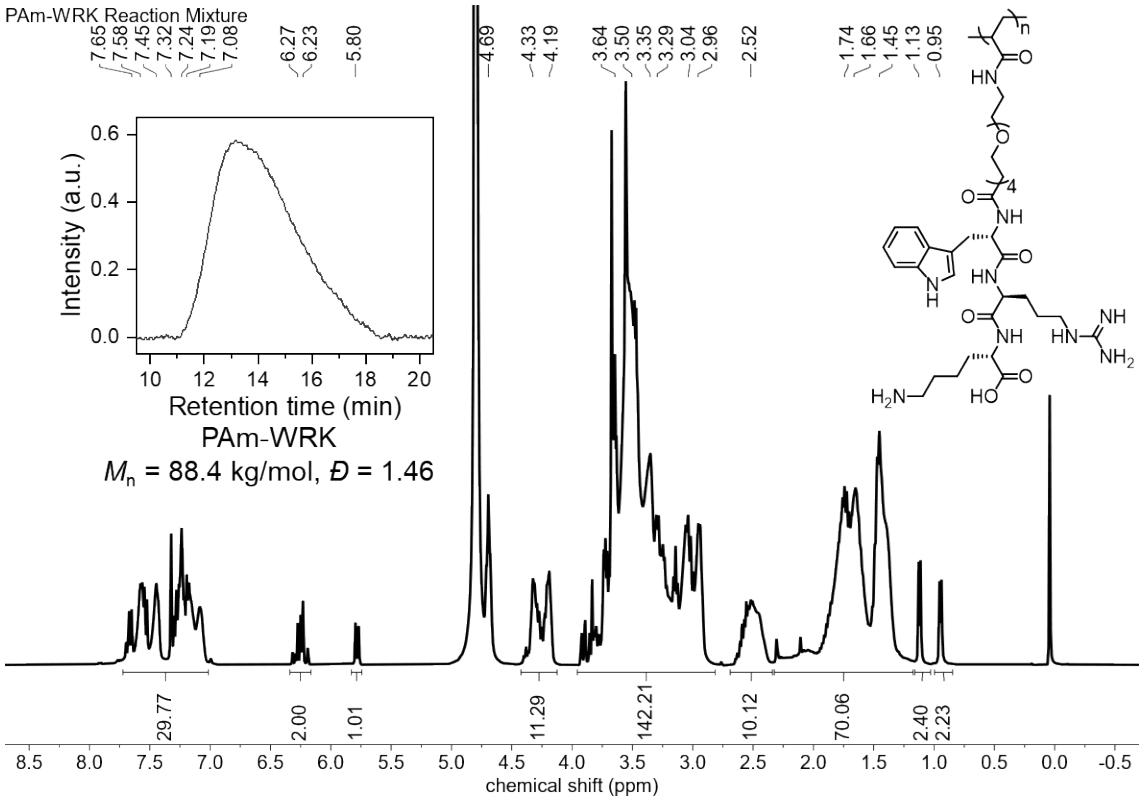

Figure S2. ^{1}H-NMR spectrum of the reaction mixture of Am-WRK polymerization in D_2O solution.

Inset: GPC chromatograph of the isolated PAm-WRK.

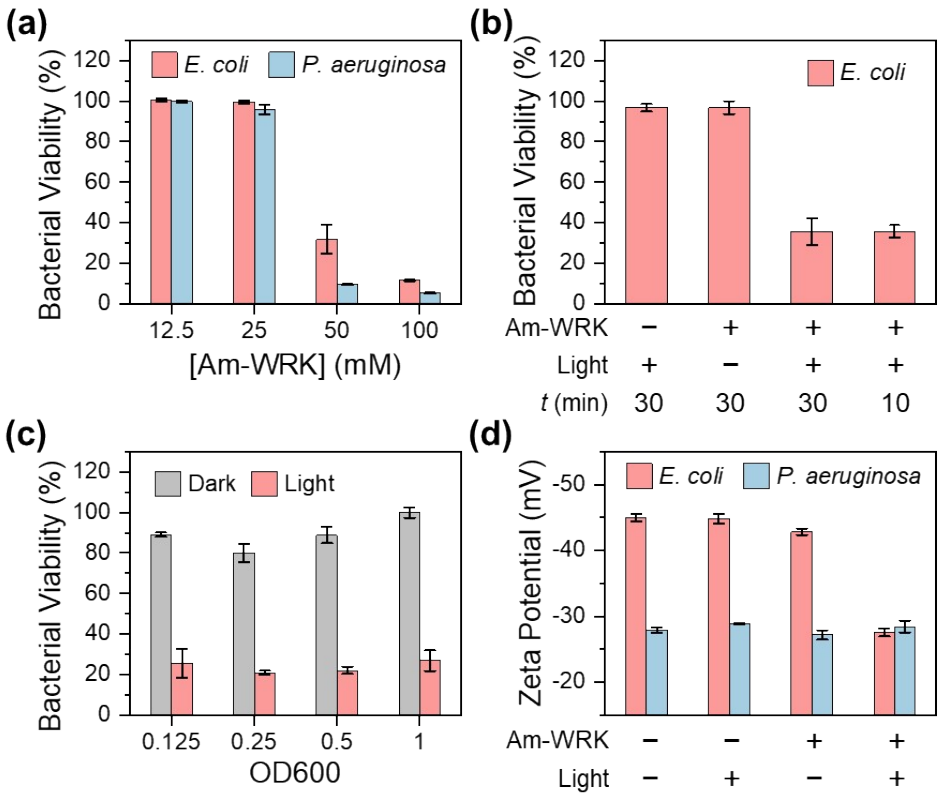

Figure S3. (a) Viability of *E. coli* and *P. aeruginosa* treated with different concentrations of Am-WRK. (b) Viability of *E. coli* under variable conditions of Am-WRK and/or light. (c) Viability of *E. coli* under different OD_{600}. (d) Zeta potential of the bacteria under the treatments of Am-WRK and/or light. [Am-WRK] = 25 mM in panels b, c, d.

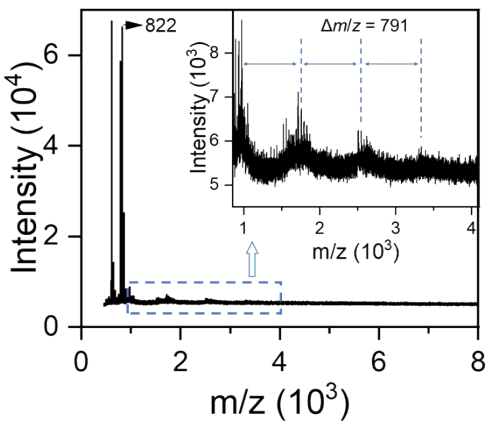

Figure S4. The mass spectrum of the supernatant from *E. coli*-mediated polymerization mixture (in presence of both Am-WRK and light irradiation).

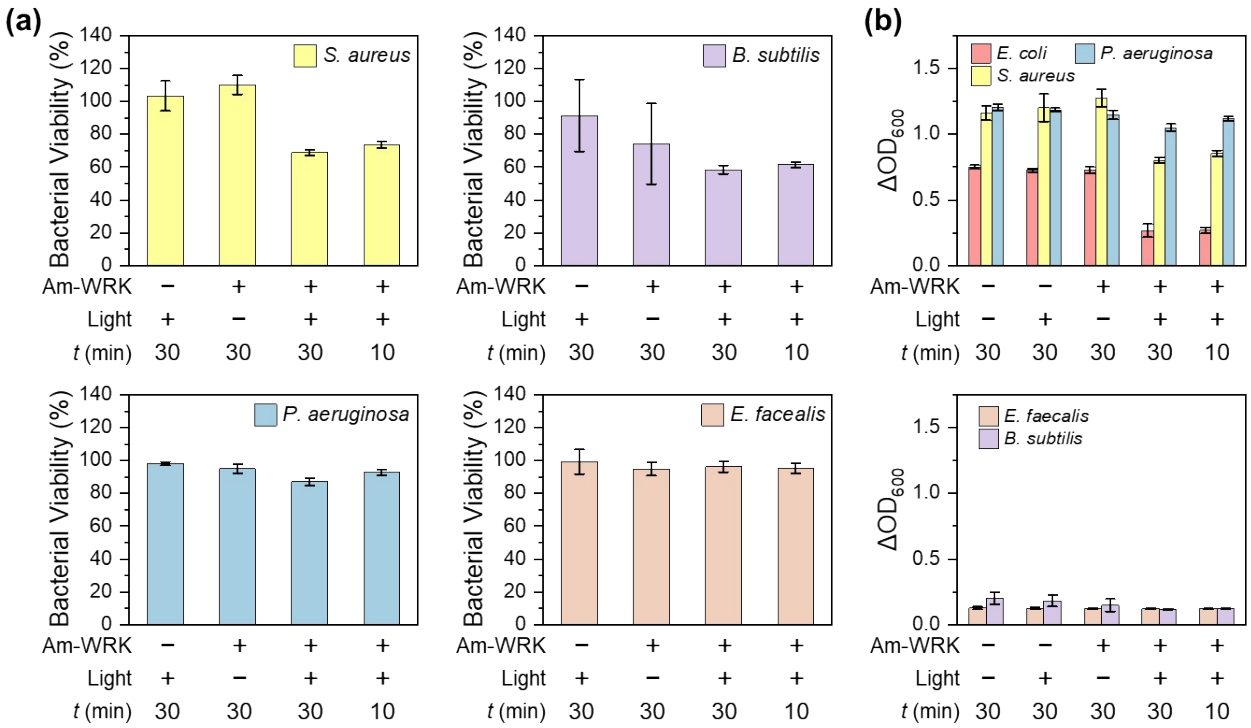

Figure S5. (a) Viability of *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. faecalis* under variable conditions of Am-WRK and/or light. [Am-WRK] = 25 mM. (b) ΔOD_{600} of bacterial species under variable conditions of Am-WRK and/or light.

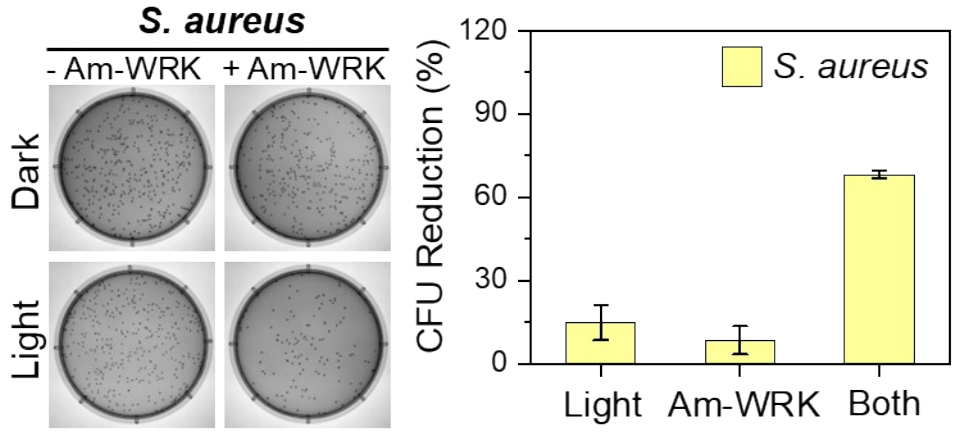

Figure S6. The growth inhibition efficiency of *S. aureus*-mediated in-situ polymerization.

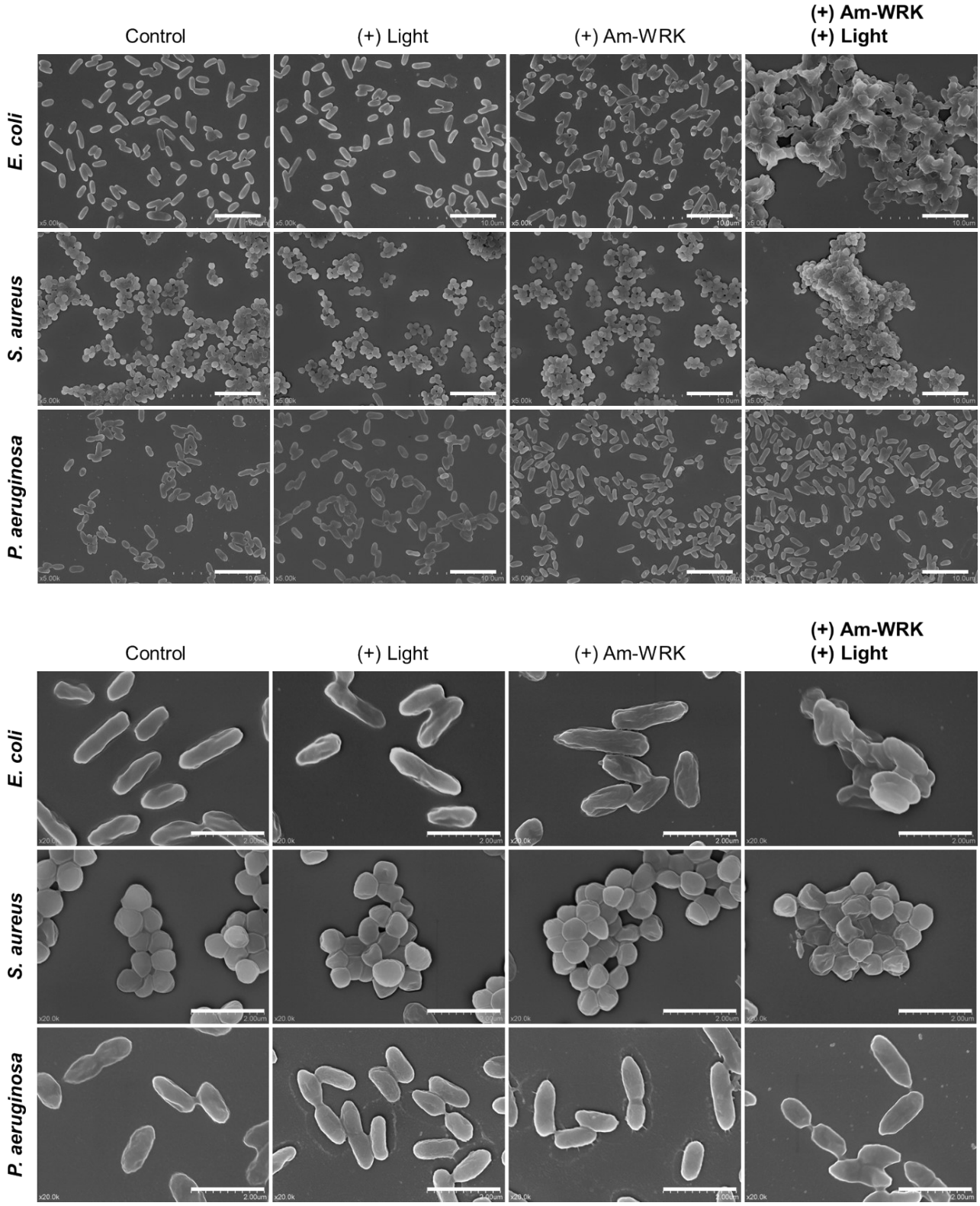

Figure S7. SEM images of *E. coli*, *S. aureus,* and *P. aeruginosa* under variable conditions of the bacteria-mediated Am-WRK polymerization. Scale bar, 5 μm for the top group (some images are also shown in Fig. 2c), 2 μm for the bottom group.

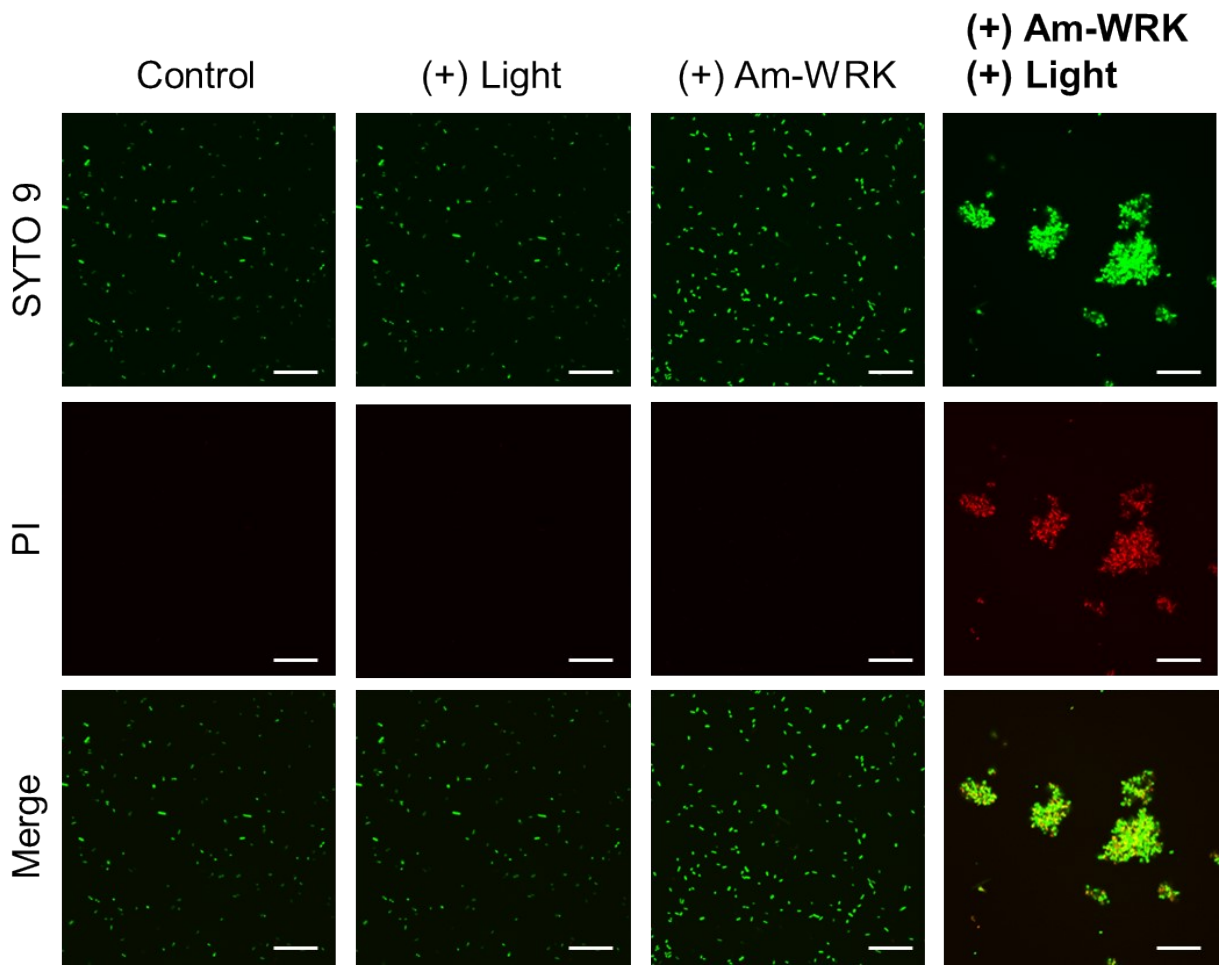

Figure S8. CLSM images of *E. coli* under variable conditions of the bacteria-mediated Am-WRK polymerization. Scale bar, 20 μm.

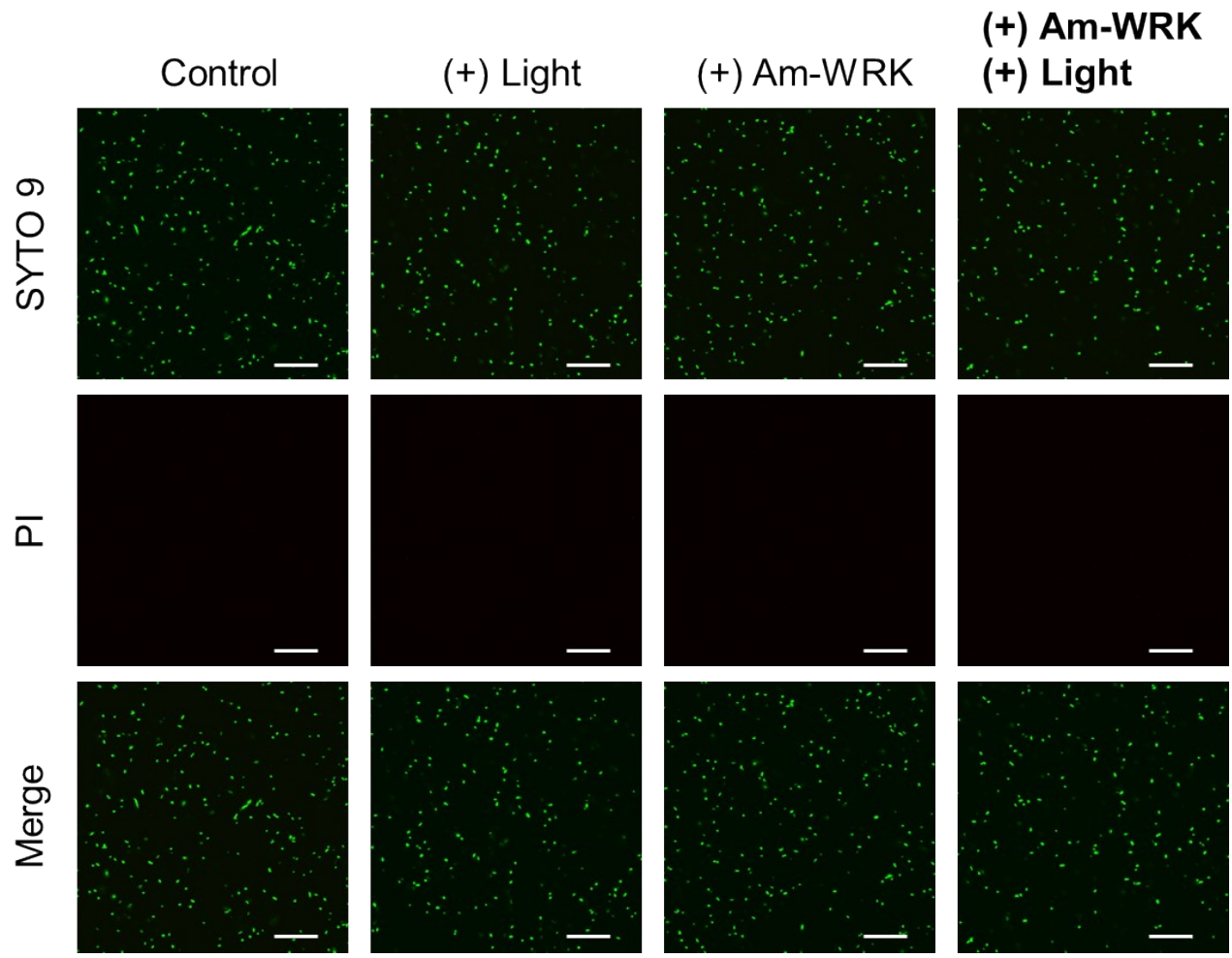

Figure S9. CLSM images of *P. aeruginosa* under variable conditions of the bacteria-mediated Am-WRK polymerization. Scale bar, 20 μm.

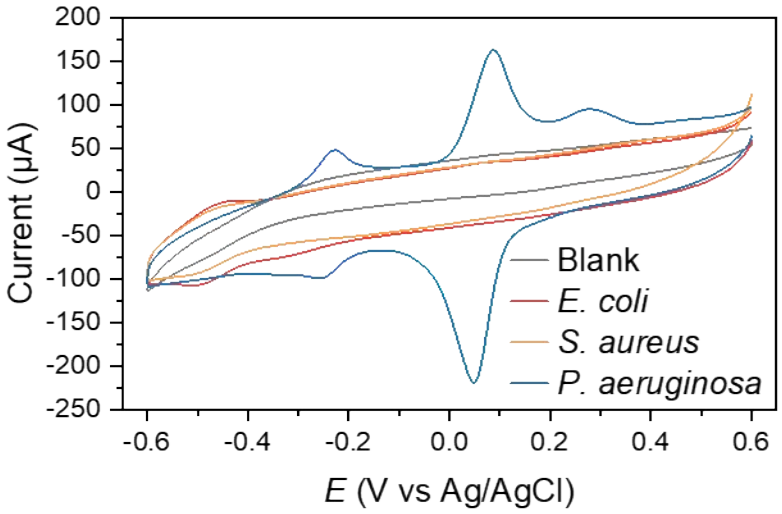

Figure S10. Cyclic voltammograms of *E. coli*, *S. aureus*, and *P. aeruginosa*.