Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supporting Information

Plasmonic Photoreactors-Coated Plastic Tubings as Combined-Active-and-Passive Antimicrobial Flow Sterilizer

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S1. Supplemental Data Items

Table S1. Hydrodynamic Diameters and ζ-Potentials of Photoreactors and Controls.

Groups	Hydrodynamic Diameter (nm)	ζ-Potential (mV)
Ag NPs	43.5±1.2	-28.4±6.8
L-Ag	56.1±8.5	-36.6±0.9
Photoreactor Nanocomposites	57.3±6.8	-30.5±7.8

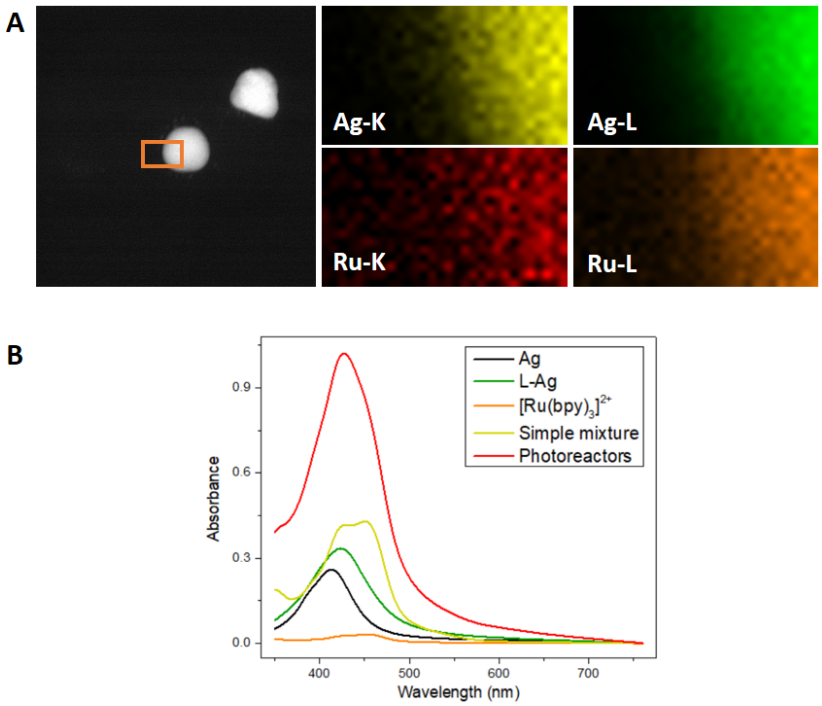

Figure S1. Supplemental Characterization of Plasmonic Photoreactors.

(A) STEM image with element mapping area (orange rectangle) and Ag and Ru element maps on a single plasmonic photoreactor particle. Both Ag and Ru are found to coexist within the particle boundary, which evidences the successful integration of the $[Ru(bpy)_3]^{2+}$ photocatalyst around the Ag NP by the hybrid lipid membrane.

(B) UV-vis absorbance spectra of a water suspension of plasmonic photoreactors and control groups. All samples were prepared to have the identical Ag and/or Ru concentration as measured from the photoreactors (**Table S2**), except for $[Ru(bpy)_3]^{2+}$, which was normalized to this concentration to avoid inaccuracies in the spectra due to very low input concentration. Significant enhancement in the absorption band around 430 nm is observed for the photoreactors due to the resonant enhancement of the Ag LSPR and the metal-to-ligand charge transfer band of $[Ru(bpy)_3]^{2+}$.

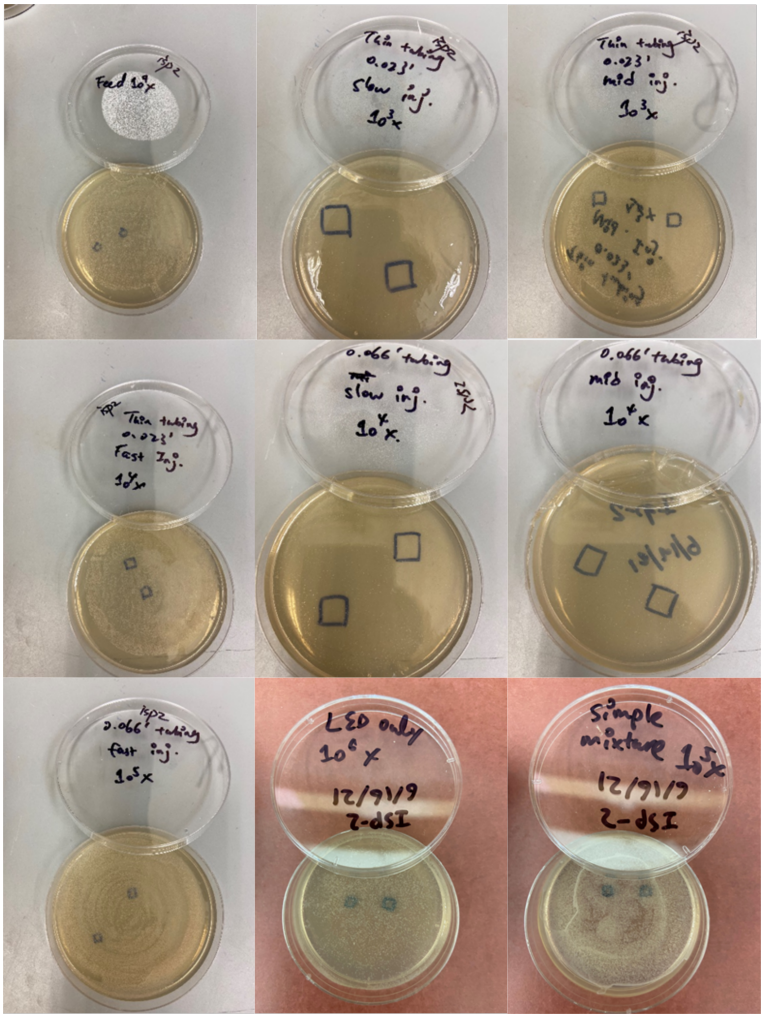

Figure S2. Pictures of Agar Plates for Representative Groups and Controls after Flow Inactivation of *Arthrobacter sp*.

Top row: *Arthrobacter sp.* kept in dark for 30 min, dilution factor (DF) 10^7 (left); *Arthrobacter sp.* after flow through inactivation in flow sterilizer with 0.023'' tubing at 3.33 µL/min with DF of 10^3 (middle), or at 6.67 µL/min with a DF of 10^3 (right). All samples were irradiated at 430 nm.

Middle row: *Arthrobacter sp.* after flow through inactivation in flow sterilizer with 0.023'' tubing at 10 µL/min with a DF of 10^4 (left); *Arthrobacter sp.* after flow through inactivation in flow sterilizer with 0.066'' tubing at 20 µL/min with a DF of 10^4 (middle), or at 50 µL/min with a DF of 10^4 (right). All samples were irradiated at 430 nm.

Bottom row: *Arthrobacter sp.* after flow through inactivation in flow sterilizer (430 nm irradiation) with 0.066'' tubing at 100 µL/min with a DF of 10^5 (left); *Arthrobacter sp.* after flowing through uncoated tubing (0.023'', 10 µL/min) with 430 nm irradiation (LED only control) with a DF of 10^6 (middle); *Arthrobacter sp.* after flowing through tubing (0.023'', 10 µL/min) coated with a simple mixture of L-Ag NPs and $[Ru(bpy)_3]^{2+}$ irradiated at 430 nm and at identical concentrations as in the photoreactors (simple mixture control) with a DF of 10^5 (right).

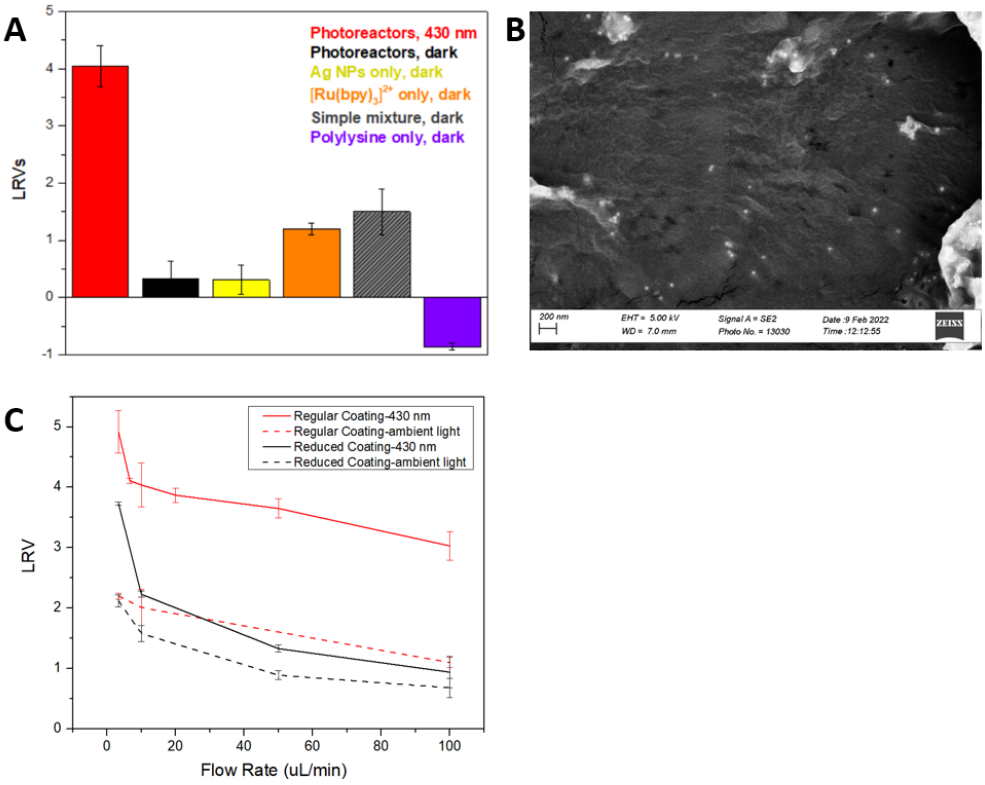

Figure S3. Supplemental Characterizations of Flow Inactivation.

(A) Comparison of LRVs of *Arthrobacter sp.* with LDPE tubing (ID=0.023'') Coated with Plasmonic Photoreactors or Control Groups. Flow rate for all groups: 10 µL/min. Error bars: Mean ± standard deviation of 3 independent tests.

(B) SEM of plastic surface coated with halved photoreactor density. A coating density of (5.62 ± 0.09) x 10^8 NP/cm^2 on the plastic surfaces was achieved as calculated from SEM images, which is approximately halved compared to 1.07 x 10^9 NP/cm^2 for the regular coating density used in this work.

(C) Comparison of LRVs of *Arthrobacter sp.* with LDPE tubing (ID=0.023'') coated with plasmonic photoreactor (antimicrobial flow sterilizers) at regular coating density (1.07 x 10^9 NP/cm^2, red) or halved coating density (5.62 x 10^8 NP/cm^2, black) at different flow rates with 430 nm (solid lines) or ambient (dashed lines) illumination.

Table S2. MP-AES Results of Ag and Ru Concentrations of Plasmonic Photoreactors, and of Flowed-Through Bacteria Suspensions Plotted in Fig. 5A, B and Fig. S6, S7.

Groups		Ag Conc. (ppb)	Ru Conc. (ppb)
Plasmonic Photoreactors (33 pM NP)		5511.1±769.6	755.0±160.7
Thin Tubing (0.023''), Dark	3.33 µL/min	122.4±64.9	30.6±1.6
	6.67 µL/min	110.5±45.8	32.3±13.6
	10 µL/min	89.7±27.6	23.8±7.6
	20 µL/min	73.5±54.6	15.3±1.2
	50 µL/min	50.9±33.4	9.1±2.6
	100 µL/min	40.8±38.0	9.8±3.2
Thick Tubing (0.066''), Dark	3.33 µL/min	128.5±3.33	55.8±9.7
	6.67 µL/min	131.9±42.5	73.0±10.0
	10 µL/min	135.7±35.4	52.4±5.1
	20 µL/min	127.3±36.3	30.8±1.6
	50 µL/min	75.1±73.0	27.8±1.5
	100 µL/min	99.7±33.8	14.5±3.5
Thin Tubing (0.023''), Ambient Light	3.33 µL/min	277.5±130.5	33.2±21.6
	6.67 µL/min	295.2±92.6	28.0±21.1
	10 µL/min	237.2±100.2	17.2±14.8
	20 µL/min	223.4±98.9	9.2±4.8
	50 µL/min	125.6±47.5	3.6±1.6
	100 µL/min	88.8±42.1	3.4±2.1
Thin Tubing (0.023''), 430 nm Illumination	3.33 µL/min	411.2±171.9	120.9±77.2
	6.67 µL/min	230.8±135.2	96.7±64.9
	10 µL/min	229.1±199.3	102.0±64.0
	20 µL/min	158.6±50.4	73.3±33.2
	50 µL/min	132.3±54.6	38.8±25.0
	100 µL/min	111.2±37.3	31.4±19.7
Thick Tubing (0.066''), Ambient Light	3.33 µL/min	233.5±63.6	81.4±28.7
	6.67 µL/min	249.5±108.0	74.4±11.2
	10 µL/min	267.3±94.4	35.2±5.0
	20 µL/min	217.3±66.2	22.2±2.8
	50 µL/min	120.0±37.3	9.6±1.7
	100 µL/min	122.6±41.4	3.4±2.3
Thick Tubing (0.066''), 430 nm Illumination	3.33 µL/min	788.5±297.6	476.6±166.0
	6.67 µL/min	615.7±294.0	426.7±168.1
	10 µL/min	520.4±190.0	264.5±119.0
	20 µL/min	342.4±114.4	163.0±123.6
	50 µL/min	187.6±48.0	96.5±51.7
	100 µL/min	161.9±49.7	81.9±51.8

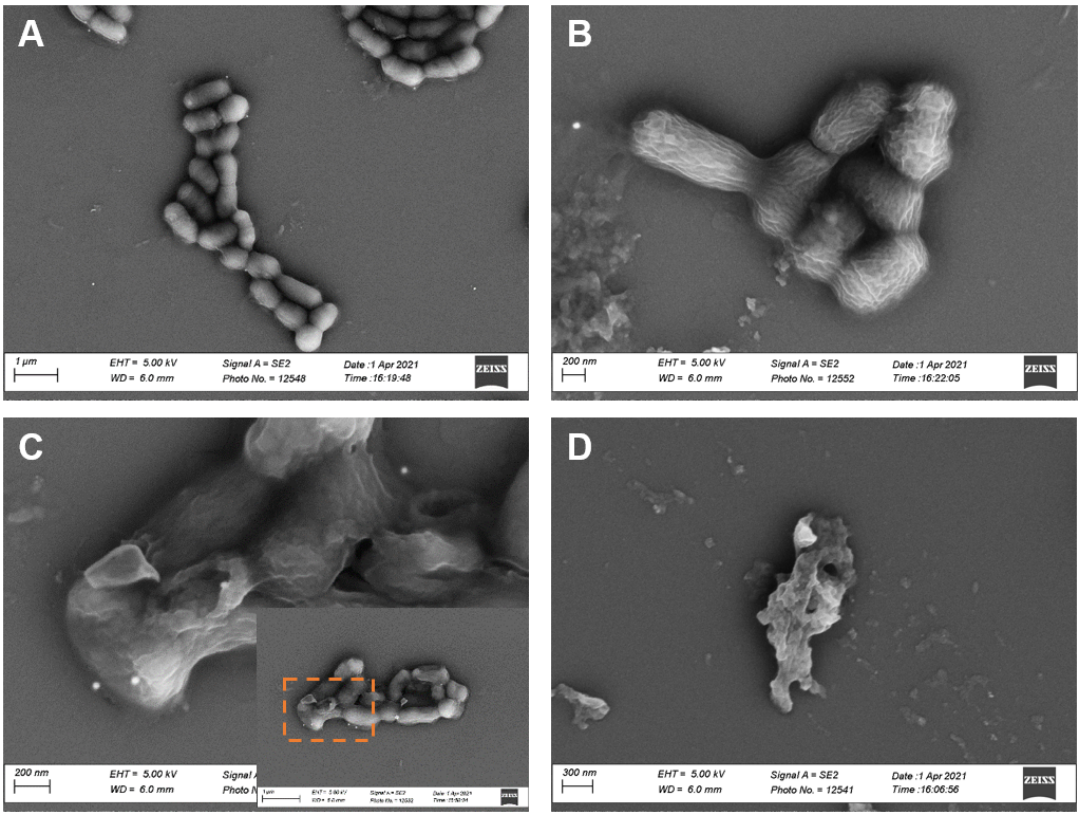

Figure S4. Supplemental SEM Images of *Arthrobacter sp*.

(A, B) SEM image of *Arthrobacter sp.* collected after flowing through the flow sterilizer (0.023'') at 3.33 µL/min in the dark. Intact bacteria cells and surface morphologies can be observed.

(C, D) SEM image of *Arthrobacter sp.* collected after flowing through the flow sterilizer (0.023'') at 3.33 µL/min with 430 nm illumination. Damages to surface morphology and cell disintegration can be observed after flow inactivation.

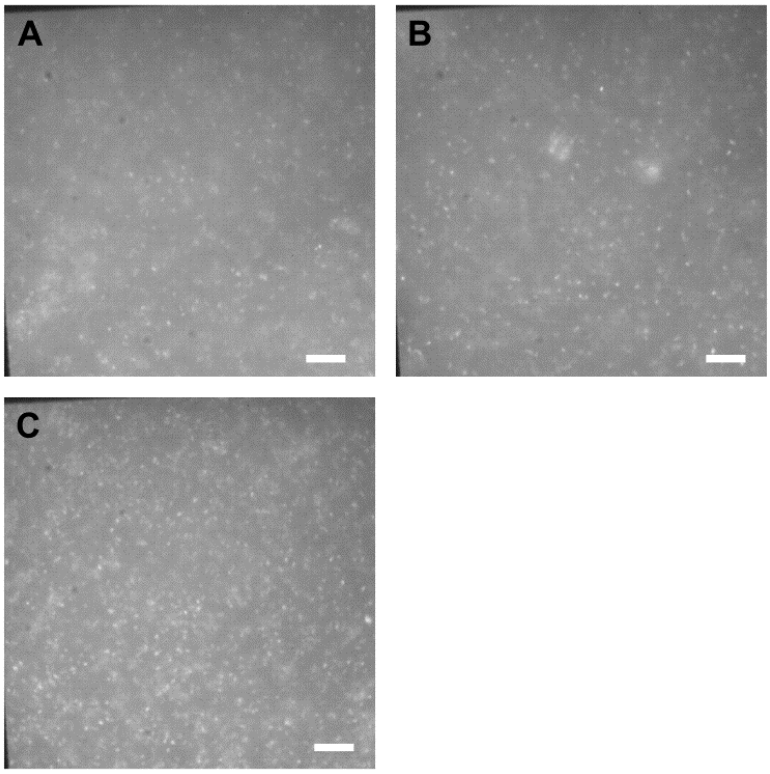

Figure S5. Supplemental Characterizations of Bacterial Growth Inhibition on Plastic Surfaces.

(A-C) Darkfield images of plastic slide surfaces that are coated with plasmonic photoreactors (A), with Ag NPs control (B), or with L-Ag controls (C) after seeding with 10^7 bacteria/mL *Arthrobacter sp.* and left at RT for two days in dark. Scale bars = 10 μm.

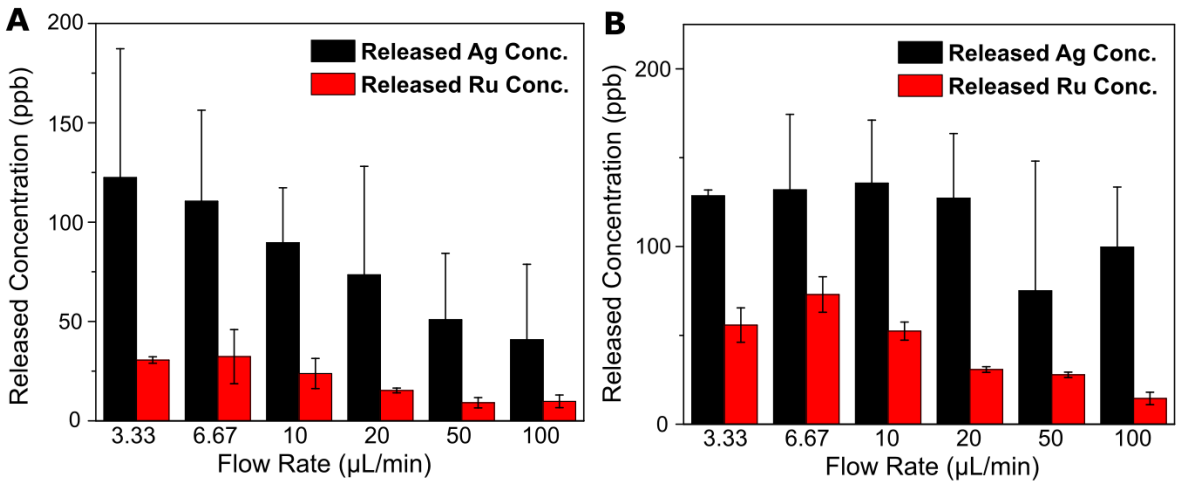

Figure S6. Measurement of the Released Ion Concentrations in Dark.

(A-B) MP-AES results of released Ag and Ru concentrations in the bacteria suspension after flow inactivation assay with plasmonic photoreactor-coated LDPE tubings (antimicrobial flow sterilizers) with inner diameters (ID) of 0.023'' (A) and 0.066'' (B) in dark.

Error bars: Mean ± standard deviation of 3 independent measurements.

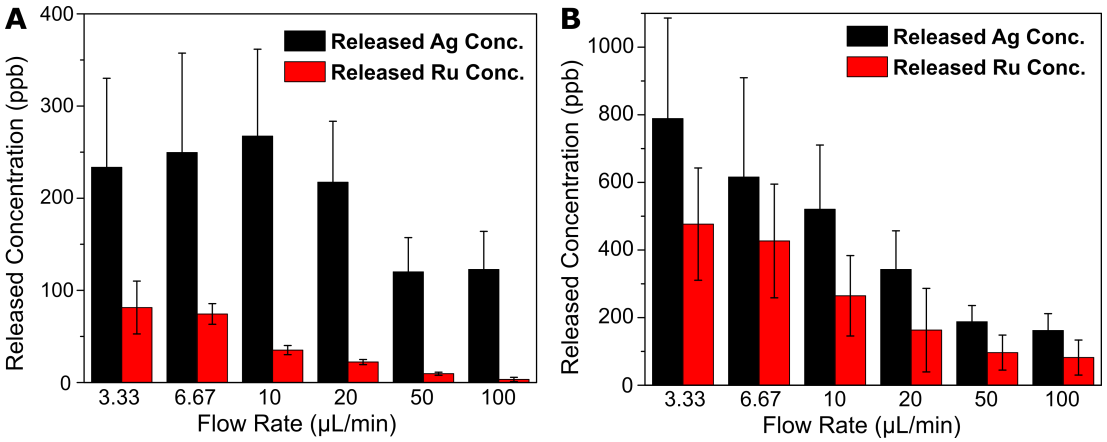

Figure S7. Measurement of the Released Ion Concentrations from the 0.066'' Tubing.

(A-B) MP-AES measurements of released Ag and Ru concentrations in the bacteria suspension after flow inactivation assay with plasmonic photoreactors-coated LDPE tubing (ID=0.066'') at different flow rates with ambient light (A) or with 430 nm illumination (B).

Error bars: Mean ± standard deviation of 3 independent measurements.

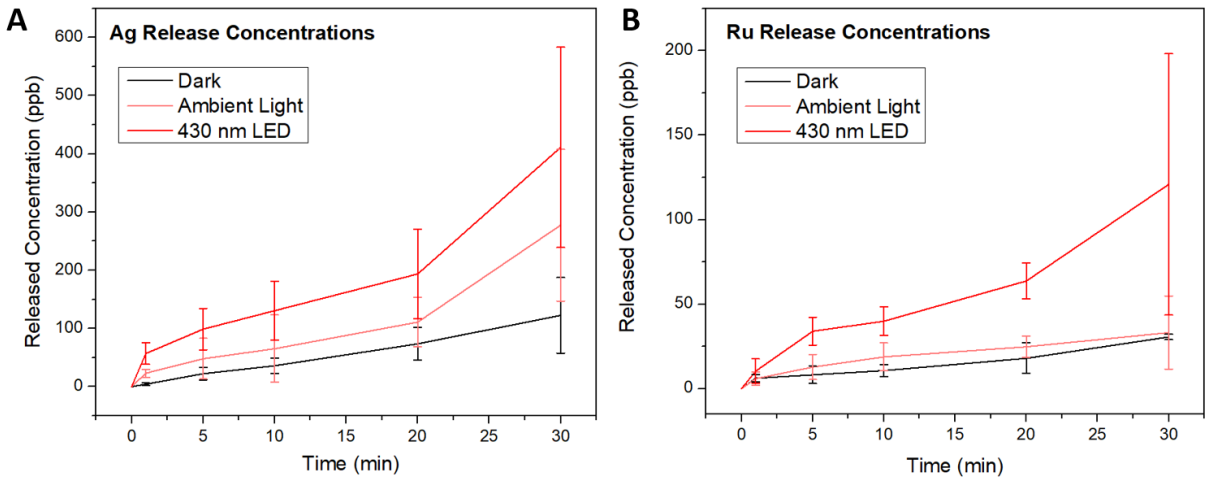

Figure S8. Measurement of the Released Ion Concentrations from the 0.023'' Tubing at Different Times.

(A, B) Released Ag (A) and Ru (B) concentrations from photoreactors-coated 0.023'' tubing (flow sterilizer) after 100 µL bacteria suspension were flushed through the flow sterilizer at 3.33 µL/min; flowed-through suspension were collected at different times.

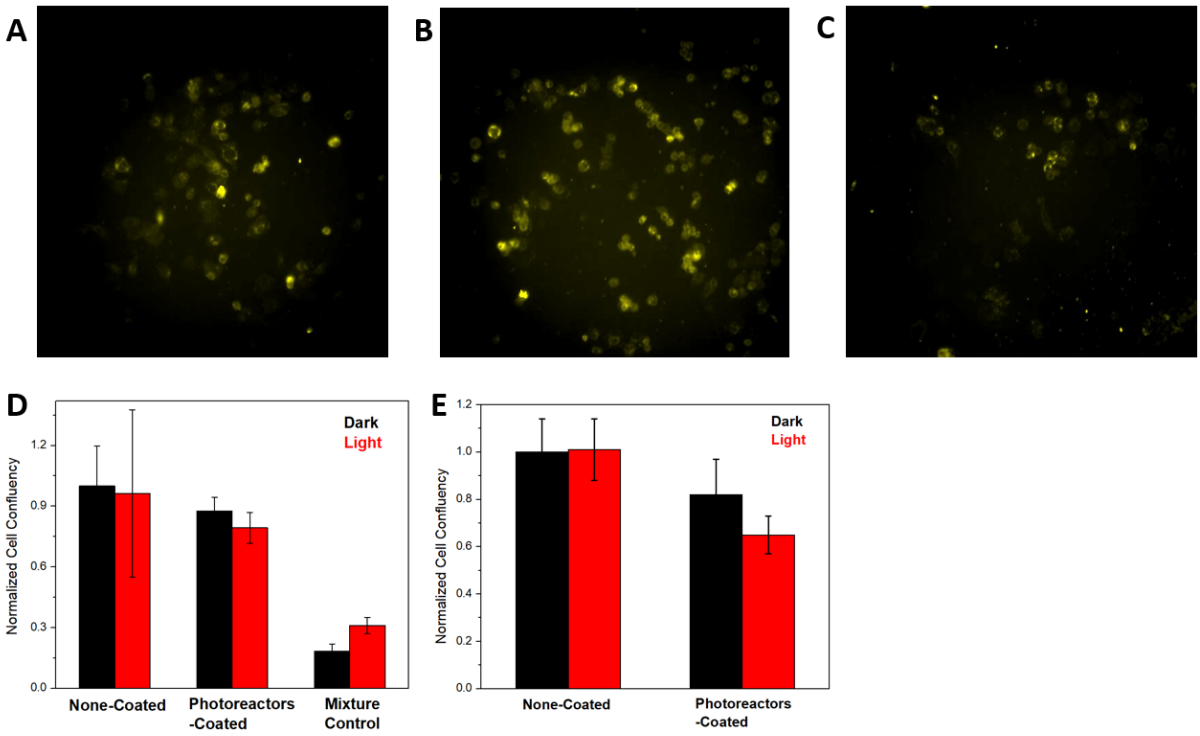

Figure S9. Characterization of Cancer Cell Confluency on Photoreactor-Coated Slides and Controls.

(A-C) Darkfield images of the surfaces of PVC slides that are uncoated (A), coated with plasmonic photoreactors (B), or coated with the same concentration of a simple mixture control of Ag NPs and $[Ru(bpy)_3]^{2+}$ (C) after culturing with MDA-MB-468 cells with ambient light for 2 days at 37 °C.

(D) Normalized cell confluency of MDA-MB-468 cells after culturing on PVC slides coated with photoreactor nanocomposites or controls with (red) or without (black) ambient light for 2 days at 37 °C.

(E) Normalized cell confluency of MDA-MB-231 cells after culturing on PVC slides that are none-coated or coated with photoreactor nanocomposites with (red) or without (black) ambient light.

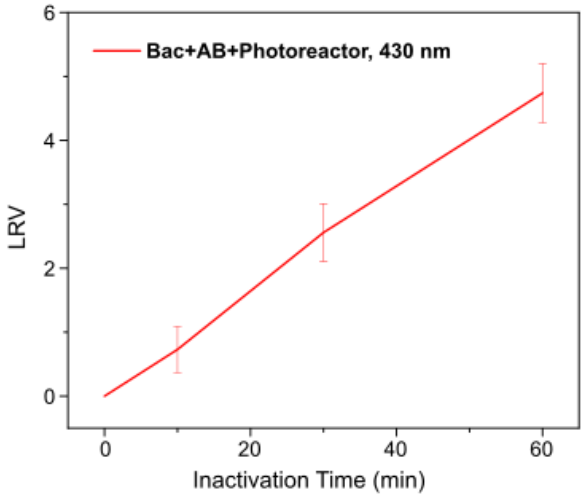

Figure S10. LRVs of *E. coli* CFUs After Inactivation Assay with Plasmonic Photoreactors in the Presence of EGFR Antibody (AB).

S2. Supplemental Experimental Procedures

Microscopic Characterizations of the Photoreactors and Coated Plastic Surfaces

Transmission Electron Microscopy (TEM), High Resolution TEM (HRTEM), Scanning TEM (STEM) and Energy Dispersive X-Ray Spectroscopy (EDX) were performed with a Tecnai Osiris TEM with a Super-X EDX detection system at 200 kV acceleration voltage. Element scanning of Ag and Ru K and L edges is performed with EDX under STEM mode with spot size of 6. Scanning Electron Microscope imaging was carried out on a Zeiss Supra 40 VP SEM with 5 eV EHT. Photoreactors-coated PVC slides or sliced-open LDPE tubings were laid onto silicon wafer substrates (<100>, University Wafer) for the imaging. For calculation of bound particle area density, at least 30 images were obtained from 3 independent experiments for both the slides and tubings.

For the correlated DF/FL imaging of plastic slides coated with dye-labelled photoreactor nanocomposites, 3 mol% DSPE-PEG(2000)-Biotin (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000](ammonium salt), Avanti Polar Lipids) was included in the preparation process of the nanocomposites, and the DPPC was reduced to 44 mol% to keep the total lipid amount constant. The remaining synthesis procedures are identical to described in the Methods section of the manuscript. 17 μL 1 mg/mL solution of a streptavidin Alexa Fluor 594 (ThermoFisher) dye in 0.5x Phosphate Buffered Saline (PBS) was then added to 50 μL of the nanocomposite suspension and incubated in dark at RT for 2 hours. After centrifuging and washing twice with 0.5x PBS, the nanocomposites were used to incubate the PVC slides as described above. Subsequently, the slides were observed under an Olympus IX71 Inverted Microscope for DF and FL imaging with an Olympus 60x oil objective (NA=0.65-1.25).

Spectroscopic Characterizations of the Photoreactors and Coated Plastic Surfaces

UV-vis absorbance spectra were collected on a Cary 5000 Spectrometer in 350-760 nm range with a scan rate of 10 nm/s. A 1 cm light path Quartz cell (Starna) was used in the measurement for all groups. All control groups were prepared to possess an identical Ag and/or Ru concentration as measured in **Table S2**, except for the $[Ru(bpy)_3]^{2+}$ only group, which was measured at 1 mM concentration and normalized to the identical concentration in order to avoid inaccuracies in the absorption spectra measured at a low concentration.

Hydrodynamic diameters were determined by Dynamic Light Scattering (DLS); and ζ-potentials were measured with a Malvern Zetasizer. Element concentrations of Ag and Ru in the photoreactor composite were measured by Microwave Plasma-Atomic Emission Spectroscopy (MP-AES), which was performed on an Agilent 4200 MP-AES with SPS 4 Auto sampler. Each sample was run in quadruplicate in each measurement, and was repeated in 3 independent experiments. Emission wavelengths of Ag (328.068 nm), Ag (338.289 nm), Ru (349.894 nm), Ru (372.803 nm) were used. A photoreactor suspension with a particle concentration of 33 pM was used for the measurement of Ag and Ru concentrations. Ag^+ and $[Ru(bpy)_3]^{2+}$ release concentration measurements were also conducted with the above-mentioned MP-AES system. For these experiments, flow sterilizers with 0.023'' or 0.066'' tubings coated by 100 pM photoreactor suspensions were used for flow inactivation of *Arthrobacter sp.* at different flow rates with or without irradiation. The resulting flowed-through suspensions were gathered for the measurements.

Preparation of Bacteria

The *Arthrobacter sp.* (NRRL B3728) was grown on the ISP2 agar plate for 24 hours at 28 °C. One colony of *Arthrobacter sp.* (NRRL B3728) was inoculated in ISP2 media and incubated at 28 °C for 24 hours at 180 rpm on a shake incubator. The concentration of bacteria in growth media was

determined based on their absorbance (optical density (OD) at 600 nm) (OD_{600} of 1: 8×10^8 CFU/mL). The culture of *E. coli* (BL21 (DE3) was performed following similar approach except that the cells were cultured in LB media (or LB agar plate) and incubated at 37 °C for 16 hours at 180 rpm.

Flow Inactivation and Characterizations

For SEM imaging of *Arthrobacter sp.*, the cell suspensions were first diluted with different dilution factors based on estimated concentrations of each groups, and then fixed with 4% formaldehyde for 10 min. The suspensions were then centrifuged and washed twice with PBS before being drop-casted on Si wafer substrates for SEM imaging on a Zeiss Supra 40 VP SEM with 5 eV EHT.

For Live/Dead staining, BacLight bacteria viability kit (ThermoFisher) was used. Bacteria suspensions collected after passing the flow reactor were stained for 20 min, centrifuged and washed twice with PBS, and redispersed in 1 mL PBS solution. The suspensions were imaged under an Olympus IX71 Inverted Microscope for DF and FL imaging with an Olympus 60x oil objective (NA=0.65-1.25). For FL imaging, images from Live and Dead channels were obtained separately before being merged in ImageJ for comparison.

Biofilm Growth Inhibition Experiments

For biofilm growth inhibition characterization, PVC slides coated with photoreactor nanocomposites or controls were immersed in an *Arthrobacter sp.* suspension in medium with an estimated bacteria concentration of 10^9 Bac/mL from absorbance optical density. The suspensions were kept either in the dark or under ambient illumination at RT for 2 days, before the coated plastic slides are retrieved, washed with PBS, and imaged under the Olympus IX71 Inverted Microscope for DF imaging with an Olympus 60x oil objective (NA=0.65-1.25). The bacterial surface densities for each group were calculated from at least 6 images from 3 independent experiments, and log reductions were calculated by comparing the experiment groups with the density on a plastic surface control coated with polylysine only, but not with any bactericidal components.

Characterization of Effect of Photoreactors on Monoclonal Antibody

To characterize whether the bactericidal effect could still occur in the presence of mammal proteins, biotinylated Epidermal Growth Factor Receptor (EGFR) antibody cocktail (ThermoFisher, MA5-13266) was added to the inactivation colloid. In this experiment, 100 µL *Arthrobacter sp.* or *E. coli* suspension with an estimated concentration of 10^{10} Bac/mL by the absorbance OD_{600}, 100 µL media, 100 µL 33 pM photoreactor nanocomposite suspension, and 100 µL 0.05 mg/mL antibody were added to a glass cuvette, mixed, and illuminated under stirring with the 430 nm LED for 1 hour. The reaction mixture was fetched at 10, 30 and 60 min to be serial-diluted, plated, colony counted and calculated for LRVs as described above.

To characterize if the functionality of the antibody is preserved after the inactivation assay, a reaction mixture of 100 µL PBS, 100 µL 10 or 30 pM photoreactor nanocomposite suspension, and 100 µL 0.05 mg/mL antibody were added to a glass cuvette, mixed, and illuminated under stirring with the 430 nm LED for 30 min. To prevent contamination to the cell culture, no bacteria were added in this step. Afterwards, the antibody mixtures after treatment with photoreactors, as well as an untreated control, were centrifuged to remove the photoreactor nanocomposites, redispersed in PBS, and then incubated with EGFR-overexpressing MDA-MB-468 (ATCC, HTB-132) breast cancer cells at 37 ^{o}C for 3 hours for specific binding. MDA-MB-468 cells were cultured

in advanced Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v) fetal bovine serum (FBS), 2 mM L-glutamine, and 50 units/mL penicillin-streptomycin, and grown in a 37 ^{o}C incubator with 5% CO_2 and 95% relative humidity. Prior to experiments, cells were starved in serum-free media for 24 hours. Experiments were performed when cells reached 80% confluency. After incubation with the treated antibodies or controls, the cells were washed with HBSS buffer and incubated with 0.5 mg/mL Streptavidin-Alexa Fluor 594 dye (ThermoFisher, S11227) for 1 hour at 37 ^{o}C. Subsequently, the cells were washed with HBSS and detached from plates using Accutase cell dissociation reagent (ThermoFisher) and fixed with 4% (w/v) pierce formaldehyde (ThermoFisher) for 10 min before washed with PBS buffer. The fluorescence intensity of the cells were measured by flow cytometry.

Characterization of Cancer Cell Viability on Coated Plastic Slides

PVC slides were sterilized with 75% ethanol and coated as described above. MDA-MB-468 or MDA-MB-231 (ATCC, HTB-26) cells were seeded at a density of 1 x 10^5 cells/mL in advanced Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v) fetal bovine serum (FBS), 2 mM L-glutamine, and 50 units/mL penicillin-streptomycin on uncoated slides, photoreactors-coated slides, and slides coated with a simple mixture control of Ag NPs and $[Ru(bpy)_3]^{2+}$ at identical concentrations as in the nanocomposites. The cells were cultured in a 37 ^{o}C incubator in dark or with ambient light for 2 days and imaged under the Olympus IX71 Inverted Microscope for DF imaging with an Olympus 10x air objective. Cell confluency were measured by the area density of the cells from 4 independent DF images, and confluency percentages were calculated normalized by dividing by the area density of the cells on the none-coated plastic slides cultured in dark.