Supporting Information

Hybrid Plasmonic Photoreactors as Visible Light-Mediated Bactericides

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S1. Supplemental Data Items

S1.1 Structural Characterizations of the Photoreactor Bactericides

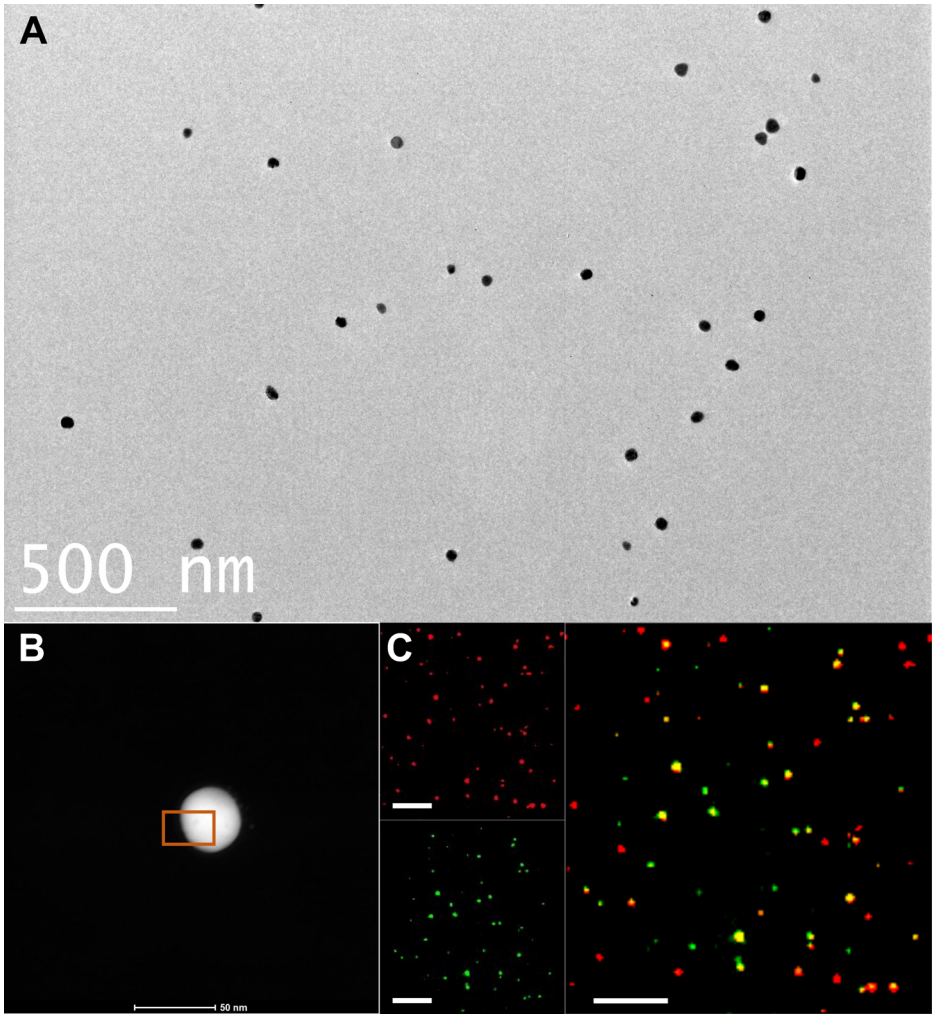

Figure S1. Supplemental Structural Characterizations of the Photoreactor Bactericide.
(A) TEM image of an area with photoreactor bactericide nanocomposites.
(B) STEM image of a photoreactor particle and EDX scan area (orange rectangle) for element mapping. Scale bar=50 nm.
(C) Correlated Darkfield (Upper Left) and Fluorescence (Lower Left) images and merge (right) of an area with photoreactor bactericide nanocomposite suspensions. Scale bars=2 μm.

S1.2 Characterization of the Visible Light-Controlled Cationic Release and Inactivation Properties of the Photoreactor Bacteriocides

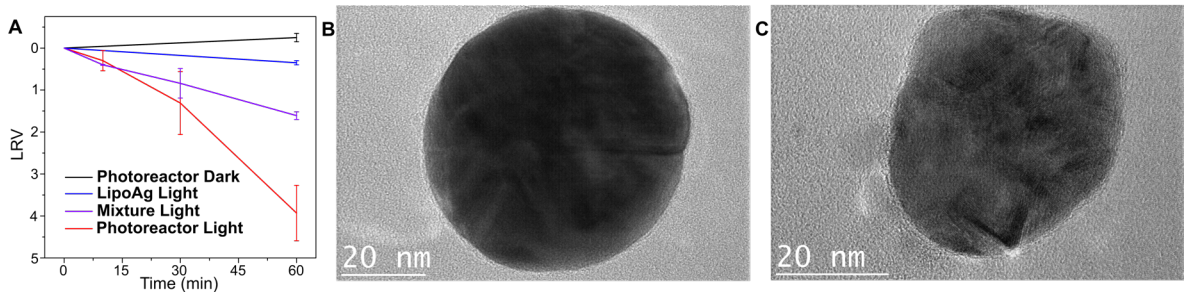

Figure S2. Characterization of Photoinduced Cationic Release Concentration over Time.
(A) Inactivation curves of *E. coli* for photoreactor bactericide and controls.
(B, C) TEM images for LipoAg particles before illumination (B) and after 3 hours illumination (C) with 430 nm LED. A visible lipid membrane can be observed for both samples.

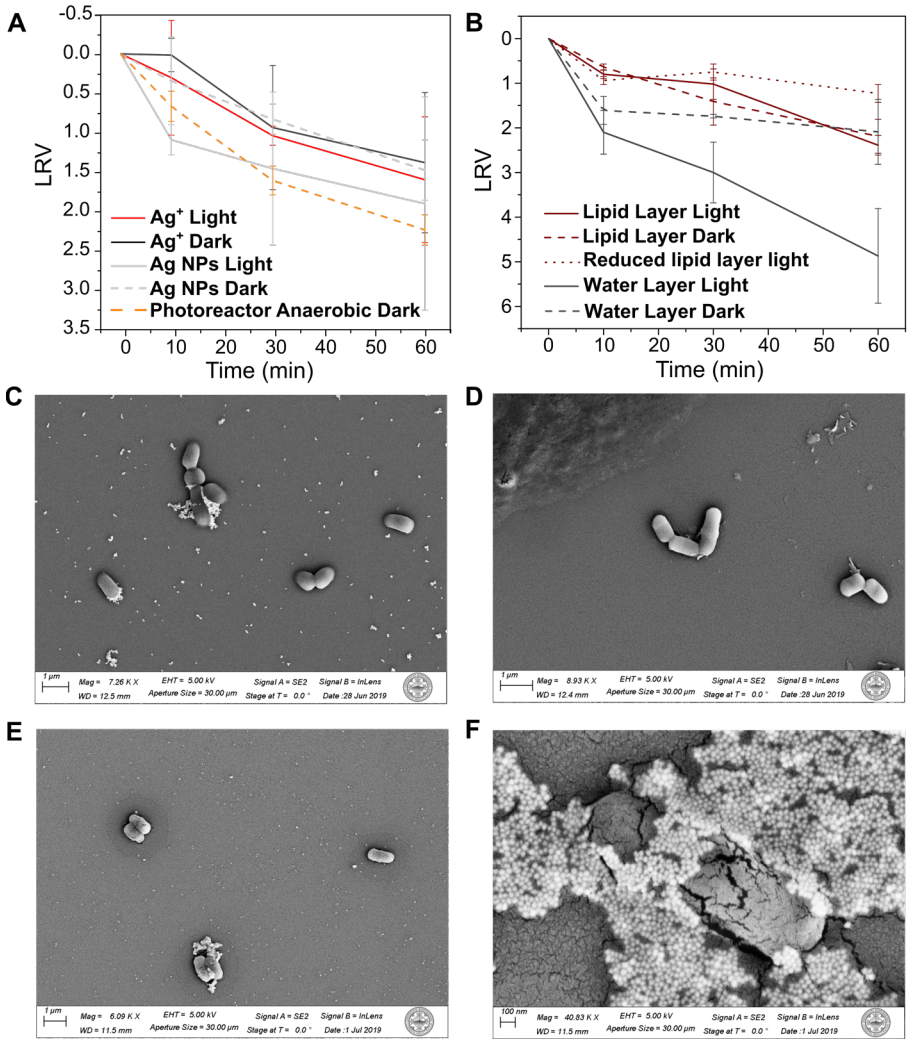

Figure S3. Supplemental Characterization of Inactivation Efficacy and Bacterial Surface after Inactivation.

(A) Inactivation of *Arthrobacter sp.* with Ag NPs and Ag^{+} cations at equivalent concentration as in the photoreactor bactericide with or without light.

(B) Inactivation of *Arthrobacter sp.* with stripped lipid layer and leftover water layer with and without light.

(C-F) SEM images of *Arthrobacter sp.* inactivated with Ag NPs at a NPs: Bacteria ratio of 100:1 (C); with 52785 ppb $[Ru(bpy)_3]^{2+}$ solution (D); and with Ag NPs at a NPs: Bacteria ratio of 10000:1 (E, F).

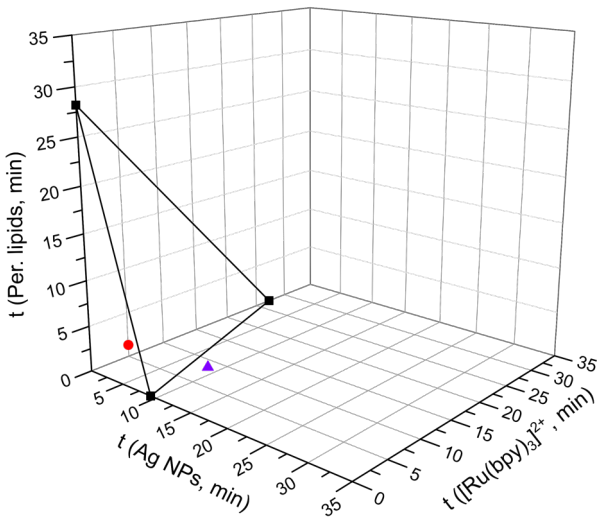

Figure S4. 3-D Isobologram for Synergistic Effect in Photoreactor Antibacterial Effect.
Axes: time to reach LRV=1 for Ag NPs, peroxidized lipids and $[Ru(bpy)_3]^{2+}$ under 430 nm LED illumination with *Arthrobacter sp.* Data points: time to reach LRV=1 for Photoreactors (red dot) and a simple mixture control of Ag NPs and $[Ru(bpy)_3]^{2+}$ (purple triangle).

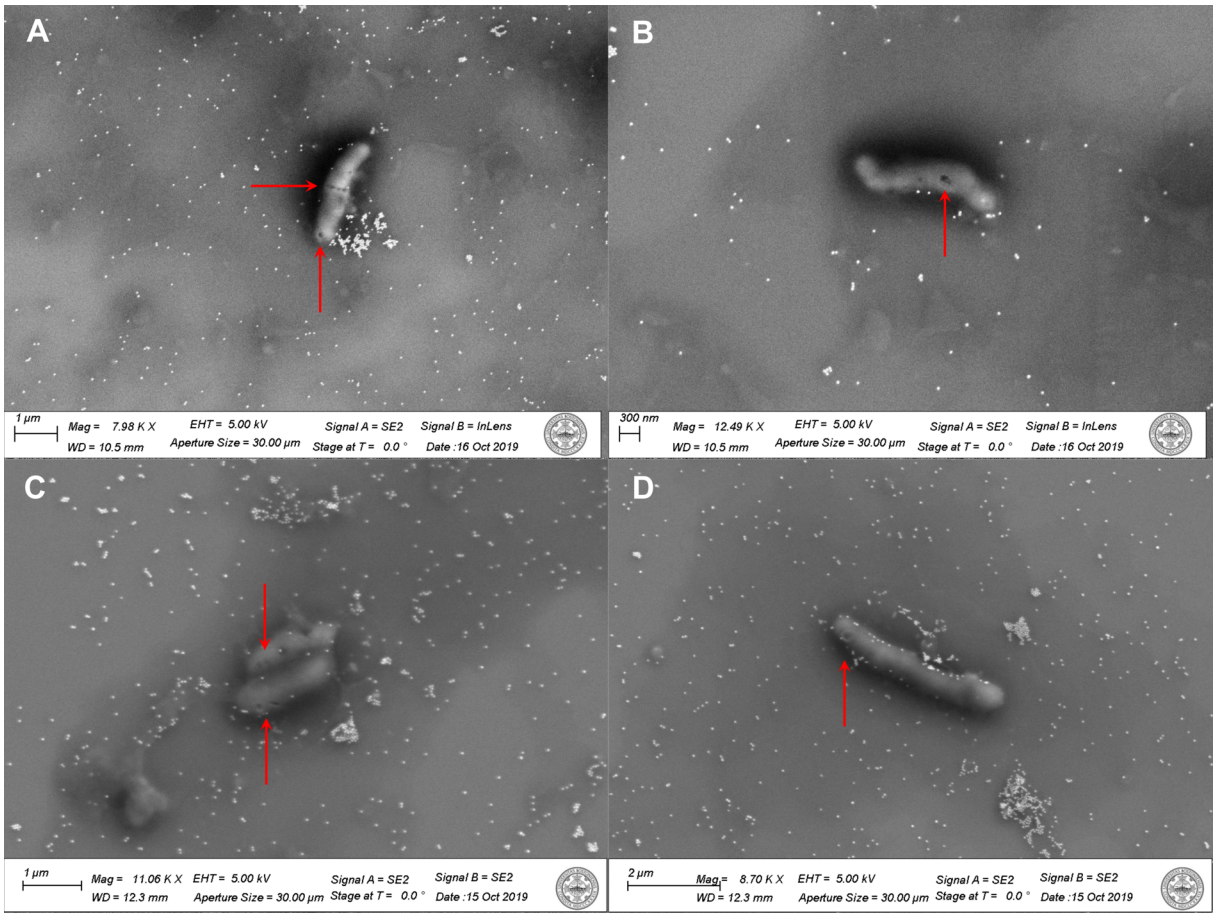

Figure S5. Supplemental SEM Images for formation of holes on *Arthrobacter sp.* surfaces.

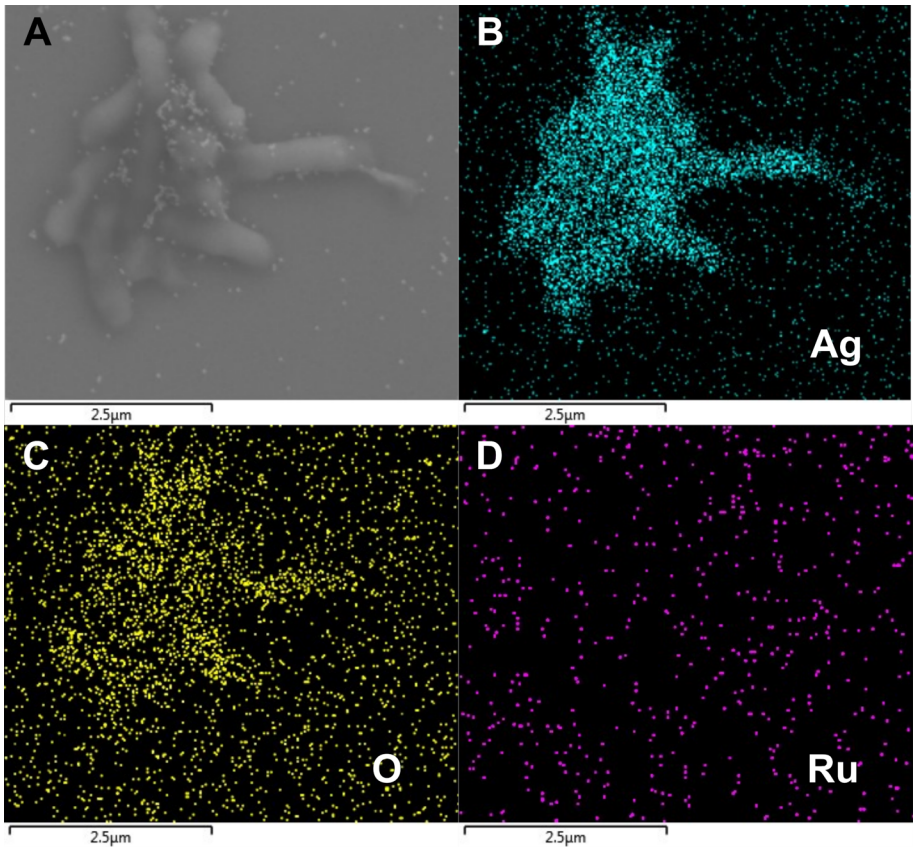

Figure S6. Characterization of Elemental Composition of *Arthrobacter sp.* After Inactivation.
(A) SEM image of the EDX scan area.
(B-D) EDX element maps for Ag (B), O (C) and Ru (D)

S1.3 Characterization of the Inactivation of *Arthrobacter sp.* Biofilm

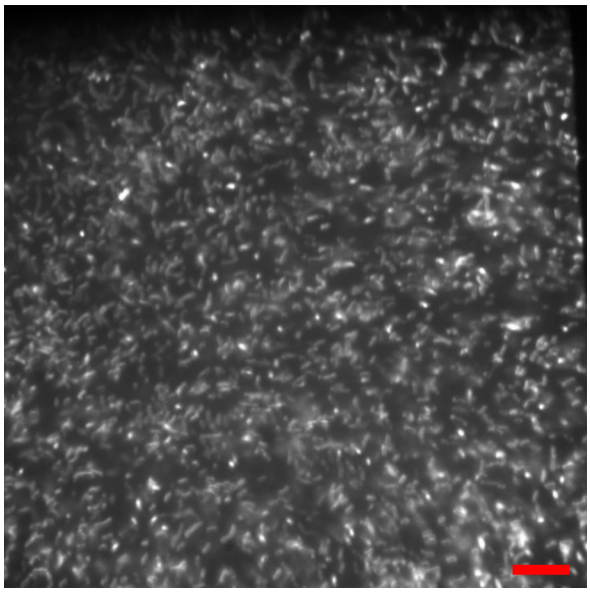

Figure S7. Darkfield Image of the Bacterial Biofilm Before Inactivation. Scale bar = 10 μm.

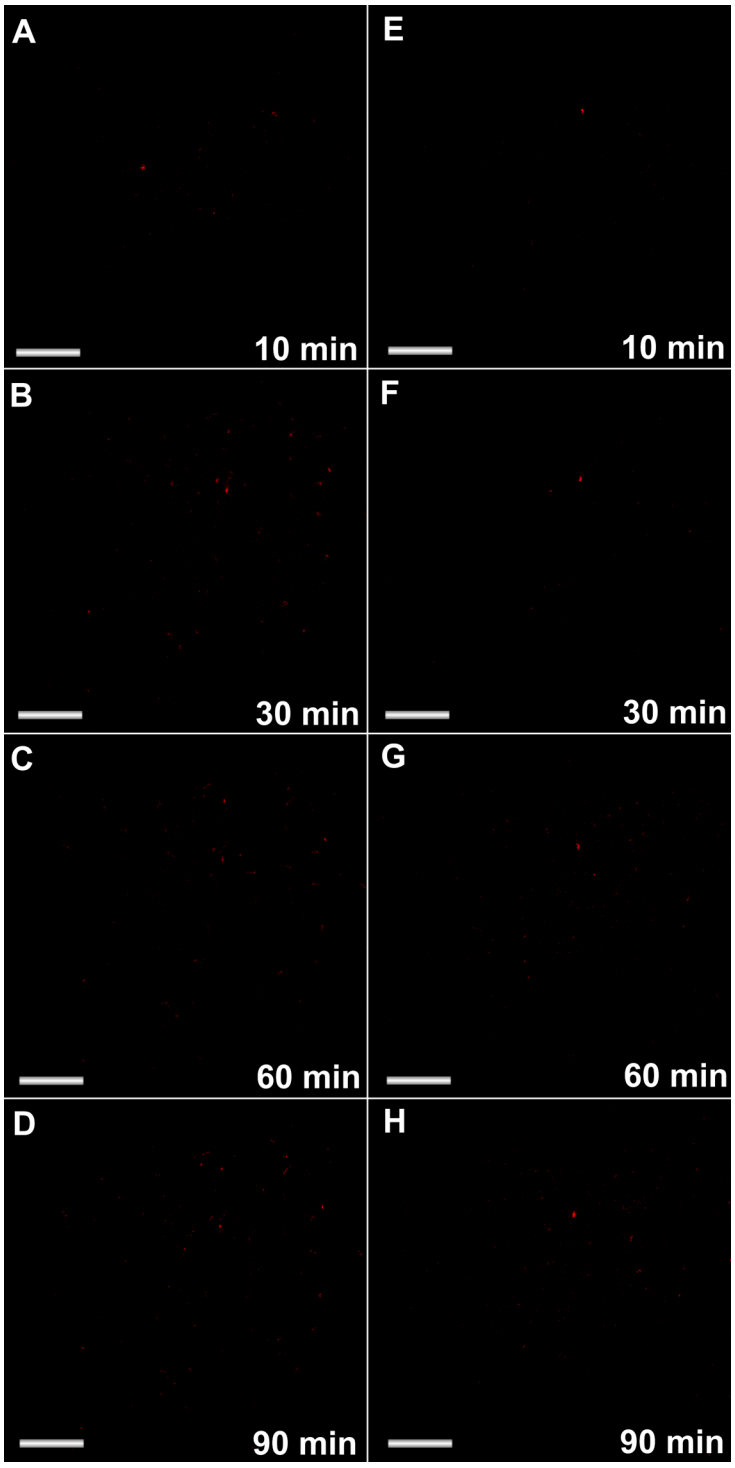

Figure S8. Characterization of Effect of Control Groups on *Arthrobacter sp.* Biofilm

(A-D) Fluorescence images of the biofilm with light but with no photoreactor nanocomposites taken at 10 min (A), 30 min (B), 60 min (C) and 90 min (D).

(E-H) Fluorescence images of the biofilm with photoreactor nanocomposites in dark taken at 10 min (E), 30 min (F), 60 min (G) and 90 min (H). Scales bars are 4 μm in A-H.

S2. Supplemental Experimental Procedures

Characterizations of the Plasmonic Photoreactors

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 Au and Fe K and L edges is performed with EDX under STEM mode with spot size of 6 and magnification of 410 kx. Hydrodynamic diameter results were measured through Dynamic Light Scattering (DLS) with a Malvern Zetasizer in polystyrene cuvettes with 1 cm light path. Element concentrations of Ag and Ru in the photoreactor composite were measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) on a VG Plasma Quad ExCell ICP-MS equipped with a Merchantek LUV213 Laser Ablation system. The samples were prepared, washed and combined so that each group contained $2x10^{10}$ Particle and 2.3 μmol of lipid membrane components (DPPC, DOPS, Cholesterol) in 0.5 ml total volume for ICP-MS measurement of element concentrations.

Optical colocalization results (**Fig. S1B**) of Darkfield and Fluorescence images were taken on an Olympus IX71 Inverted Microscope and analyzed with Image J. For these tests, a small amount of 2 mol % lipid dye (16:0 Liss Rho PE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium salt, Avanti Polar Lipids) was added to the lipid mixture during the preparation of the photoreactor bactericides nanocomposites. DPPC was adjusted to 45 mol % to maintain the same input amount of lipids. The lipid dye has excitation/emission wavelengths of 560/583 nm. UV-vis absorbance spectra was collected by 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.

Ag^+ and $[Ru(bpy)_3]^{2+}$ release concentration measurements were also conducted with the abovementioned ICP-MS system. For these experiments, photoreactors nanocomposites (528 ppb ^{101}Ru), lipid-wrapped Ag NPs without the photocatalyst $[Ru(bpy)_3]^{2+}$ (LipoAg), a simple mixture of unwrapped Ag NPs and $[Ru(bpy)_3]^{2+}$ solution, a modified photoreactor control A with higher $[Ru(bpy)_3]^{2+}$ content (857 ppb ^{101}Ru, as opposed to 528 ppb in the photoreactors), and another modified photoreactor control B with less cholesterol content (20 mol %, as opposed to 35 mol % in the photoreactors) are investigated. In control B, the amount of DPPC is increased by 20 mol % to have the same total amount of membrane components. The samples were prepared, washed and combined so that each group contained $2x10^{10}$ Particle and 2.3 μmol of lipid membrane components (DPPC, DOPS, Cholesterol) in 0.5 ml total volume. 100 μl of the abovementioned groups are fetched, diluted to 300 μl by water, and are either illuminated or kept in the dark for 1 hour. 75 μl of solution is fetched for each group at time 0, 10 min, 30 min and 1 hour into the experiments. All samples are centrifuged, and 50 μl supernatant solution is collected for analysis of released ion concentrations. The pellet is carefully avoided to not include element concentration increase from the NPs or nanocomposites. Released Ag^+ concentrations are represented by the ^{107}Ag concentration in the supernatant, and $[Ru(bpy)_3]^{2+}$ concentrations are represented by the ^{101}Ru concentrations. The ion concentrations at time 0 is subtracted from all other time points in the same group to correct for the initial ion concentration in the supernatant.

Preparation of Bacteria and Inactivation Assays

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

of photoreactor nanocomposites was analyzed before lipid wrapping based on the absorbance at 450 nm.

To investigate the effect of Reactive Oxygen Species (ROS), 100 µl 10 mM sodium azide (scavenger for $^1O_2$, Sigma Aldrich), MnTBAP (scavenger for O_2^-, Sigma Aldrich) and mannitol (scavenger for ·OH, Sigma Aldrich) were mixed with 100 µl *Arthrobacter sp.* suspension and 100 µL photoreactor suspension to achieve a mixture containing 10^{10} NPs and 10^8 bacteria. The rest of the procedure is identical to the regular inactivation experiment described in **Materials and Methods**.

To measure the inactivation effect of peroxidized lipid species, the peroxidation product was separated through solvent extraction. The photoreactor bactericides water suspension was first illuminated for 1 hour with 430 nm LED under the same condition as in inactivation assays, but without the bacteria. The total volume of this solution was made up with milliQ water to the same 300 µL as in the inactivation. Chloroform was then added, and the stratified mixture was tip-sonicated and shaken for extraction of the lipid components from the water suspension into the chloroform layer. The Chloroform layer was then gathered and rotary evaporated at 34 ^oC to get rid of all solvent, and then re-dispersed with water through tip sonication. The water layer was also gathered and rotary evaporated at 34 ^oC until a clear orange liquid suspension is left, to remove all leftover chloroform. Both groups were used in inactivation assays with and without photoactivation.

To prepare the group with reduced peroxidized lipid product, twice the amount of lipid-wrapped photoreactor particles was used, and the abovementioned lipid separation procedure was followed. After the lipid products were re-dispersed with water, an equal volume of 10 mM sodium ascorbate solution was added to the dispersion to achieve the same lipid concentration as used for inactivation. The solution was shaken and incubated for 1 hour before being used for inactivating *Arthrobacter sp.* with light.

Scanning Electron Microscope imaging was carried out on a Zeiss Supra 40 VP SEM with 5 eV EHT. *Arthrobacter sp.*/photoreactor mixture 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 mixture was washed twice and dropped onto silicon wafer (<100>, University Wafer) substrates for SEM imaging. Energy Dispersive X-Ray Spectroscopy (EDX) element mapping results in **Fig. S6** were obtained on a Zeiss Supra 55 VP SEM with an EDX detector at 30 µm aperture, 5 kV EHT for 30 seconds.