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Supplemental Information

2D Graphdiyne Oxide Serves as a Superior New Generation of Antibacterial Agents

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Transparent Methods

Materials

Concentrated sulfuric acid H_2SO_4, (98 wt %) was supplied by Beijing Chemical Works. Hydrogen peroxide (H_2O_2, 30 wt %) and ethylenediaminetetraacetic acid ferric sodium salt (Fe(II)) were provided by Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. Tert-butanol was obtained from Tianjin Yongda Chemical Reagent Co., Ltd. Glutaraldehyde was provided by Beijing Leagene Biotechnology Co., Ltd. Isopropanol alcohol (IPA) was purchased from Tianjin Fuyu Fine Chemical Co., Ltd. 4-Hydroxy-2,2,6,6-tetramethylpiperidinyloxy (TEMPOL) and *p*-hydroxyphenylacetic acid were supplied by Aladdin Co., Ltd. Sodium azide (NaN_3) was obtained from MRC Co., Ltd. PCX50C Discover as visible light source (420-760 nm). Sodium chloride NaCl was purchased from Tianjin Beilian Fine Chemicals Development Co., Ltd. Distilled water was generated on a Millipore system (Millipore Inc.). Without further purification, all the chemicals were utilized as received. Yeast extract powder was bought from Beijing Aoboxing Biotech Co., Ltd. Beef cream was obtained from Guangdong Huankai Biotech Co., Ltd. Tryptone was purchased from Oxoid Co., Ltd. Agar was provided by Biosharp Co., Ltd. All of the above used in biological tests were biological-reagent grade.

Preparation of GDY and GDYO

GDY was prepared according to the cross-coupling reaction detailed in our previous report (Li et al., 2010). Typically, the monomer of hexaethynylbenzene was synthesized in a yield of 62 % by addition of tetrabutylammonium fluoride (TBAF) to tetrahydrofuran (THF) solution of hexakis[(trimethylsilyl)ethynyl]benzene for 10 min at 8 ^{o}C. The graphdiyne was successfully grown on the surface of copper foil in the presence of pyridine by a cross-coupling reaction of the monomer of hexaethynylbenzene for 72 h at 60 ^{o}C under nitrogen atmosphere. GDYO was synthesized by the simple oxidation of GDY powder using a mixture of H_2O_2 and H_2SO_4 as a complex oxidant. Briefly, 20 mg of GDY was ground for 10 min using an agate mortar, and a GDY dispersion was achieved by the assistance of an ice bath sonication of the GDY powder in 30 mL of distilled water for about 10 hours. Ultrasonicated GDY was obtained by freeze-drying. Subsequently, 20 mg of the ultrasonicated GDY was gradually stirred into 30 wt % H_2O_2 solution (2 mL) and 98 wt % H_2SO_4 (6 mL) under an ice-water bath for 5 h. Finally, GDYO was obtained by ultrasonication for 4 hours, followed by a freeze-drying treatment.

Antibacterial Assay

The antibacterial properties of GDY and GDYO were determined via the colony-counting method using *Escherichia coli* (*E. coli*, 8099 Gram-negative bacteria) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538 Gram-positive bacteria) as the bacterial models. Typically, bacterial strains were expanded in a shaking table with a shaking speed of 220 rpm for 12 hours in a liquid medium at 37 ^{o}C to obtain a strain concentration of 10^8-10^9 CFU·mL^{-1} and were stored at 4 ^{o}C. Prior to the antibacterial tests, 1.0 mL of bacterial culture was centrifuged at ~4000 rpm, the as-centrifuged strains were washed three times with a 0.8 wt % sterile aqueous solution of sodium chloride, then gradually diluted to 10^6 CFU·mL^{-1}. 3 mg of GDY and GDYO were dispersed separately in 900 μL of sterilized distilled water, vortexed, and then sonicated for 30 min. For an antibacterial test, 100 μL of bacterial suspension was added into 900 μL of sample suspension, mixed well, and incubated under constant shaking. At different time intervals, aliquots of the mixture above were gathered and then diluted to 10^2 CFU·mL^{-1} using the sterilized saline solution, and then spread

uniformly on nutrient agar plate and incubated at 37 ^{o}C for 24 h. To evaluate the effect of visible light irradiation on antibacterial efficiency, a visible light simulator—a 300 W Xe lamp filtered through a UV cutoff (k < 420 nm)—was used as the light source during the antibacterial procedure, and the control experiments in the dark were conducted in the same way, minus the light. All the inactivation experiments were performed in triplicate. Survival of bacterial colonies was calculated based on the following equation: % survival = (A/B), where A is the number of surviving colonies of the sample and B is the number of surviving colonies of the control.

Impact of ROS on Antibacterial Efficiency

The roles of •OH, $\bullet O_2^-$, $^1O_2$, and H_2O_2 in the antibacterial process were verified by the active oxygen scavenging experiment. The scavengers used in this study were IPA (0.5 mM) for •OH, TEMPOL (2 mM) for $\bullet O_2^-$, NaN_3 (0.077 M) for $^1O_2$, and Fe (II) (2.4 mM) for H_2O_2. The scavenging experiments were carried out under the same conditions as the antibacterial assay described above.

Detection of H_2O_2

The *p*-hydroxyphenylacetic acid could be degraded by H_2O_2, and so the existence of H_2O_2 could be qualitatively detected using the fluorescence spectrophotometry. In this test, 200 μL of GDY dispersion or GDYO dispersion (3 mg·mL^{-1}) was irradiated with visible light for 4 h, followed by the dilution to 3 mL. After the pH was adjusted to 9, 50 μL of *p*-hydroxyphenylacetic acid was added into the dispersion to measure the fluorescence spectrum. *p*-Hydroxyphenylacetic acid (50 μL) was dissolved in 3 mL of distilled water, and the corresponding fluorescence spectrum was measured as a control. In addition, the fluorescence spectra of *p*-hydroxyphenylacetic acid (50 μL) in the presence of H_2O_2 (200 μL and 2200 μL, respectively) were measured to confirm the degradation of *p*-hydroxyphenylacetic acid by H_2O_2.

Bacterial Morphology Observation

GDYO (10 mg) was dispersed in distilled water (1 mL), sonicated for 5 min, mixed with 10^7 CFU·mL^{-1} of bacterial cells, and then irradiated under visible light for 4 h. The mixture above was centrifuged at 4000 rpm for about 10 min and washed at least three times with PBS solution. After that, 1 mL of glutaraldehyde fixative was added into the mixture, and it was then left to stand for 12 h at 4 ^{o}C. The as-obtained mixture was centrifuged at ~4000 rpm, washed with PBS at least three times, and dehydrated with 20 %, 50 %, 80 % or 100 % ethanol. Afterwards, the mixture was washed twice with *t*-butanol, then dissolved in *t*-butanol and allowed to stand at 4 ^{o}C for more than 30 min. Finally, the morphology of bacteria and their interaction with the samples were characterized using SEM, TEM, and STEM mapping.

Dispersity Evaluation

In this test, the dispersity of GDY and GDYO were evaluated as a function of their standing periods. Briefly, GDY and GDYO dispersions (3 mg·mL^{-1}) were obtained by sonicating GDY and GDYO in deionized water using a bath sonicator at 37 kHz for 5 min. Then the GDY and GDYO dispersions were placed separately in cuvettes, and the corresponding photographs were recorded after the cuvettes had been standing for 12 h and 36 h.

Inhibition Zone Test

3 mg of GDY and GDYO were well dispersed in 1 mL of distilled water using an ice bath sonicator. The as-obtained GDY and GDYO dispersions were evenly dropped and loaded completely onto filter paper. After a bacterial solution of 10^5 CFU·mL^{-1} was evenly spread on the solid medium and dried,

the sample-loaded filter paper was placed on the medium and incubated at 37 ℃ for 12 h in the dark. For the visible light irradiation group, the sample-loaded filter paper was placed on the medium and irradiated under visible light for 4 h, then incubated at 37 ℃ for 12 h. The antibacterial capabilities against *E. coli* were confirmed by measuring the inhibition zones.

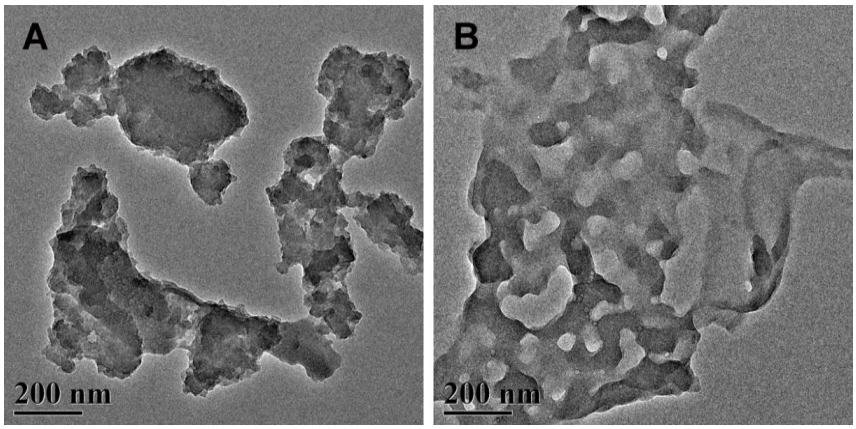

Figure S1. TEM images of (A) GDY and (B) GDYO with low magnification. Related to Figure 1.

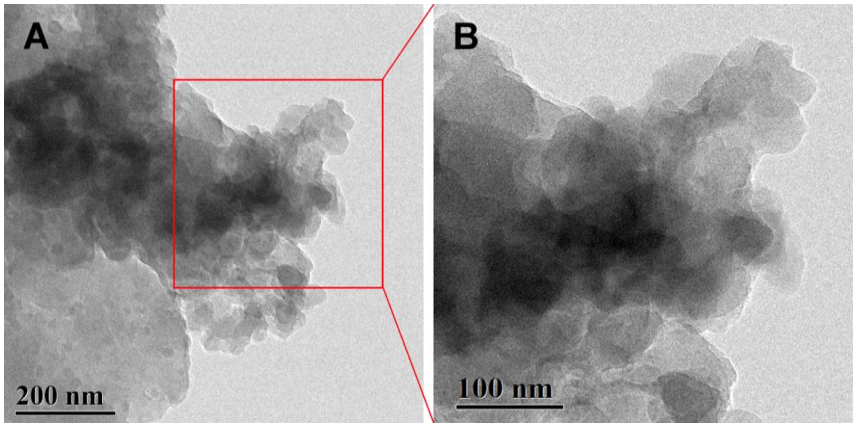

Figure S2. TEM images of ultrasonicated GDY at different magnifications. Related to Figure 1.

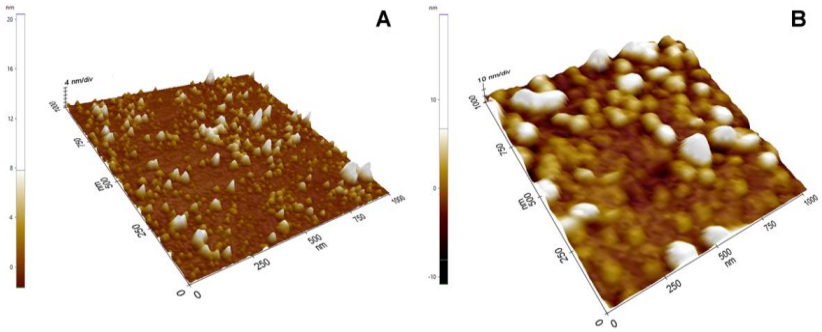

Figure S3. AFM images of (A) GDY and (B) GDYO. Related to Figure 1.

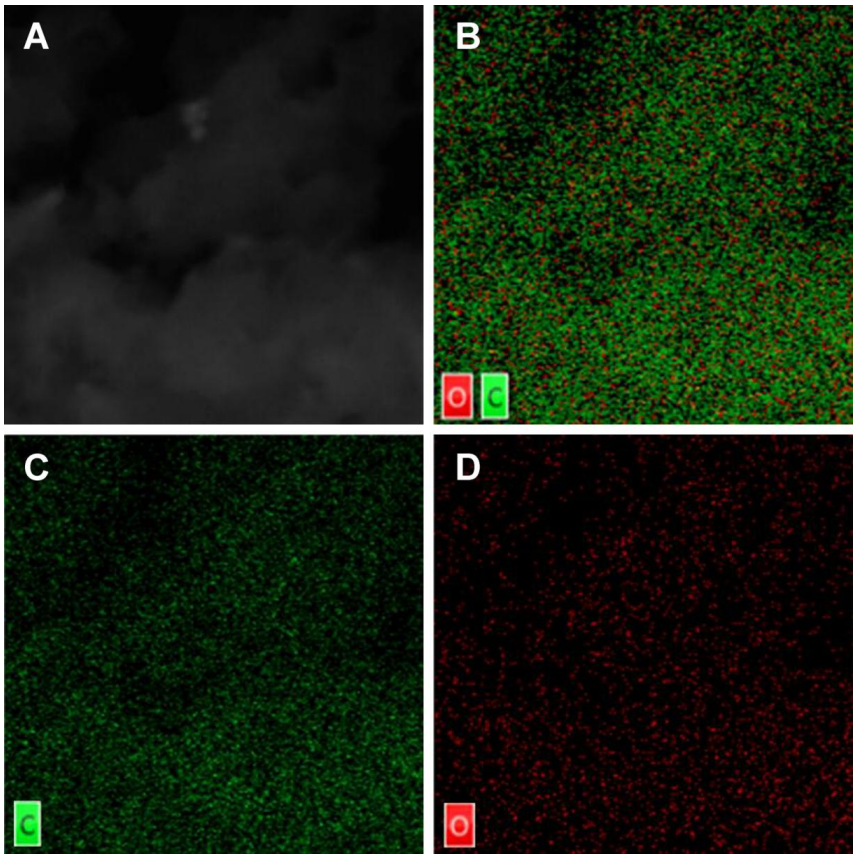

Figure S4. Representative STEM mapping of ultrasonicated GDY. Related to Figure 2.

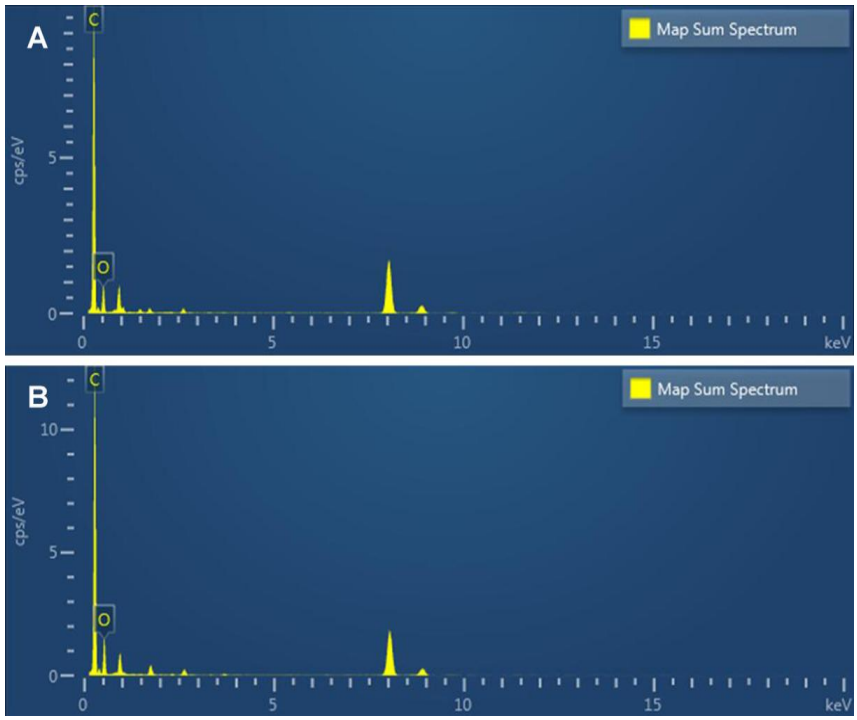

Figure S5. EDX spectra of (A) GDY and (B) GDYO. Related to Figure 2.

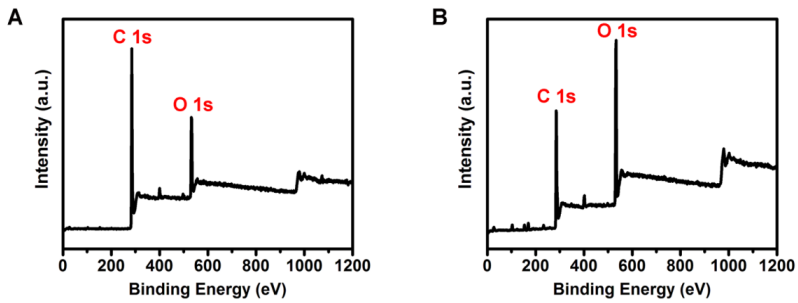

Figure S6. XPS survey spectra of (A) GDY and (B) GDYO. Related to Figure 2.

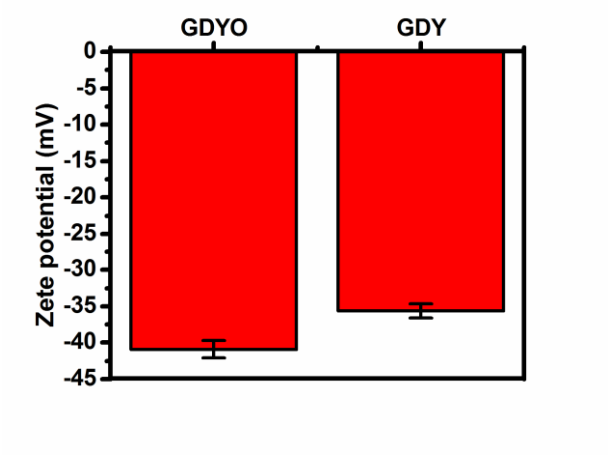

Figure S7. Zeta potentials of GDYO and GDY. Related to Figure 2.

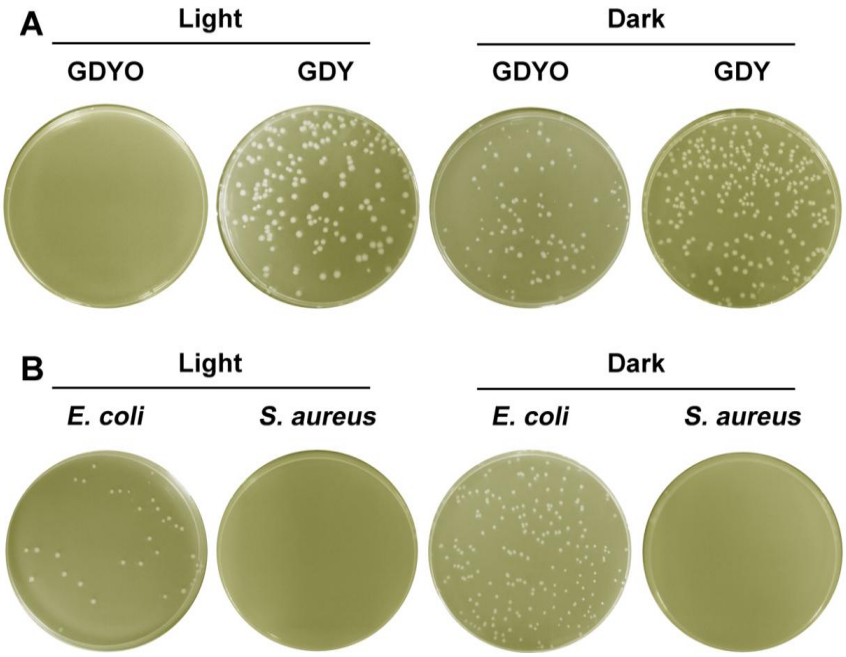

Figure S8. (A) Photographs of the bacterial culture plates of *E. coli* after treatment with GDY or GDYO for 120 min in the dark or under visible light irradiation. (B) Photographs of the bacterial culture plates of *E. coli* and *S. aureus* after treatment with GDYO for 60 min in the dark and under visible light irradiation. Related to Figure 3.

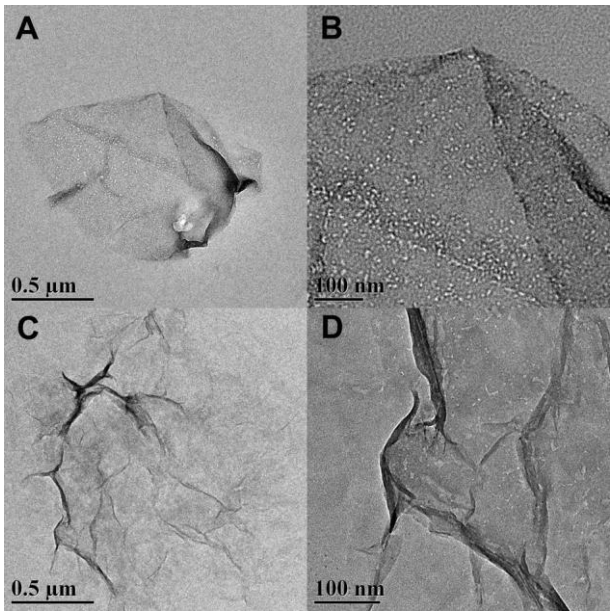

Figure S9. Representative TEM image of GN (A and B) and GO (C and D). Related to Figure 3.

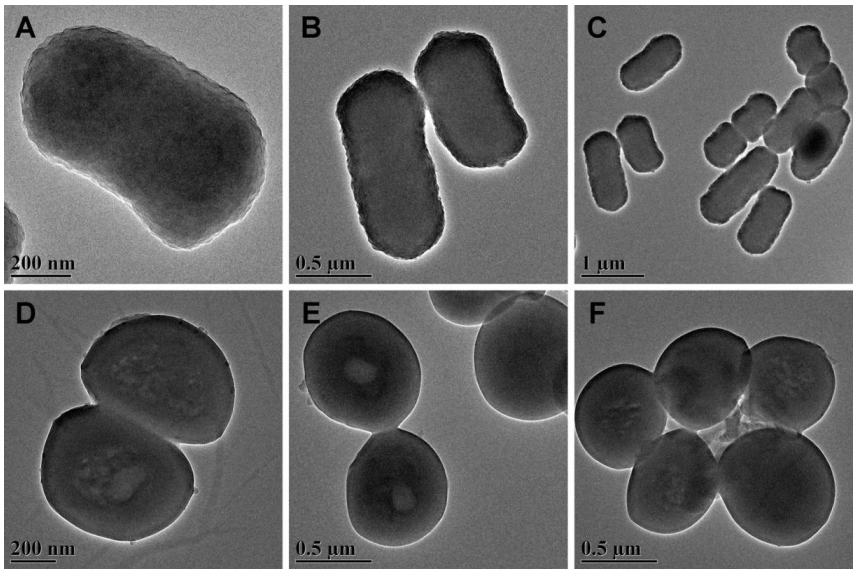

Figure S10. TEM images of (A–C) *E. coli* and (D–F) *S. aureus* after incubation with saline solution. Related to Figure 4.

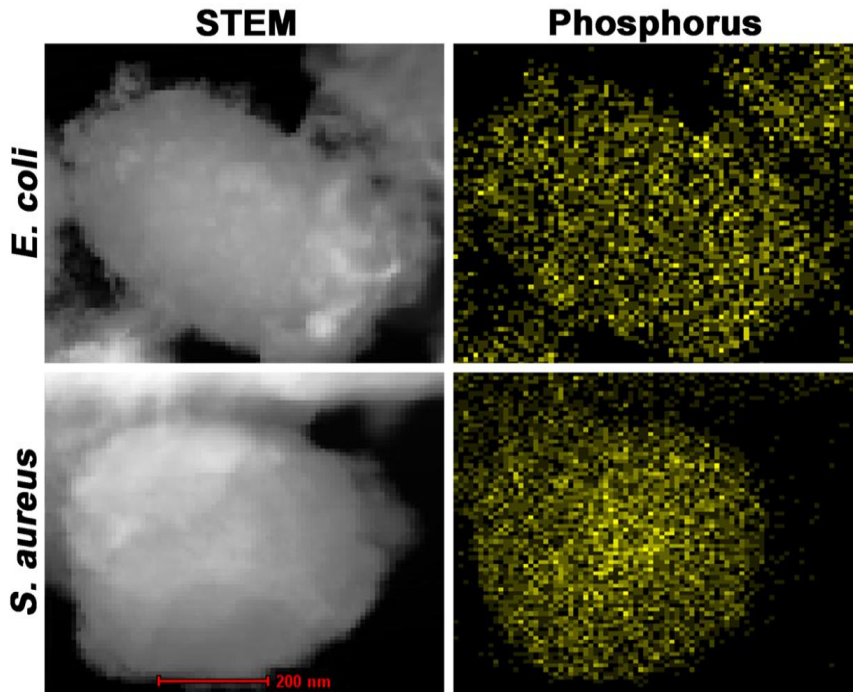

Figure S11. STEM mappings of *E. coli* and *S. aureus* after incubation with GDYO suspension under visible light irradiation for 4 h. Related to Figure 4.

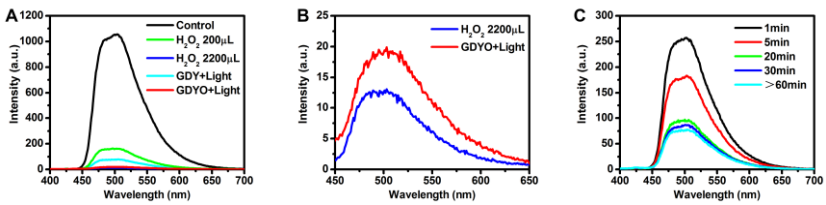

Figure S12. (A and B) The fluorescence spectra of *p*-hydroxyphenylacetic acid in the presence of H_2O_2, GDY and GDYO under visible light irradiation. (C) The fluorescence spectra of *p*-hydroxyphenylacetic acid in the presence of GDYO under visible light irradiation, as a function of aging time. Related to Figure 5.

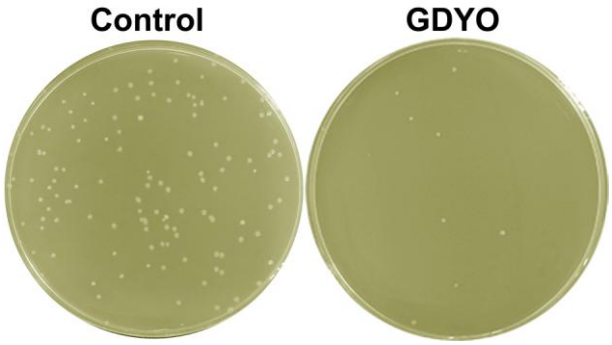

Figure S13. Pictures showing the bacterial survival after direct contact between GDYO with *E. coli*. Related to Figure 7.

Supplemental References

Li, G., Li, Y., Liu, H., Guo, Y., Li, Y., Zhu, D. (2010). Architecture of graphdiyne nanoscale films. Chem. Commun. *46*, 3256-3258.