Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Bi_2O_3/TiO_2@reduced graphene oxide with enzyme-like properties efficiently inactivates *Pseudomonas syringae* pv. tomato DC3000 and enhances abiotic stress tolerance in tomato

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Supplementary material

Methods：

Method S1

Nanomaterial preparation: Firstly, at room temperature(25 ℃), add $Bi(NO_3)_3{\cdot}5H^2O$ and citric acid monohydrate in a 2:1 ratio to 10 mL HNO_3 (0.04 M) and stir for 1 h. After complete dissolution, add Pluronic® P-123 (0.008 g) and continue stir for 4 h. Add P25 and stir for 3 h. Put the obtained product into a crucible and held at 300 ℃ for 24 h, then cool naturally to room temperature to obtain Bi_2O_3-TiO_2.

Secondly, graphite oxide (GO) was made from graphite (325 mesh, was supplied from Henan Yixiang New Material Co., Ltd) by Hummers' method.

In the third and final step, GO was dispersed in 40 mL of ethanol and sonicated for 90 min. Thereafter, P25 and Bi_2O_3-TiO_2 powder respectively was added and sonicated for 90 min. This suspension was added to a 50 mL polytetrafluoroethylene-

lined autoclave and kept at 180 ℃ for 8 hours. The reaction simultaneously reduced GO and loaded P25 or Bi_2O_3-TiO_2 onto the graphene sheets. The product was collected and washed with DI water, followed by lyophilized to obtain rGO-TiO_2 or rGO-(Bi_2O_3-TiO_2). Add GO according to 4% of Bi_2O_3-TiO_2 mass percentage.

Ellman's Assay: Specifically, GSH (0.8 mM, 600 μL) and bicarbonate buffers solution (50 mM, pH = 8.7) were mixed with nanomaterials aqueous solutions (1280 $\mu g \cdot mL^{-1}$, 600 μL) in 2 mL centrifuge tube. Aluminium foils were used to cover these tubes to prevent radiation of light. After incubated for 4 h, Tris-HCl solution (50 mM, pH = 8.5, 314 μL) and DTNB (100 mM, 6 μL) were added into 180μL of the mixtures. Nanomaterials were removed by centrifugation at 10,000 rpm for 10 min. Then, 150 μL of supernatant (removed nanomaterials) was transformed into 96-wellplate. The absorbance (As) was monitored at a wavelength of 414 nm by a Microplate reader (ST-360, China). Meanwhile, ultrapure water was mixed with GSH solution as the negative control (An). Moreover, the loss of GSH was calculated according to the following equation:

Loss of GSH (%) = ((An - As) / An) × 100%

Peroxidase-Like Property: The peroxidase-like property of nanomaterials was evaluated using TMB as substrate after incubation for 4.5 h at room temperature. Three groups of assays including TMB + H_2O_2, TMB + H_2O_2 + nanomaterials (dark), and TMB + H_2O_2 + nanomaterials(light) were investigated at pH = 7. Specifically, of nanomaterials aqueous solution (1280 $\mu g \cdot mL^{-1}$, 100 μL) with NaAc / HAc buffer (0.2 M: 0.2 M), TMB solution in DMSO (2 mM, 100 μL), and H_2O_2 (10 mM, 100 μL) were mixed within 4.5 h. The absorbance of different mixtures for TMB at the wavelength of 630 nm was measured using a Microplate reader (ST-360, China)

Superoxide Dismutase-Like Property: The final concentration of NBT nitrogen blue tetrazole (75 μM, 0.3 mL), riboflavin (2.0 μM, 0.3 mL), Met (13 mM, 0.3 mL, EDTA (10 μM, 0.3 mL) mixed solution and suspended nanomaterial (1280 $\mu g \cdot mL^{-1}$, 1.5 mL) were mixed. Illuminate the mixed solution with visible light for 10 minutes. Immediately after irradiation, the absorbance was measured at 560 nm. Take no nanomaterials as a blank control. The inhibition rate was calculated according to the

following formula:

Inhibition rate (%) = (A_0-A/A_0)×100, where A_0 and A are the absorbance without with nanomaterials 560 nm respectively.

Method S2

King's B (KB) medium (glycerol 1%, peptone 20 g·L^{-1}, K_2HPO_4 1.5 g·L^{-1}, $MgSO_4 \cdot 7H_2O$ 1.5 g·L^{-1}) containing rifampicin 25 mg·L^{-1} was employed to culture a mono colony of *Pst*.DC3000 at 28°C for 24 h on a shaker. The bacterial suspension (10 mL, 10^{10} CFU·mL^{-1}) was mixed with different proportions nanomaterials (10 mL, 1000 μg·mL^{-1}) in petri dishes. Then the mixed solution was incubated under visible light and dark at 30 °C for 150 min. Take samples every 30 minutes, dilute the solution 10^6, 10^7, 10^8 times with PBS buffer solution. 100 μL of bacterial dilution was spread on the KB solid medium plate. After incubating at 30°C for 24 hours, the number of CFU was counted and recorded to perform quantitative and time-dependent tests respectively.

Method S3

Take the mixture directly to measure its conductivity and mark it as Si. The cells were disrupted by ultrasound (4°C, 4 min, broken 5 s, gap 9 s) and then shakeup. The measured conductivity was expressed as Sn, and the conductivity in the absence of light and nanomaterials was the control (CK).

The relative conductivity formula was as follows:

$$L = \frac{S_i}{S_n} \tag{1}$$

The formula for cell damage was as follows:

$$cell\ damage\ (\%) = \frac{L_t - L_{ck}}{1 - L_{ck}} * 100\% \tag{2}$$

Dissolve Coomassie Brilliant Blue (G-250) (1 g) with 90% ethanol (50 mL) and 85% orthophosphoric acid (100 mL) to a volume of 1L for the reaction solution. Mix the sample (0.5 mL) with the G-250 solution (5 mL) and let it stand for 5 minutes, and measure the photometric value at 595 nm.

Method S4

The tomato seeds (Five Star Grape, F1) used in this study were purchased from

Shouhe Seed Industry Co., Ltd. (Shandong, China). The seeds were planted in a petri dish with two layers of sterilized filter paper, adding 5 mL of sterilized deionized water or nanomaterial suspension (320, 640, 1280, 2560 μg·mL^{-1}), and keeping them in the dark for 6 days.

For plant cultivation, seeds were soaked in warm water at 55°C temperature for 20 min, and placed in an incubator at 28°C with shaking at 200 rpm for 2 days. After budding 0.5-1 cm, sown into a mixed substrate of turfgrass and vermiculite (volume ratio of 3:1). The growth condition was maintained at 25°C ± 2°C, with a 16/8-light/dark photoperiod and 130-180 mmol·m^{-2}·s^{-1} light intensity. When the tomato grew to the early seedling stage, the growth and development were measured, and when the tomato grew to the late seedling stage, the photosynthetic function and enzyme activity was measured.

Method S5

Crude enzyme extraction: Leaves were collected at 0, 12, 24, 48, 72, 96, and 120 h of inoculation, and stored at -70°C for later using. 0.5 g of frozen sample for each treatment was mixed 3 mL phosphate buffer (50mM, pH=7.8, containing 0.2 mol·L^{-1} EDTA, 1% PVP) and ground evenly, then centrifuged at 12000 rpm for 20 min at 4℃. The supernatant was collected as the crude enzyme solution.

PAL: The borate buffer (1 mL, 0.05 M, pH=8.8), L-phenylalanine (1 mL, 0.02 M), distilled water (1 mL) was added to crude enzyme solution (1 mL) in sequence, mixing well. The boiled enzyme solution was used as a blank control. After incubating in a water bath at 30°C for 30 min, HCl (1 mL, 6 M) was added to terminate the reaction. The absorbance at 290nm was measured, and one PAL unit activity was defined as the amount of enzyme whose absorbance increased by 0.01 per g of fresh weight per minute.

PPO: Briefly, phosphate buffer (1.5 mL, 0.05 M) and catechol (1.5 mL, 0.1 M) was mixed to 20 μL crude enzyme solution. The boiled enzyme solution was as a blank control. After incubating in a water bath at 30°C for 2 min, HCl (1 mL, 6 M) was added to terminate the reaction. The absorbance at 398 nm was measured, and one PPO unit activity was defined as the amount of enzyme whose absorbance increased by 1 per g

of fresh weight per minute.

POD: Phosphate buffer (2.9 mL, 0.05 M), H_2O_2 (1 mL, 2%) and guaiacol (1 mL 0.05 M) was added to 1 mL crude enzyme solution in sequence. The boiled enzyme solution was as a blank control. The absorbance at 470 nm was measured immediately after mixing. One unit of POD activity was defined as the amount of enzyme that increases the absorbance by 0.01 per g of fresh weight per minute.

Method S6

According to the manufacturer's instructions, the total RNA was extracted from the bacteria and tomato leaves (DP430, Tiangen Biotech (Beijing) Co., Ltd. China). DNAse was used to remove genomic DNA contamination. The concentration and purity of RNA were determined by (NanoDrop® ND-2000, Thermo Scientific, United States) for quality inspection. Each gene's primer specificity and amplification efficiency were verified through melting curve analysis and agarose gel electrophoresis. RNA was reverse transcribed into cDNA (PrimeScript™ RT reagent Kit with gDNA Eraser, TaKaRa, China) for quantitative PCR analysis.

The qRT-PCR reaction system was a 20 μL reaction mixture which contains 2 μL of cDNA sample, 10 μL of 2x Greenstar qPCR Master Mix (Bioneer, Korea), 0.5 μL of each forward and reverses primer (10 μM) (Table 1) and 7 μL of sterile water. The qRT-PCR reactions were performed by the following PCR cycling condition in the CFX96™ Real-Time system (Bio-Rad, Hercules, CA, USA): 95°C initial denaturation for 30 seconds, followed by 40 cycles of 95°C denaturation for 5 seconds, and 60°C annealing and extension for 40 seconds in the process. 16srRNA and actin genes were used as internal controls for normalizing target gene expression in bacteria and tomato, respectively.

Tables：

Table S1 Primer sequences of related genes in *Pseudomonas sringae* pv. *tomato*

Primer name		Primer sequence (5'to3')
recO	F	AAACGTCGGTCGTATGGAGA
recO	R	CCATTCAAACGAACGCAACA

omlA	F	CTCTTGCTAACCAGTTTCAC
omlA	R	GCTATACAGGTAATCCCACC
sodC	F	CTCAGCGTTCAGGCAGCATC
sodC	R	CTCGCAACTACCGTTTTCAT
katB	F	GTTACAGCGAGCTGCCAATC
katB	R	TCTGCCTTGTAGAGGAACGA
gshB	F	GTTACAGCGAGCTGCCAATC
gshB	R	TCTGCCTTGTAGAGGAACGA
hrpS	F	CTCCAGAGTCGGTTATGTCG
hrpS	R	TTTCCAGCACCCTCAGCAAT
corS	F	GTCGTGAGTCTTAGGGTGGG
corS	R	GATGAAGGGCGTAGGAAAGG
iaaL	F	AAAAGGTCGTCTTCAATGGC
iaaL	R	GAGGTGATGAGGATGCTGTC
flgG	F	TCTGGAGCCTGCGATTGTGG
flgG	R	GTCTGGATGTTGCCGATGAT
16s	F	CAGCMGCCGCGGTAATWC
16s	R	CCGTCAATTCMTTTRAGTTT

Table S2 Primer sequences of related genes in *Solanum lycopersicum* L.cv.

Primer name		**Primer sequence (5'to3')**
PR-2	F	GCGGTGTTCAGCCTGGATG
PR-2	R	AGCATGAGCAAGTATGTTGTG
PR-10	F	TGTAGGCAAAGGGAAATCCA
PR-10	R	CCGTGTCATACAGATTGGAAAA
SOD	F	CAAAAATGGTGAAGGCCGTC
SOD	R	GCTTAACCCTGGAGGCCAAT
PR-13	F	ATGGCTCGATCCTTGTGCTTCATG
PR-13	R	TTAGTTATCCATCATCTCTTC
GSTZ1	F	ACCCTGAGCCACCTTTGTTA

GSTZ1	R	TAACCCAGGCAGTCTTCTCC
Actin	F	AGGCACACAGGTGTTATGGT
Actin	R	AGCAACTCGAAGCTCATTGT

Figures：

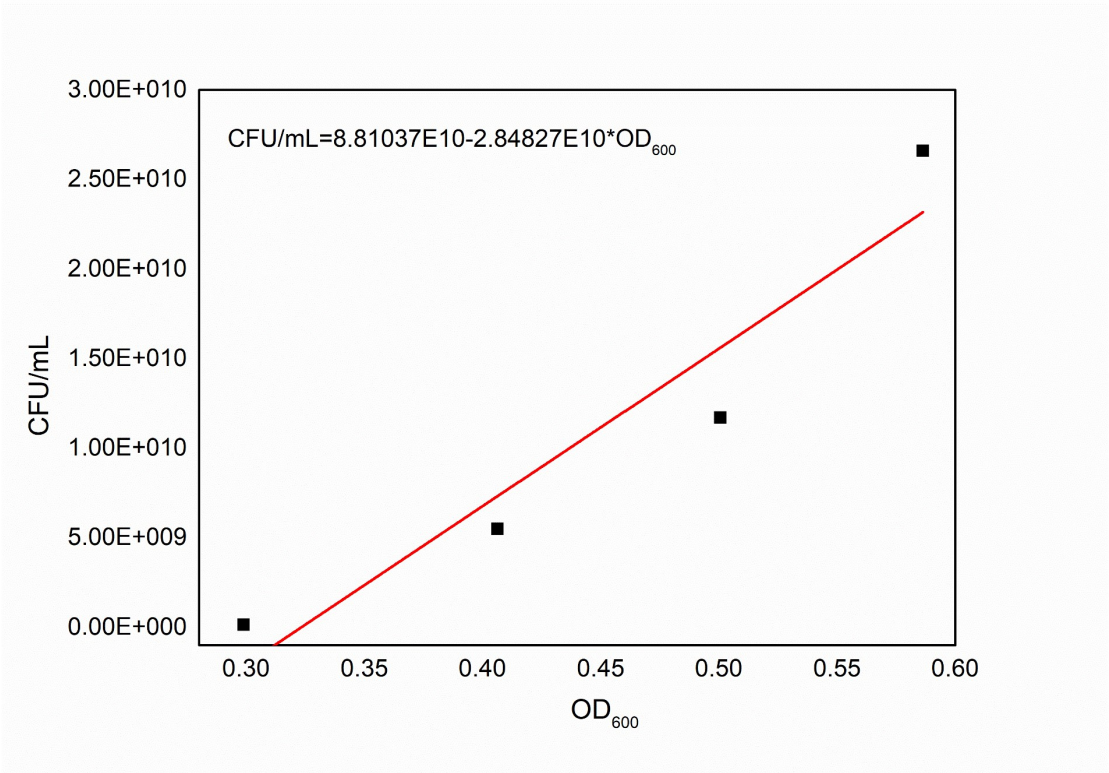

Figure S1 CFU - OD_{600} standard curve.

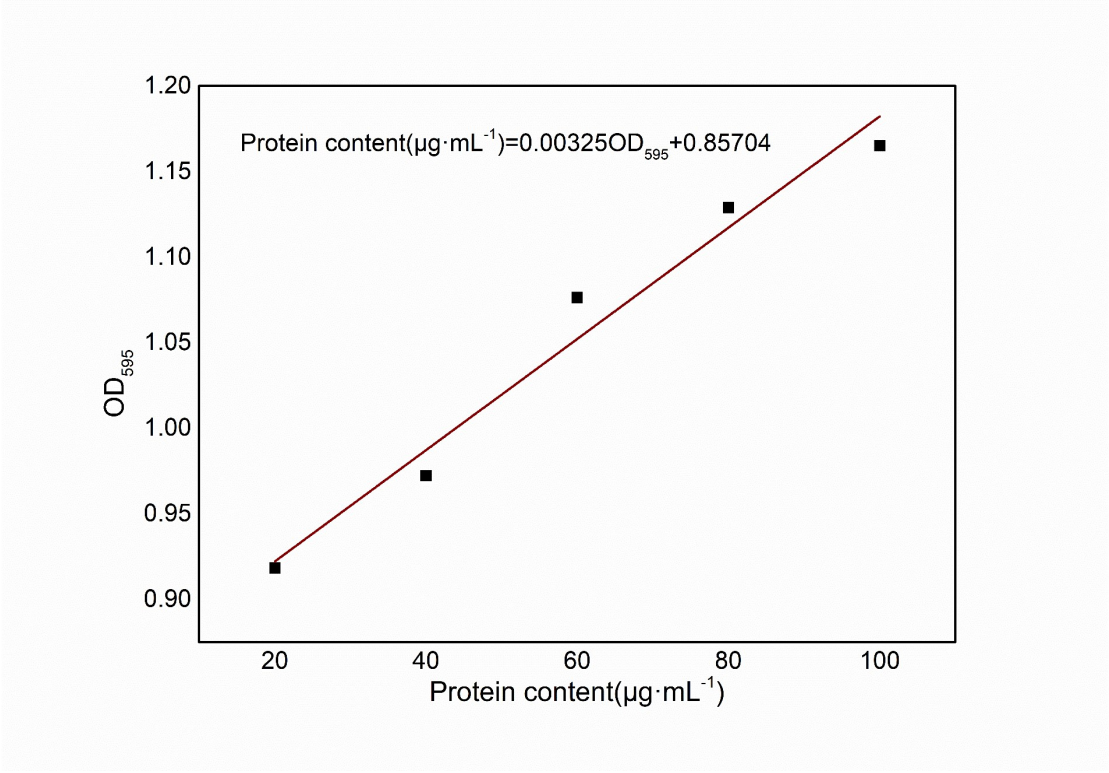

Figure S2 Protein standard curve.

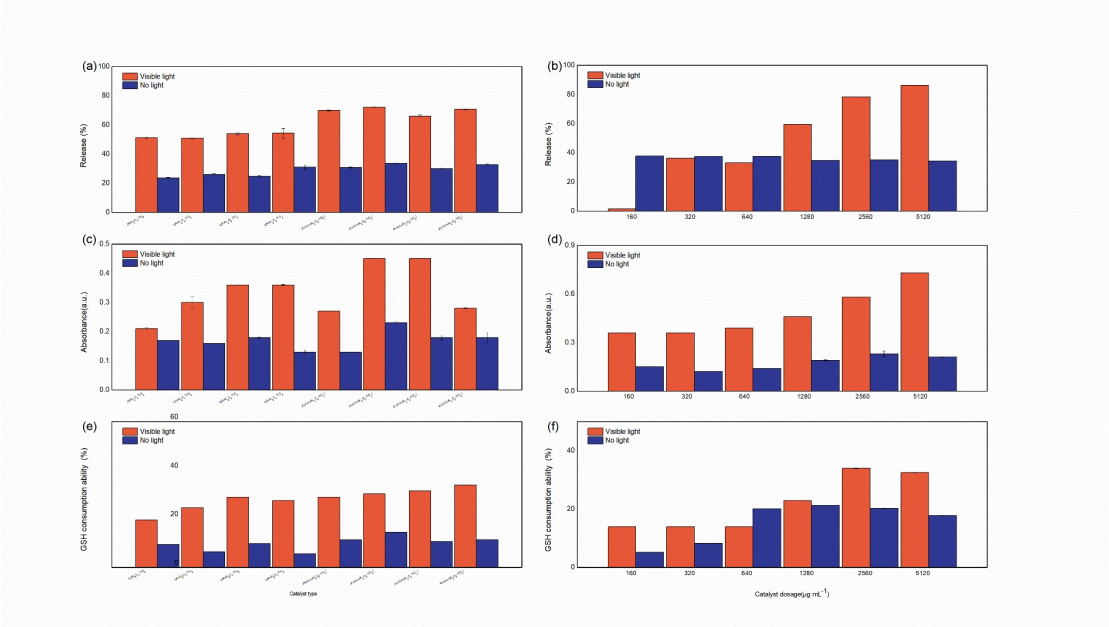

Figure S3 Enzyme-like properties of 4%rGO-(Bi_2O_3-TiO_2).

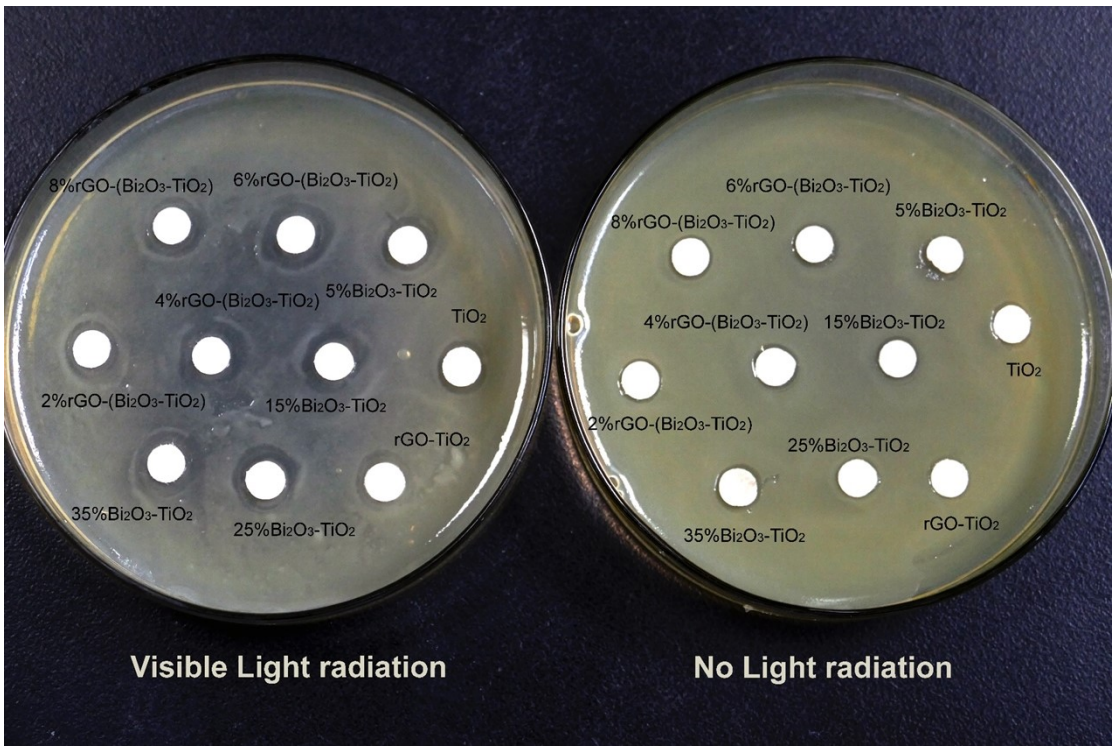

Figure S4 Inhibition zone of different nanomaterials.

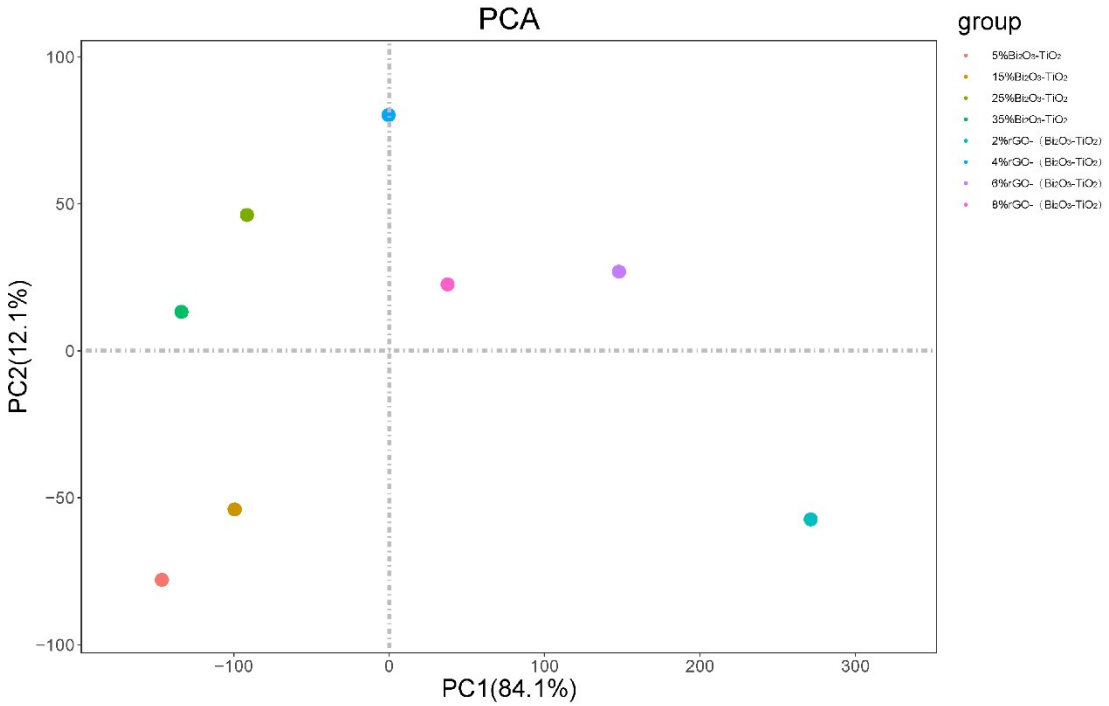

Figure S5 PCA analysis of FTIR data.

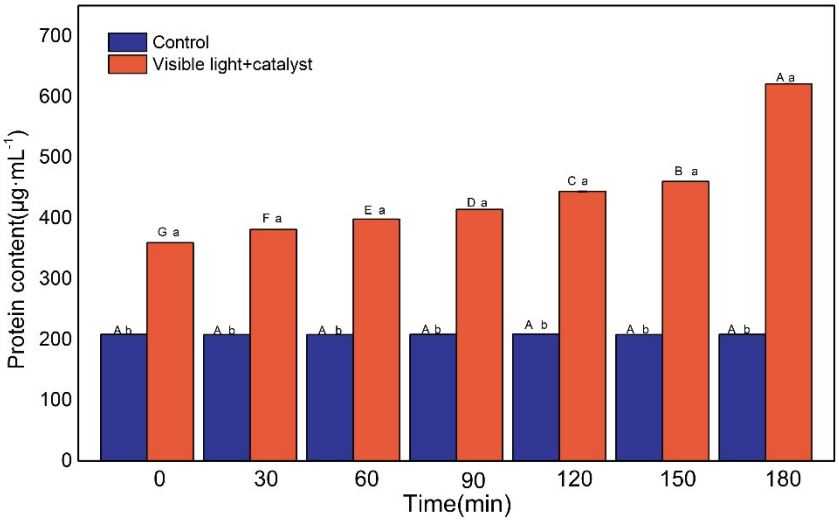

Figure S6 Changes in protein content.

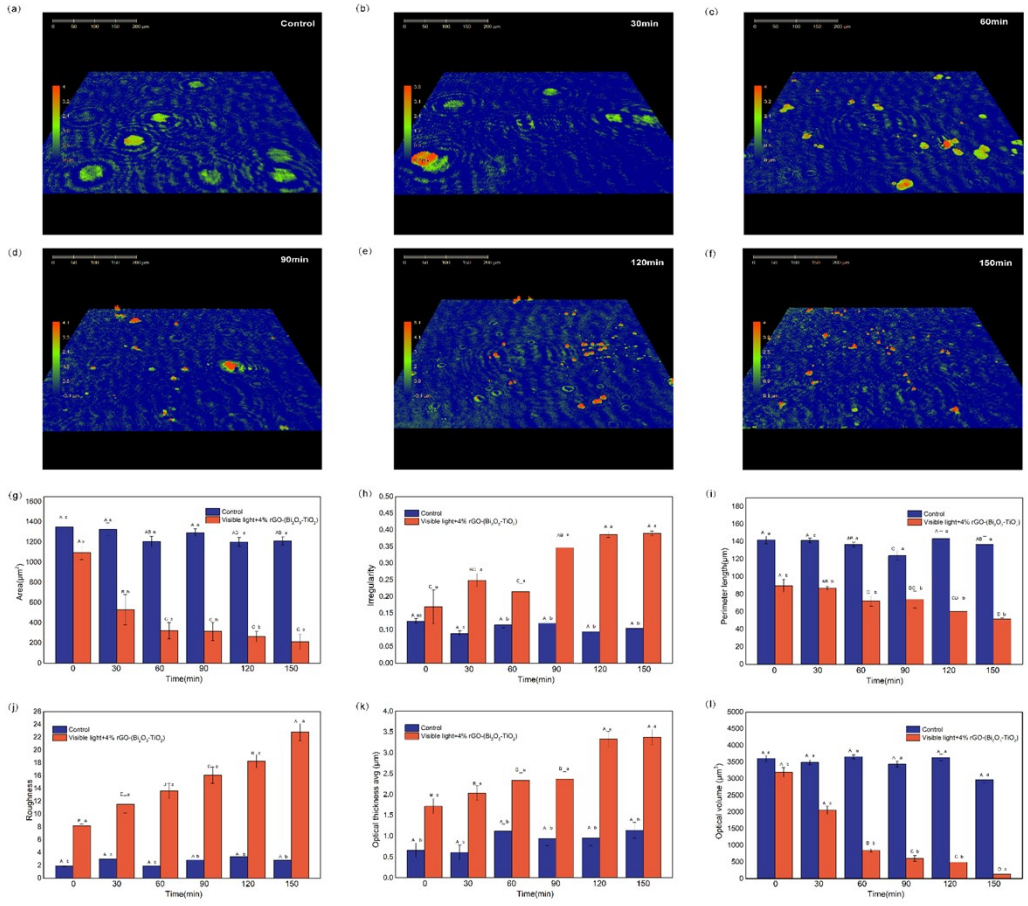

Figure S7 3D image of bacterial aggregation treated with 4%rGO-(Bi_2O_3-TiO_2) (a-f).

Changes in various optical characteristics of bacterial aggregation (g-l).