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Supporting Information

Synthesis of Enediyne Carbon-Allotrope Surface for Photo-Thermal Degradation of DNA

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Materials and Methods.

Materials.

1,2-Diiodobenzene, Phenylacetylene, Copper iodide, Tetrakis(triphenylphosphine)palladium(0), polystyrene-block-polyacrylic acid (PS-b-PAA, (2:1), Mn 8,700) and Whatman filter papers were purchased from Millipore Sigma, USA. Graphene nanoplatelets were purchased from Strem Chemicals (Newburyport, MA, USA). Polyvinylidenefluoride (PVDF) membrane filter (0.22 μm) and 10 mL syringe were obtained from Millipore Sigma (Temecula, CA, USA) and centrifuge tubes were procured from Eppendorf (Hauppauge, NY, USA). Gel electrophoresis was performed using pBR322 vector DNA (New England Biolabs, Ipswich, MA) and imaged under Universal Hood III, Bio-Rad, Hercules, CA. Mixtures were sonicated at required parameters on Q700, Qsonica Sonicators (Newtown, CT).

Synthesis and chemical characterization of enediyene.

To a flask containing $Pd(PPh_3)_4$ (0.525 g, 0.455 mmol) and CuI (0.139 g, 0.727 mmol) under a nitrogen atmosphere, a solution of phenylacetylene (2.40 mL, 2.23 g, 21.8 mmol) was added via cannula. 1,2-diiodobenzene (1.19 mL, 3.00 g, 9.09 mmol) in THF (45 mL) and diisopropylamine (20 mL, 14 g, 142 mmol) which had been deoxygenated by sparging with N_2 for 10 min was then added to the suspension. The mixture was stirred for 4 h at room temperature before being diluted with CH_2Cl_2. The organic phase was washed with H_2O, 5% aq. NH_4Cl (2×), and dried over anhydrous Na_2SO_4, filtered through a short pad of silica gel (using 1:1 CH_2Cl_2/hexanes as eluent), and the solvent was removed in vacuo.

Column chromatography (silica gel, gradient 0% to 15% CH_2Cl_2 in hexanes) afforded EDE-1 (2.37 g, 94%) as a yellow liquid. R_f = 0.7 (10 % CH_2Cl_2 in hexanes). IR ($CDCl_3$ cast film): 3057 (w), 2214 (w), 1597 (w), 1494 (m), 1471 (w), 1441 (m), 1090 (w), 1068 (w), 913 (w), 750 (s), 686 (m) cm–1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65–7.58 (m, 6H), 7.41–7.31 (m, 8H). ^{13}C NMR (100

MHz, $CDCl_3$): δ 131.7, 131.6, 128.4, 128.3, 128.0, 125.8, 123.2, 93.6, 88.3. DART HRMS m/z calcd. for $C_{22}H_{15}$ ($[M + H]^+$), 279.1168, found 279.1160.

DNA cleavage in solution phase.

In order to test the degradation of DNA by enediyene, gel electrophoresis was performed on DNA samples incubated with EDE-1 under different conditions. In order to run the gel, samples were prepared using 1.5 to 2.0 mg of enediyene into 4 centrifuge tubes. We then used the bath sonicator to break the larger pellets of enediyene into a finer powder. Two of these centrifuge tubes containing samples were left in the solid state and then labeled as enediyene 1 and 2. We then added 15 µL of isopropanol into the remaining samples in order to dissolve them properly. The centrifuge tubes were vortexed for 10-15 seconds in order to ensure the complete dissolution of the samples. They were labeled as enediyne 3 and 4. The vials labeled as enediyne 1 and 3 kept at 60 °C temperature for an hour and the vials labeled as 2 and 4 kept under UV exposure for an hour in order to activate the formation of the highly reactive benzene biradical having DNA cleaving properties. After the requisite exposure, a small amount of each enediyene was added into separate centrifuge tubes with 2 µL of 1X PBR322 vector DNA and 15 µL of water. After centrifuging the vials, the DNA and enediyne were further allowed to react for an hour. Then 3 µL of 6X DNA loading dye (Thermo Scientific) was added to it and centrifuged. The gel was prepared by boiling 600 mg of agarose (Sigma Science), 6 mL of 10X Tris-EDTA Buffer (Sigma Science), and 54 mL of autoclaved water. The liquid was then poured into a gel boat with a 10-well comb and allowed to cool for 30 minutes. When the gel was cooled, it was put into an electrophoresis system and submerged in TAE buffer. The samples were then mixed in each vial in order to improve the consistency of the loading dye. 20 µL of each sample was added into separate wells and gel electrophoresis was run at 120 volts for 25 minutes. Afterwards, the gel was kept for EtBr staining for 3 minutes and washed. The gel was

then imaged. Despite the slight smearing from this process, a large amount of DNA still remained intact.

In order to cleave the remaining DNA, we decided to increase the reaction time between enediyene and DNA to 3 hours, and allowed the enediyne to react with the DNA while it is being treated under UV light or heat. In order to perform this, we had to prepare nine different samples. First, we put 0.5 mg of enediyne into 6 centrifuge tubes. The tubes were then bath sonicated to break the larger pellets. 2 µL of DNA was then added into all the 6 centrifuge tubes with enediyene and 3 additional tubes were used as controls which were not treated with enediyene. Then, the tubes were labeled 1-9 with 1-3 being controls and 4-9 containing enediyne. 5 µL of isopropanol was added into the tubes 7-9 in order to dissolve the enediyene and DNA completely. 15 µL of water was also added into the tubes 3, 6, and 9 and sealed all of the tops with parafilm in order to prevent drying up the DNA by heating. The tubes 2, 5, and 8 were then placed under UV Light; 3, 6, and 9 under heat; and 1, 4, and 7 at room temperature for 3 hours. The gel was prepared by boiling 600 mg of agarose with 6 mL of TAE buffer and 54 mL of water and then pouring it in a gel boat with a 10-well comb. 15 µL of water was then added into each tube 1, 2, 4, 5, 7, and 8. 3 µL of loading dye was further added into all the 9 tubes and centrifuged. The cooled gel was then kept into an electrophoresis system and submerged in 1X TAE buffer. 20 µL of each sample was added into each well and then ran for 25 minutes at 120 volts. The gel was then kept for EtBr staining for 3 minutes followed by washing for another 3 minutes. The gels were then imaged.

All the experiments as shown in Figure 4 with pDNA were performed with 400 ng of pBR322 DNA (100 ng/µL) and exposed under 360 nm of UV irradiation with heating at 60 °C. For figure 4, Raman measurements were taken with 532 nm laser for 60 seconds at 0.2% laser power using a 20X objective with a grating of 600 mm^{-1}. For each sample, 20 spectra were recorded and averaged.

Preparation of EDE-1 coated carbon allotrope surface.

In order to prepare graphene coated surface, a solution was prepared by adding 40 mg of graphene platelets (GRPT) and 4 mg of polystyrene-block-polyacrylic acid (PS-b-PAA) to 10 mL of autoclaved water. Mixture was then sonicated at an amplitude of 1 and duration of 30 minutes. The process of sonication was repeated to make sure the graphene platelets were evenly suspended in the water. Prepared suspension was coated on cellulose surface as model system using 125 mm Whatman filter papers. Papers were pre-incubated with water in plastic dish to hydrate it. Cellulose surface was post incubated with 10 mL of the graphene and PS-b-PAA mixture containing 20 mg of GRPT and 2 mg of PS-b-PAA. Sample were prepared in triplicates. Samples were further incubated at room temperature under controlled aeration to completely dry the surface. Finally, samples were put under heat (37 ºC) and vacuum to make sure the removal of any traces of solvent. GRPT coated cellulose surfaces were further coated with EDE-1 solubilized in THF and spin coated to a thickness of ~100 nm. These samples were used for DNA degradation studies.

Cleavage of DNA on EDE-1 coated carbon allotrope surface.

As we were able to determine that enediyne can cleave DNA, it was necessary to test the cleaving properties of enediyne[1] when coated on the prepared carbon paper as indicated above. First, two 1 x 1 cm^2 of papers were cut from the enhanced sheet of carbon filter paper. Then, 0.6 mg of enediyne was measured and mixed with 6 µL of THF. 5 µL of this solution was added onto the papers and kept for 30 minutes for the evaporation of THF. 16 µL of DNA was then mixed with 16 µL of water in order to have a 0.5X solution of DNA. 16 µL of this DNA solution was then added onto each paper. The papers were kept into the vacuum overnight to evaporate the water out. Thereafter the paper 1 was kept for three hours at room temperature and paper 2 was kept under UV light exposure for three hours. After the exposure duration was

complete, the carbon papers were put into the centrifuge tubes and 200 µL of water was added to each of the tubes. Each of the tubes was then vortexed for 10 times for 1-minute each time with a 2-minute break in between every repetition. After vortexing, the carbon filter papers were placed into separate centrifuge tubes for future use. The papers were dipped into liquid nitrogen for one to two seconds multiple times in order to freeze them. Once the DNA was frozen, they were kept into a freeze dry system for overnight to remove all of the water. Three other samples were also prepared to ensure that graphene and PS-b-PAA were not causing any DNA cleavage and one sample was prepared with DNA alone for reference. To prepare these additional three samples, 1.27 mg of graphene was added into the vials with 2 µL of DNA. 0.127 mg of Ps-b-PAA polymer was then added into a vial with 2 µL of DNA. The control was also prepared by putting 2 µL of DNA into a vial. 15 µL of water was added into all the three vials and centrifuged them. After removing the papers from the freeze dry system, 17 µL of water was added into each of the tubes. 3 µL of loading dye was added and centrifuged to bring everything at the bottom of the tubes. The gel was then prepared by measuring out 600 mg of agarose powder and putting it into a conical flask. 6 mL of 10X TAE and 54 mL of water was also added to it. The gel was boiled in the microwave for 1 minute and 30 seconds and then poured it into a boat with a 10 well comb. After letting the gel cool, it was kept under the electrophoresis system and submerged in 1X TAE buffer. 20 µL of each DNA solution was added into separate wells and ran the gel for 25 minutes at 120 volts. After putting the gel in EtBr stain and washing for 3 minutes each, the gel was imaged.

Safety study for microorganisms of waterbodies.

To evaluate the general safety of the developed system for DNA degradation, an experimental study was designed using bacterial samples. Bacillus subtilis bacteria were grown as per literature protocol till culture gained O.D. of ~0.5. Bacterial cell suspension was incubated with cellulose-graphene surface with or without EDE-1 coating and were compared with same

samples exposed to UV treatment condition as optimized above for DNA degradation. Bacterial samples were exposed under UV for 1 hour before collecting their DNA by using a DNA extraction kit. Collected DNA was run on 2% agarose gel for 1 h at 120 V along with 1 kb DNA ladder.

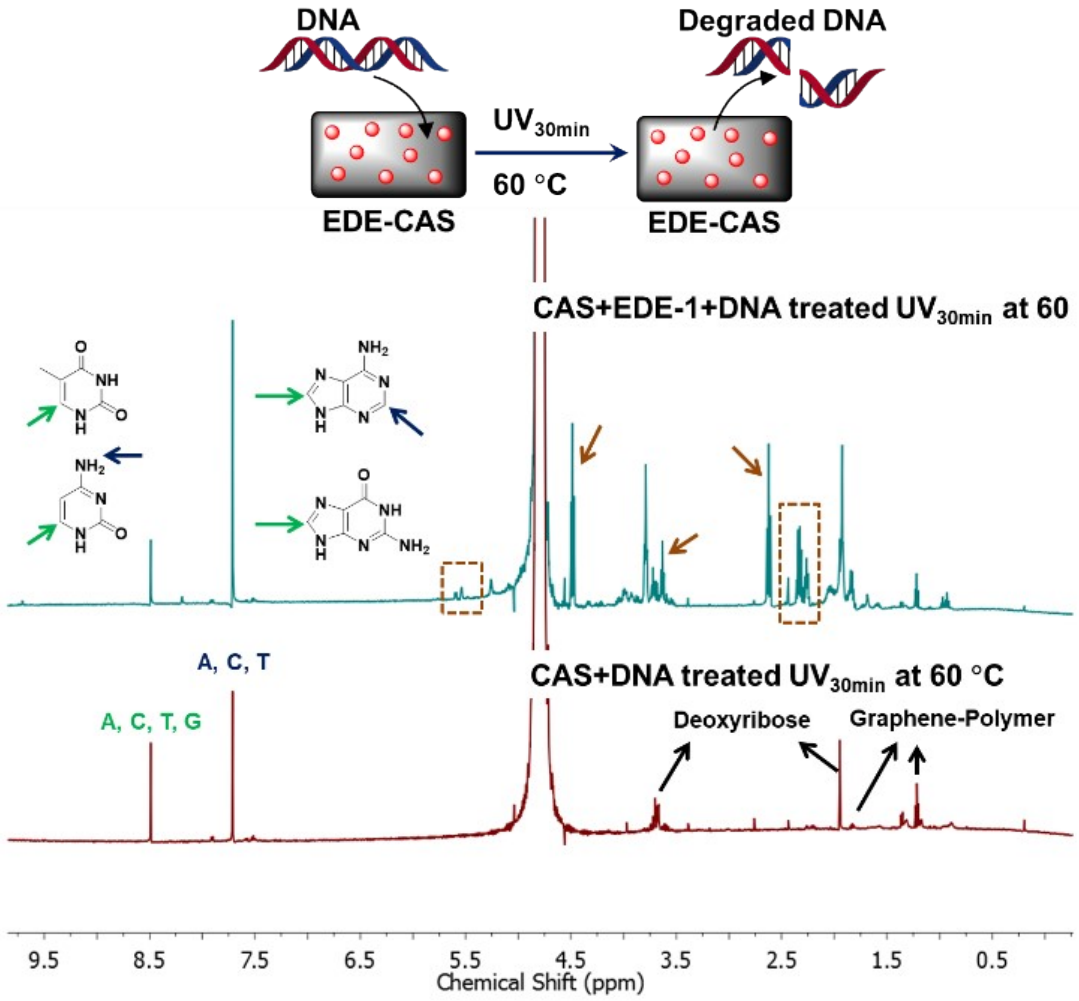


Figure S1. Proof of degradation by NMR: ^{1}H-NMR studies on CAS+DNA (lower) and EDE-CAS+DNA (upper) samples treated by 360 nm of UV irradiation for 30 min at a temperature of 60 °C. Extra peaks generated by EDE-1 activation (upper spectra) indicates the role of EDE-1 in DNA degradation. 400 ng of pBR322 DNA (100 ng/µL) was used for NMR study.

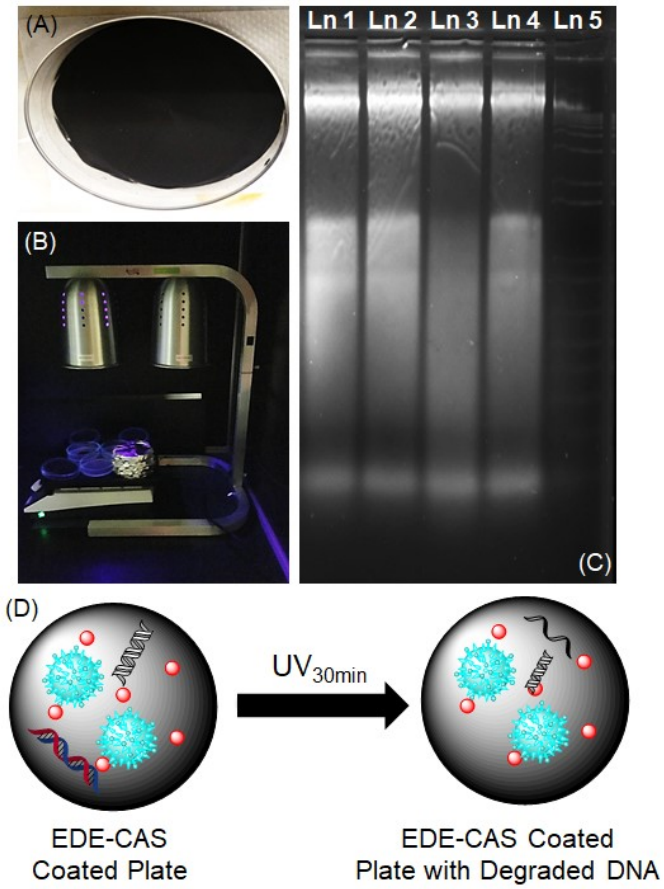


Figure S2. Proof of environmentally safe technology: (A) Surface coated petri-dishes for growing bacteria; (B) UV exposure set up for the treatment of bacterial cells incubated with EDE-CAS under continuous rotation of 120 rpm for 1h; (C) DNA extracted from bacteria samples incubated with EDE-CAS surface without (Lane 3) or with UV exposure (Lane 4) in comparison to bacteria cells growing without any interference (Lane 1) or under UV exposure (Lane 2). DNA samples were run on agarose gel (2%). Lane 5 represents DNA ladder of 1 kb. The uncropped version of Figure S2C has been shown in appendix. A schematic representing the experimental technique is shown in (D).

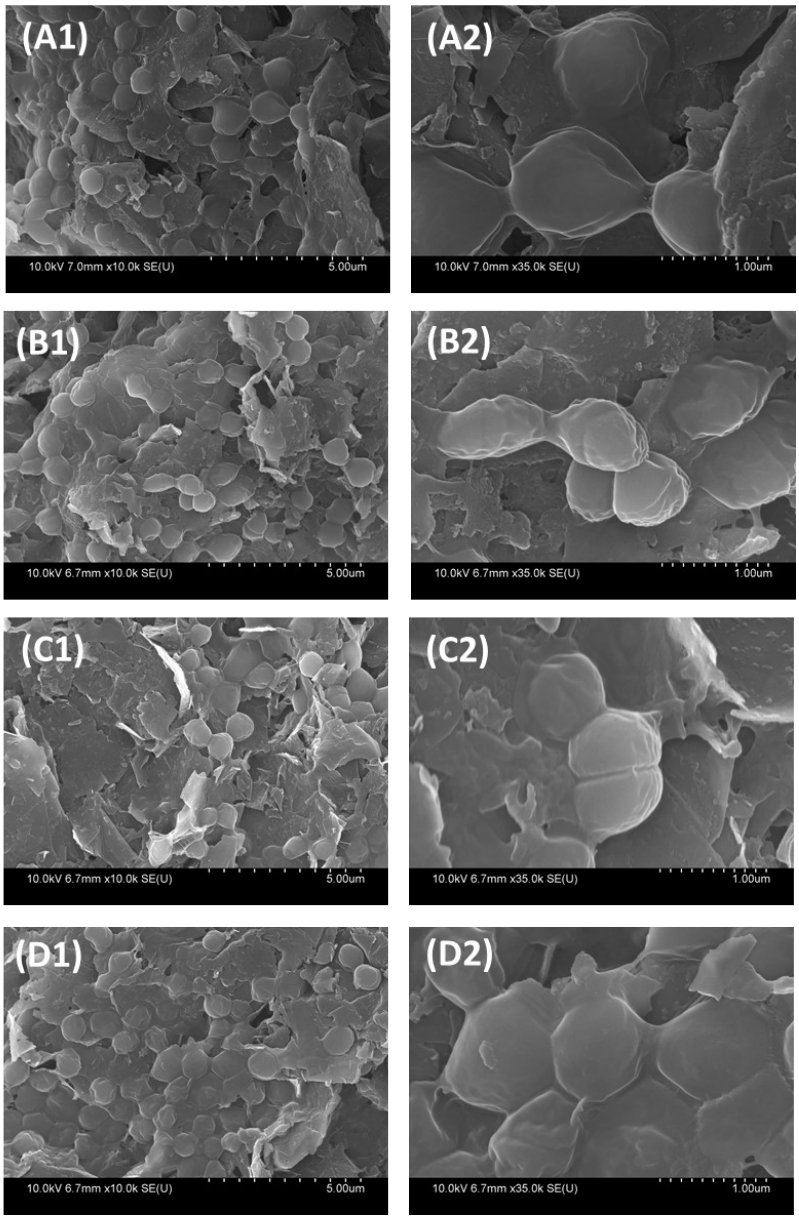
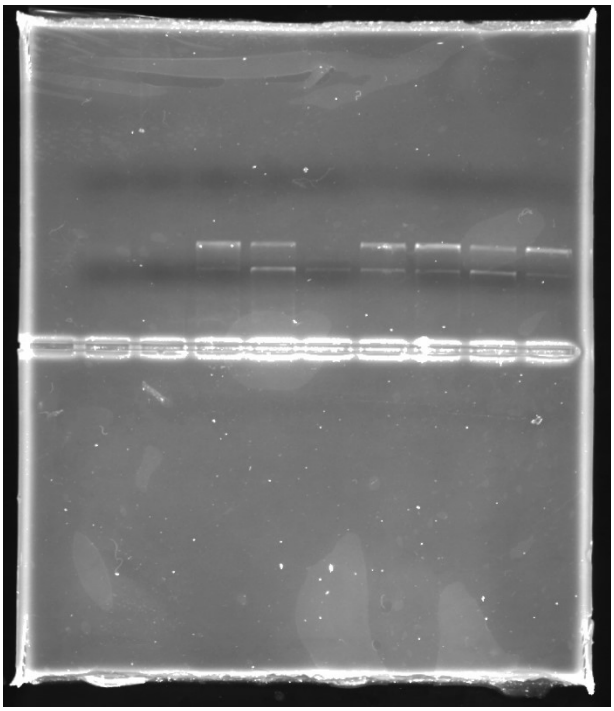


Figure S3. SEM images of bacterial populations imaged after different types of incubation on EDE-CAS with or without exposure to UV. A1-D1 and A2-D2 represent SEM images at low and high magnification, respectively for bacterial cells on CAS (A1, A2) without and (B1, B2) with UV exposure while (C1, C2) represents cell population incubated with EDE-CAS before and (D1, D2) after UV exposure. Samples were treated with 30 min of UV irradiation before fixing with 4% paraformaldehyde.

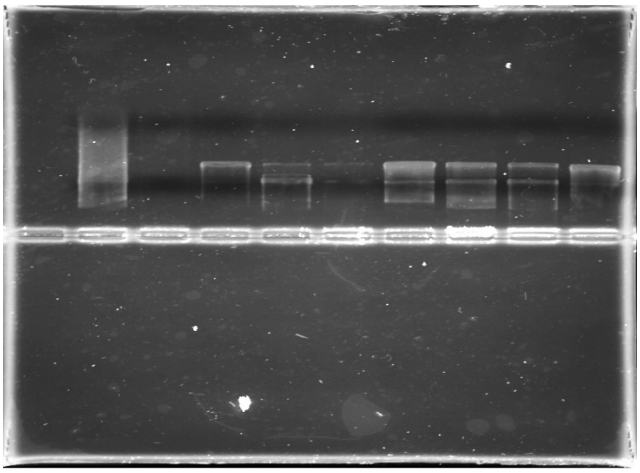
Reference.

1. M. Kar and A. Basak, *Chem. Rev.* 2007, **107**, 2861-2890.

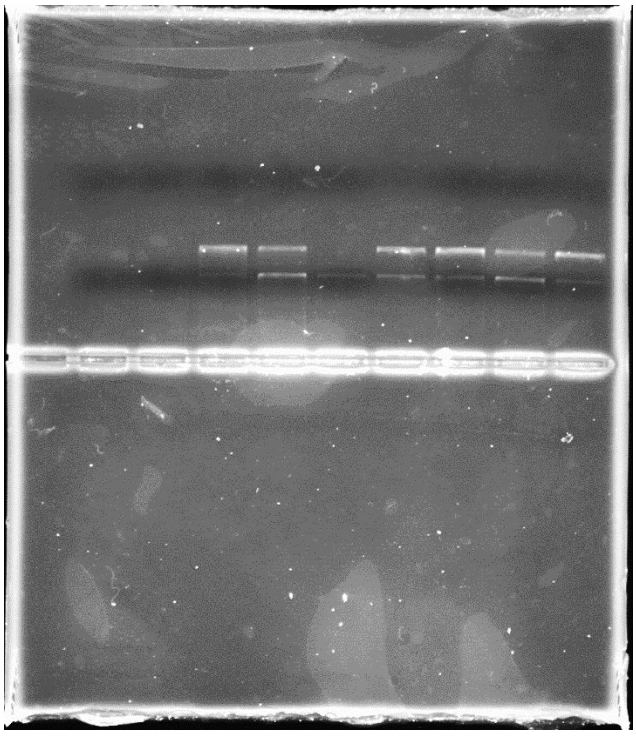
Appendix 1



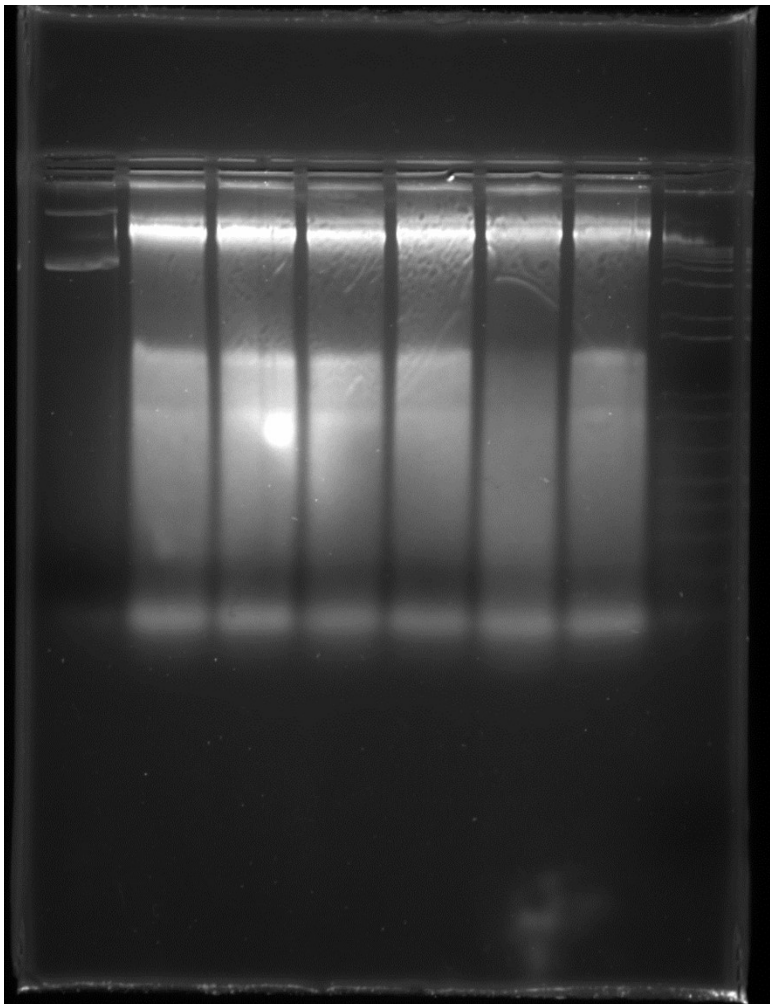
Uncropped version of Fig. 3 (A) EDE-1 samples were mixed with aqueous solution of pDNA in presence or absence of isopropanol.



Uncropped version of Fig. 3 (B) EDE-1 samples were mixed with aqueous solution of pDNA in presence or absence of isopropanol and poly-graphene.



Uncropped version of Fig. 3 (C) EDE-1 samples coated on carbon allotropic surface and incubated with aqueous solution of pDNA in presence or absence of isopropanol.



Uncropped version of Fig. S2C DNA extracted from bacteria samples incubated with EDE-CAS surface without (Lane 3) or with UV exposure (Lane 4) in comparison to bacteria cells growing without any interference (Lane 1) or under UV exposure (Lane 2). DNA samples were run on agarose gel (2%). Lane 5 represents DNA ladder of 1 kb. The last five columns have been shown in the figure.