Self-assembled Photosensitive Carbon Nanocrystals with Broad-Spectrum antibacterial bioactivity

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1. Experimental section

1.1 Library Construction and sequencing

Firstly, the rRNA was depleted from 1 microgram of total RNA using illumina MRZB12424 Ribo-Zero rRNA Removal Kit (Bacteria) (Illumina, San Diego, CA, USA). Then, the first-strand cDNA was synthesized using ProtoScript II Reverse Transcriptase (New England BioLabs, Ipswich, MA, USA) at 25 °C for 10 min; 42 °C for 15 min; 70 °C for 15 min. The second-strand cDNA was synthesized using NEBNext Second Strand Synthesis Reaction Buffer and dATP, dGTP, dCTP, dUTP mix (New England BioLabs, Ipswich, MA, USA) at 16 °C for 1 h. Resulted cDNA was purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) and end repaired with NEBNext End Repair Reaction Buffer and Enzyme Mix (New England BioLabs, Ipswich, MA, USA) at 20 °C for 30 min; 65 °C for 30 min. Sequencing adapters were ligated using NEBNext Adaptor for Illumina (New England BioLabs, Ipswich, MA, USA) at 20 °C for 15 min. The second-strand cDNA was then degraded using the USER enzyme mix (New England BioLabs, Ipswich, MA, USA) at 37 °C for 15 min and the product was purified by Agencourt AMPure XP beads (Beckman Coulter, Brea, CA). Finally, the clustering of the index-coded samples was

performed on a cBot Cluster Generation System using NEB Next Q5 Hot Start HiFi PCR Master Mix(New England Biolabs, Ipswich, MA, USA). After cluster generation, sequencing was performed using the Illumina Novaseq 6000 platform with pair-end 150 base reads.

1.2 Bioinformatic analysis

Clean reads filtering

Raw data were filtered by the folowing standards, 1) removing reads with ≥ 10 % unidentified nucleotides (N); 2) removing reads with > 50 % bases having phred quality scores of ≤ 20; 3) removing reads aligned to the barcode adapter using FASTP (version 0.18.0).[1] Quality trimmed reads were mapped to the refference genome using Bowtie2[2] (version 2.2.8) allowing no mismatches, reads mapped to ribosome RNA were removed. Retainted reads were aligned with the the refference genome using Bowtie2[2] (version 2.2.8) to identify known genes and calculated gene expression by RSEM[3].

Analysis of replicates correlation and principle component

To evaluate reproducibility between samples, the correlation coefficient among replicas was calculated. Values closer to one indicated better reproducibility. Principle component analysis (PCA) was performed with the R package gmodels (http://www.r-project.org) to reveal the relationship between samples.

Analysis of differentially expressed genes

The gene expression level was further normalized by using the fragments per kilobase of transcript per million (FPKM) mapped reads method to eliminate the influence of different gene lengths and amount of sequencing data on the calculation of gene expression. The edgeR package (http://www.r-project.org/) was used to identify differentially expressed genes (DEGs) across samples with fold changes ≥ 2 and a false discovery rate-adjusted P (q value) < 0.05. DEGs were then subjected to an enrichment analysis of GO function and KEGG pathways, and q values < 0.05 were using as threshold.

sRNA analysis

The prediction of sRNA was conducted using Rockhopper[4] (version 2.0.3) with the removal of sequences less than 50 bp. Candidate sRNAs were then screened against the sRNAMap database[5] (version 2009) and Rfam database[6] (version13) for annotation. Secondary structures of sRNA were

predicted by Vienna RNA packages[7] (version 2.3.5). The sRNA expression level was normalized by using TPM values (transcripts per million). The edgeR package (http://www.r-project.org/) was used to identify differentially expressed sRNAs across samples with fold changes ≥ 2 and a false discovery rate-adjusted P (q value) < 0.05.

Transcript structure analysis

New Transcripts were predicted using Rockhopper (version 2.0.3).[4] Cis-natural antisense transcripts (cis-NATs) are RNAs transcribed from the antisense strand of a gene locus, and are complementary to the RNA transcribed from the sense strand. According to the features, cis-NATs with overlap length to the known genes more than 30 bp were identified. Candidate cis-NATs were further verified using RNAplex[8] (version 2.0.3) and cis-NATs with minimum free energy more than -30 kcal/mol were removed. Transcription start site (TSS), transcription termination site (TTS) and operons were revealed using Rockhopper.[4] After the identification of Untranslated Regions (UTR), Shine-Dalgarno sequence was predicted using RBS finder[9] with the parameter of rbs region length of 20 bp. ρ-independent terminator was identified using Trans TermHP[10] (version 2.09).

Video 1. The conversion of color and fluorescence of nanocrystals in water, ethanol, methanol, DMF, THF, DMF, and FA solution with the same concentration under irradiation of UV-light (365 nm, 10W). Video have processed at 19× speed.

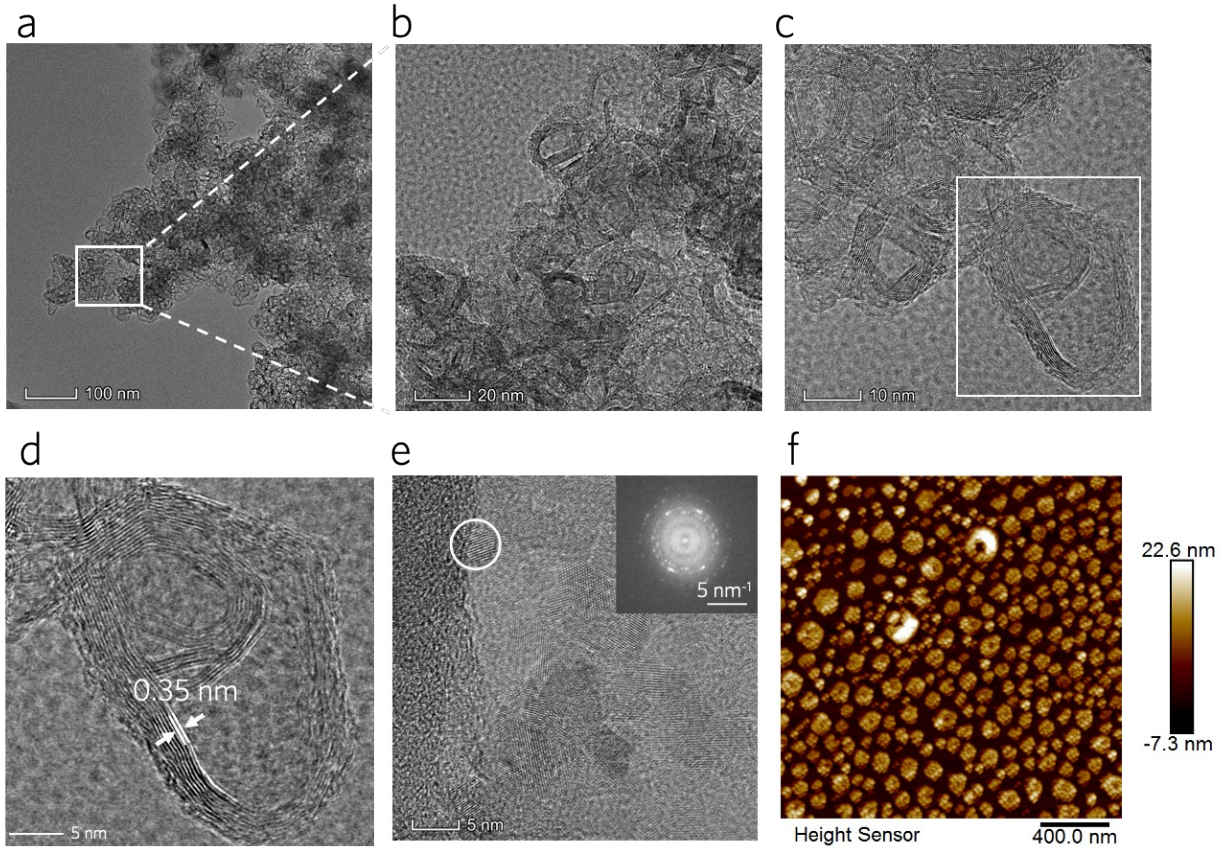

Fig. S1 HRTEM images of nanocrystal in ethanol solution. (a) Low-magnification images of nanocrystal. (b) Higher magnification images of selected areas marked with white rectangle in (a). (c) Higher magnification images of spiral nanorings. (d) Partially magnified image marked with white rectangle in (c). (e) Higher magnification images of carbon dots. (f) AFM images of nanorings.

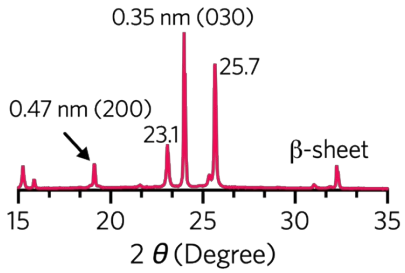

Fig. S2 XRD of nanocrystals.

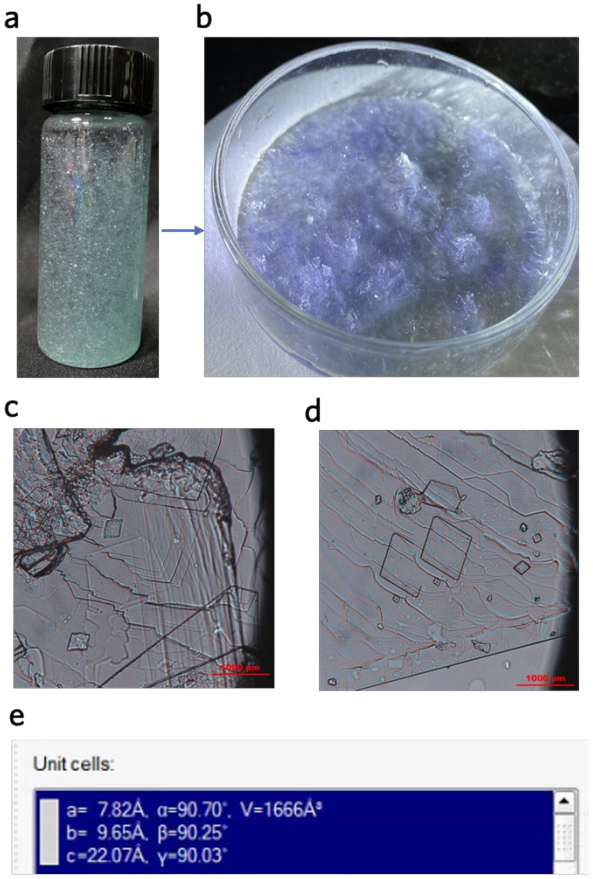

Fig. S3 (a) and (b) Images of nanocrystals after mixing the nanocrystals with absolute ethanol at for 3 min and one week, respectively. (c) and (d) Nanocrystals under a microscope. (e) Unit cell parameters of nanocrystals.

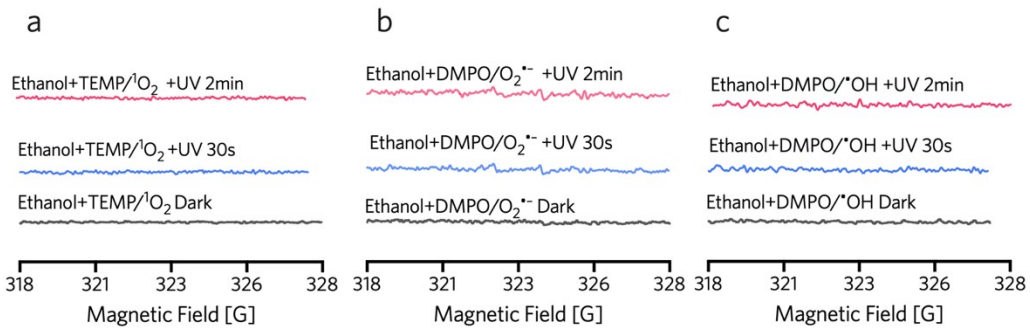

Fig. S4 (a)-(c) ESR spectra of deionized water after irradiation by UV light for different time. (a) singlet oxygen ($^1O_2$). (b) superoxide radicals ($O_2^{\bullet-}$). (c) Hydroxyl radical ($^{\bullet}OH$).

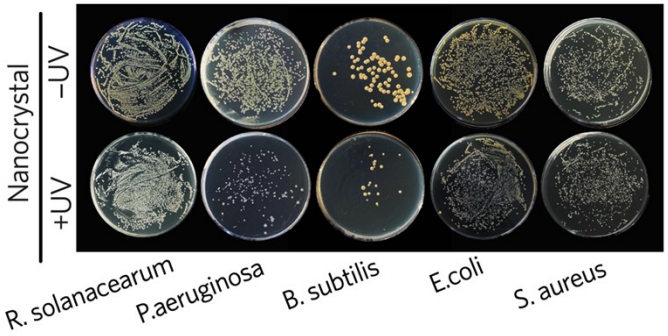

Fig. S5 Inhibition effective of nanocrystals for five common bacteria.

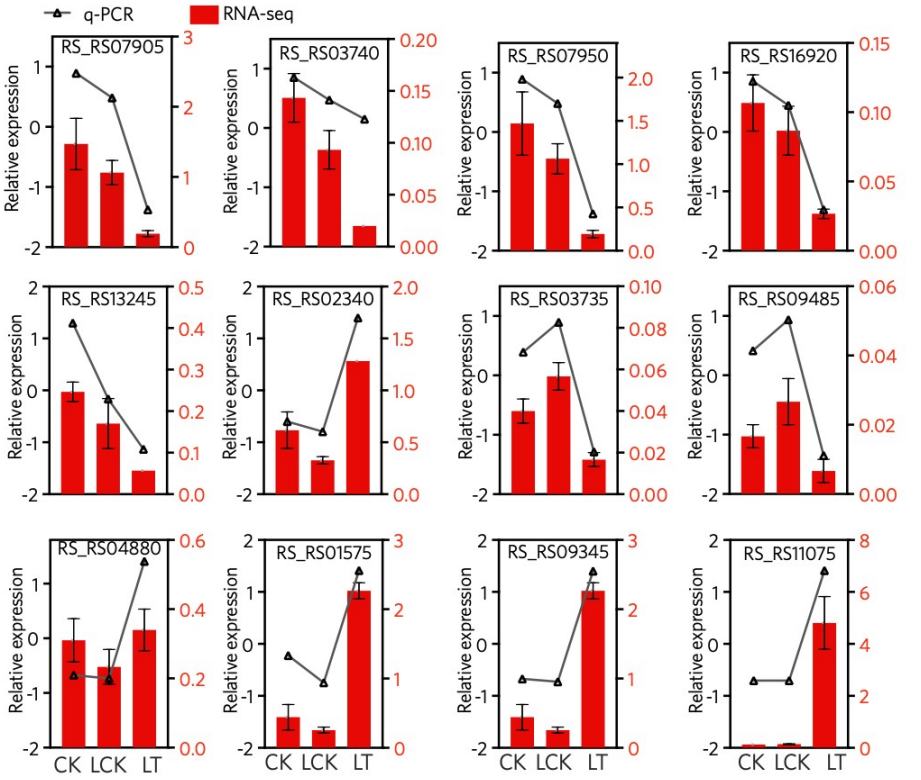

Fig. S6 The relative expression of DEGs in transcriptome and qPCR

Tab.1 A Primer sequences for qRT-PCR

Primer name	Primer sequence（5’~3’）
GAPDH-F	CGCCATCGCCAACTTCAA
GAPDH-R	GTTGACGACCAGCGTATTGC
RS_RS07905-F	GCATCCCGGCATTCTTCAC
RS_RS07905-R	ACACATCGGGTTGAAGTTGC
RS_RS09485-F	TACTACAACATCGCCTCCGC
RS_RS09485-R	CGCATTGGTCAGCACGAAG
RS_RS02340-F	AGCAGGCGAAGGAAGAAGG
RS_RS02340-R	GTTTGTTGAGCGCCACCAT
RS_RS04880-F	TGATGAGCCTGGTGAACTCC
RS_RS04880-F	ATCAGTTCGATCTCCACGCA
RS_RS01575-F	ACCGCTTTCTCCCGTCATAC
RS_RS01575-R	GTAGACATCGAACTTGGCCG
RS_RS11075-F	AAGAAATTCGCCGATGCCTC
RS_RS11075-R	CGCCCATGTAGTTGATGTCG
RS_RS13245-F	CTGCGCGACGATGAGATGGATC
RS_RS13245-R	TCCAGGCAGATCGTCAGGTAGAC
RS_RS03740-F	CCAGTTCATTGAGACCGAGCTGATC
RS_RS03740-R	GATTGACCTGCGGACCGTTGAG
RS_RS03735-F	GATCATCCGTGGGCAGCTCAAG
RS_RS03735-R	TTGGGCATGTTCCAGTCCGAAATC
RS_RS09345-F	GTGCTGGTCAACCTGTTCCTTCTG
RS_RS09345-R	ATGAAGTTCGCGTCGTCTTGCC
RS_RS16920-F	GATCATGGCGACAACGAGTACGG
RS_RS16920-R	GATGCCAGCAGATCAGCACCAG

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