Supplementary information

Gold nanoclusters augment a fluoroquinolone lethality against biofilm associated persister cells

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Keywords: Gold nanocluster, biofilms, persister cells, fluoroquinolones, Pseudomonas aeruginosa

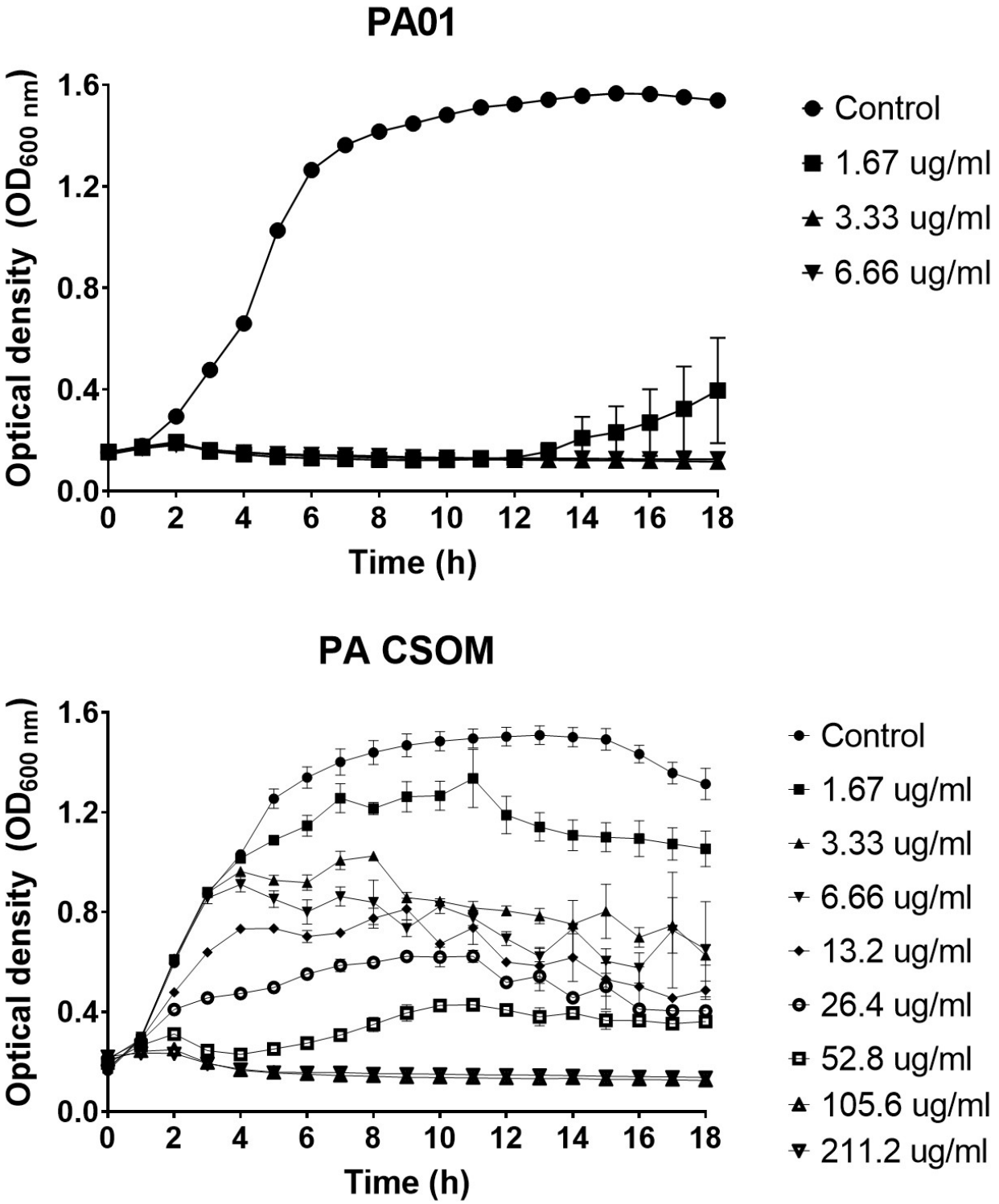


Fig. S1 Growth curves of *P. aeruginosa* strains (PA01 and PA CSOM) in the presence of varying concentrations of ofloxacin. Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth (turbidity without a pellet) of a microorganism after overnight incubation (15 h). MIC values of ofloxacin against PA01 and PA CSOM planktonic cells were 1.67 and 52.8 μg/mL, respectively.

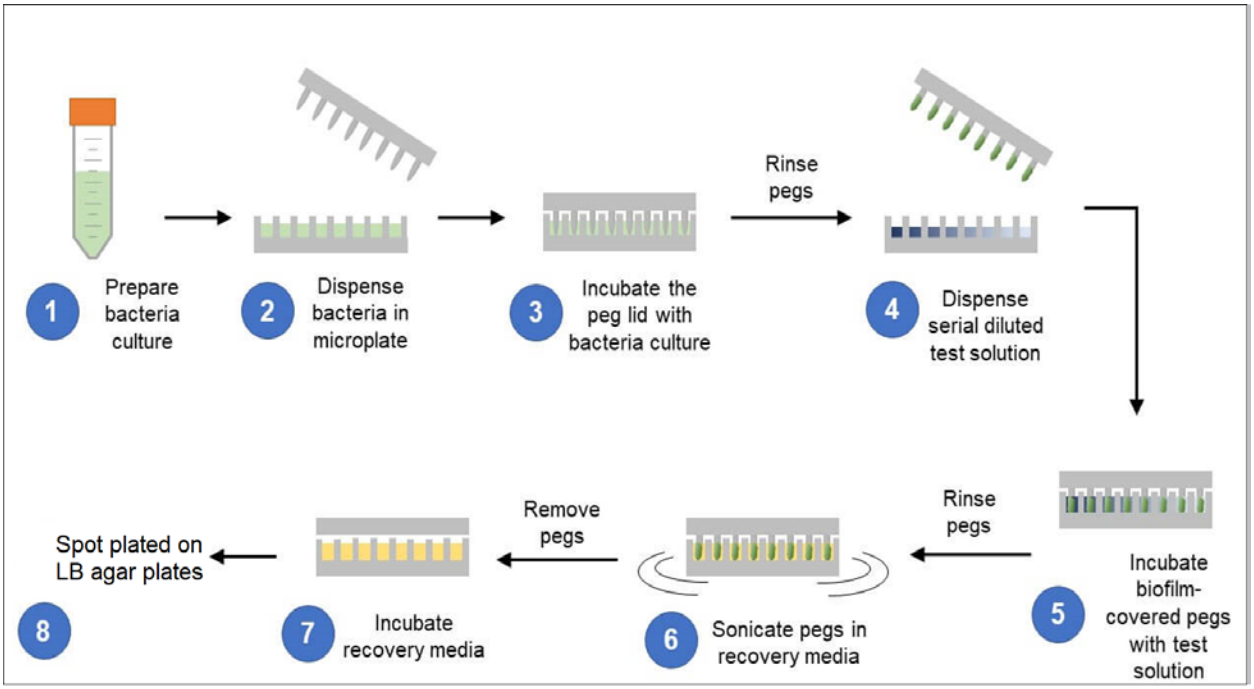


Fig. S2 Cartoon depicting the flowchart to evaluate the eradication capacities of ofloxacin, AuNC@CPP and their combination towards preformed biofilm using the Calgary biofilm device (The scheme was adapted from Emery Pharma, https://emerypharma.com/biology/biofilm-eradication/). (1-2) Bacteria culture is prepared and dispensed into a 96-well microplate. (3) The peg lid is placed in the bacteria culture and incubated to generate the biofilm. (4) The peg lid is gently rinsed to removed planktonic bacteria and a serial diluted test solution is dispensed into a new 96-well microplate. (5) The pegs covered in biofilm are incubated in the test solutions. (6) The peg lid is again gently rinsed to remove planktonic bacteria and placed in a new 96-well microplate containing recovery media. The peg lid is then sonicated to dislodge the biofilm into the recovery media. (7) Following sonication, the peg lid is replaced with a regular 96-well microplate lid and the plate containing recovery media is incubated. Following incubation, the OD_{650} absorbance is read on a spectrophotometer. Wells with an OD_{650} of less than 0.1 is evidence of biofilm eradication. (8) Spot plated on LB agar plates is used to confirm biofilm eradication.

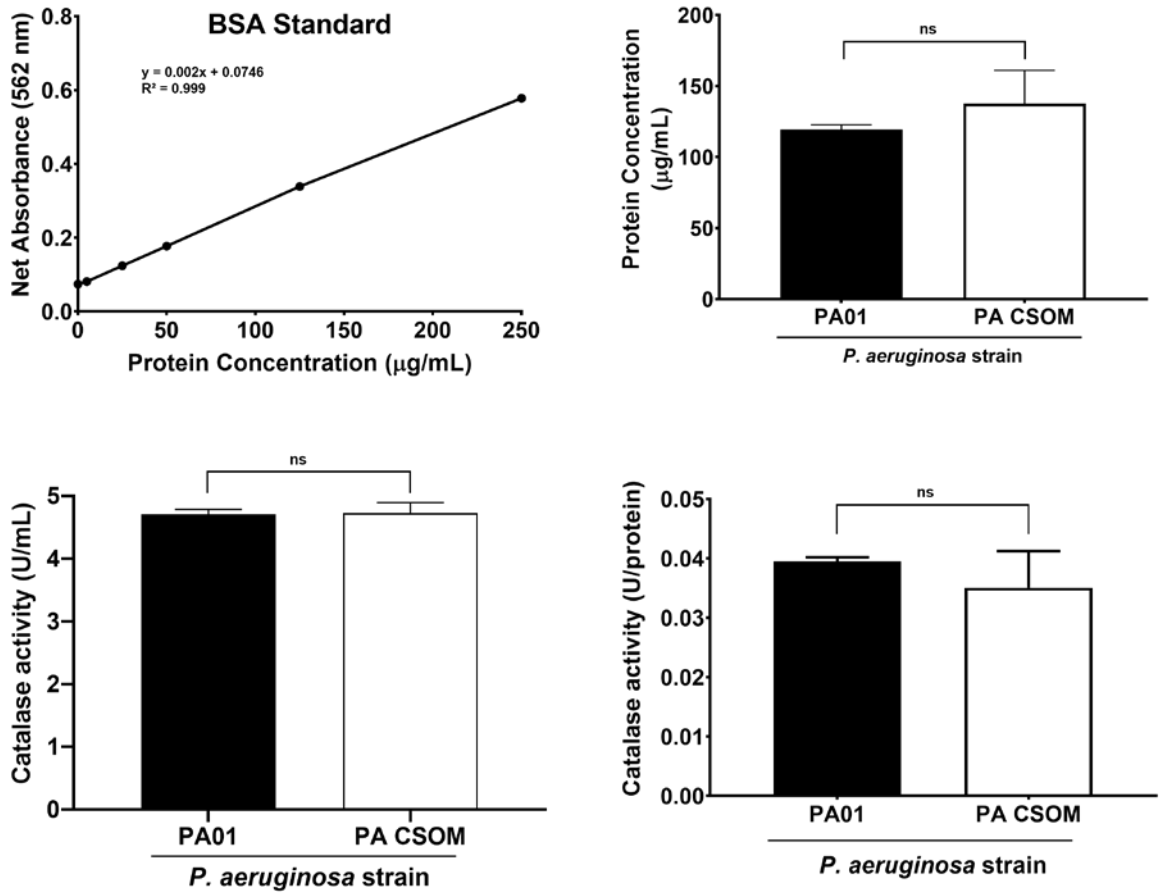


Fig. S3 Comparison of catalase activity in planktonic cells of *P. aeruginosa*. Both strains PA01 and PA CSOM exhibits similar catalase activity.

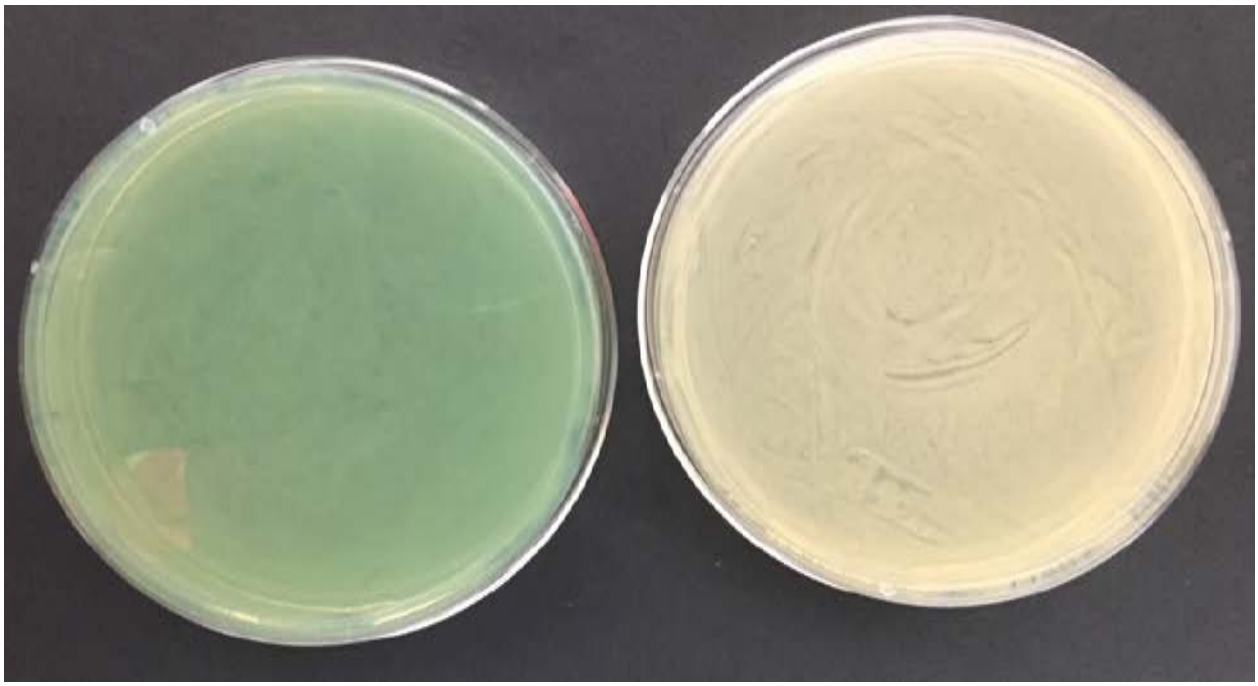


Fig. S4 3% H_2O_2 fails to eradicate planktonic stationary phase culture of *P. aeruginosa*. Agar plate showing bacterial growth 24 h after the H_2O_2 treatment of PA01 (left) and PA CSOM (right). The green collar indicates that *P. aeruginosa* PA01 produces more pyocyanin than the otopathogenic strain (PA CSOM).

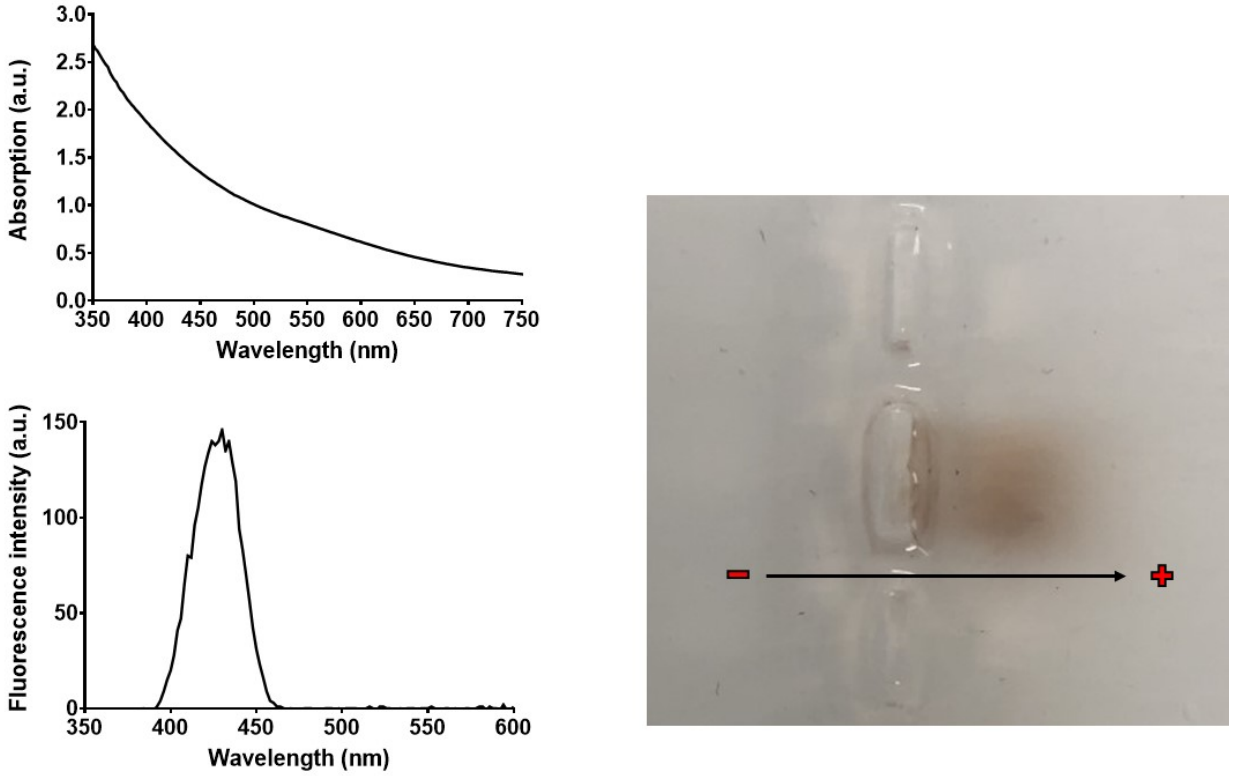


Fig. S5 UV-vis absorption and fluorescence emission spectra (with excitation wavelength of 365 nm) of AuNC@CPP. Agarose gel electrophoresis showing that AuNC@CPP is negatively charged.

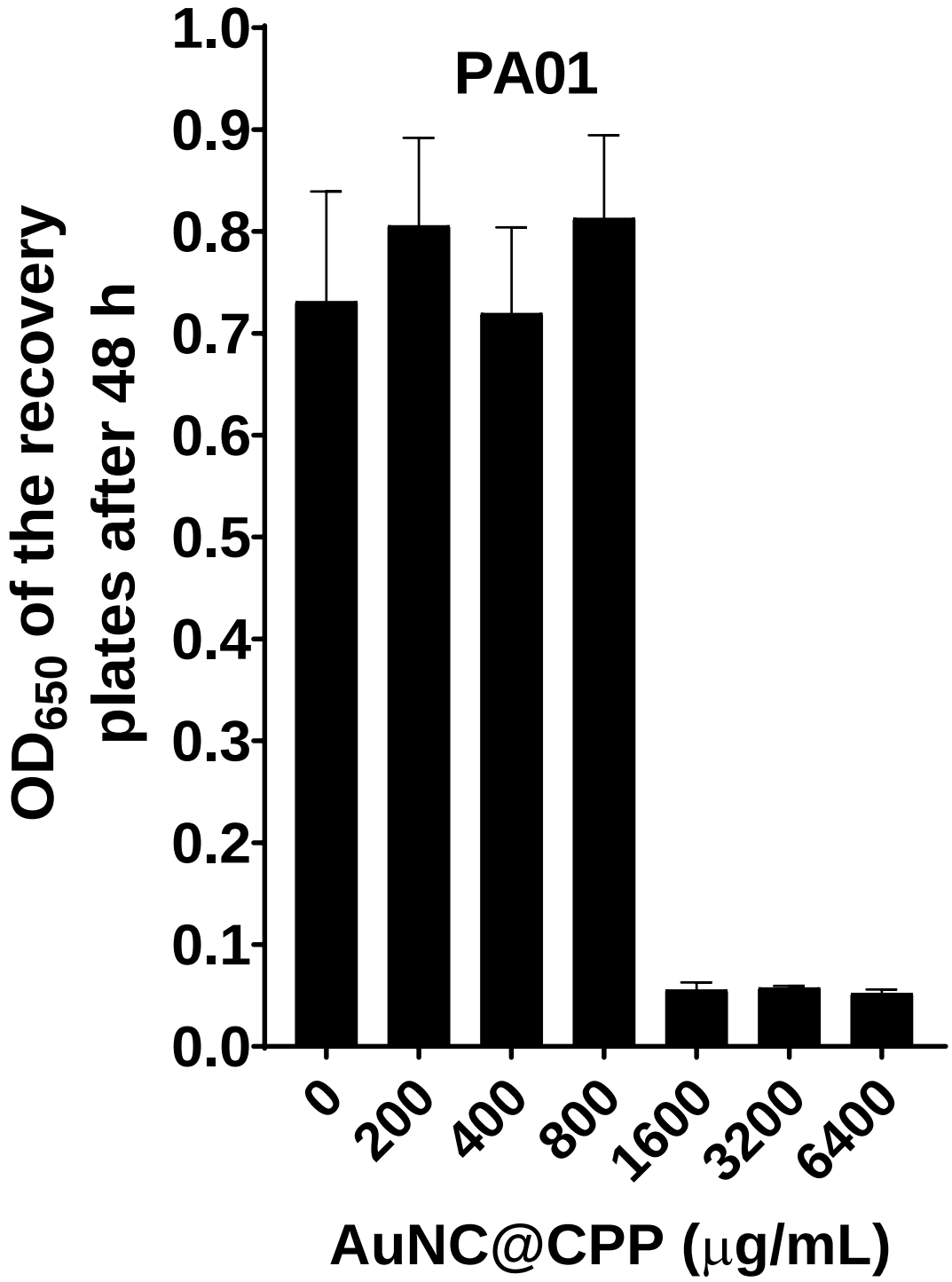
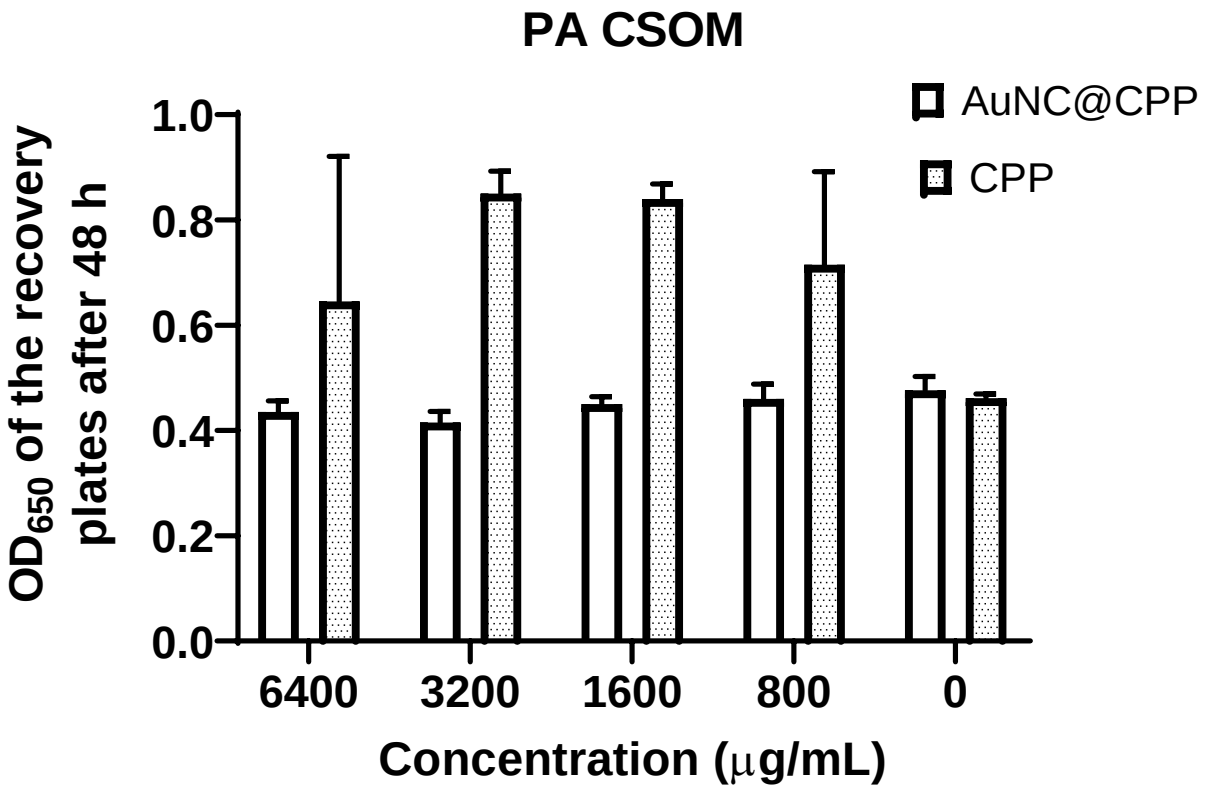


Fig. S6 Eradication of PA01 biofilm by AuNC@CPP alone. Following the treatment, the optical density (OD) from recovery plates after 48h incubation was measured at 650 nm (OD_{650}) using a spectrophotometer. Data are presented as mean ± s.d. (n= 3).



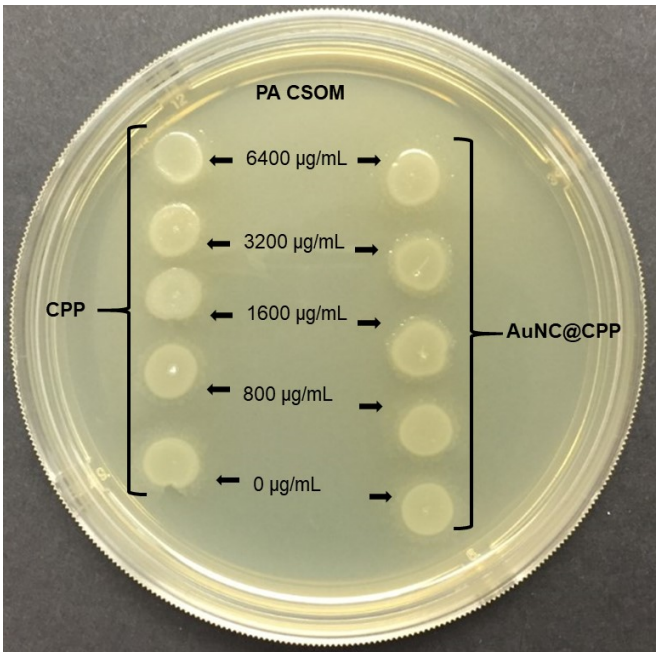


Fig. S7 AuNC@CPP and CPP alone were not able to eradicate PA CSOM biofilm at the concentrations tested. Following the treatment, the optical density (OD) from recovery plates after 48h incubation was measured at 650 nm (OD_{650}) using a spectrophotometer. Data are presented as mean ± s.d. (n= 3). Representative petri dish showing persister cells resuscitation

following AuNC@CPP and CPP treatment. LB agar plate was spotted with recovery media and incubated for 48h. Values refer to the concentrations tested (μg/mL).

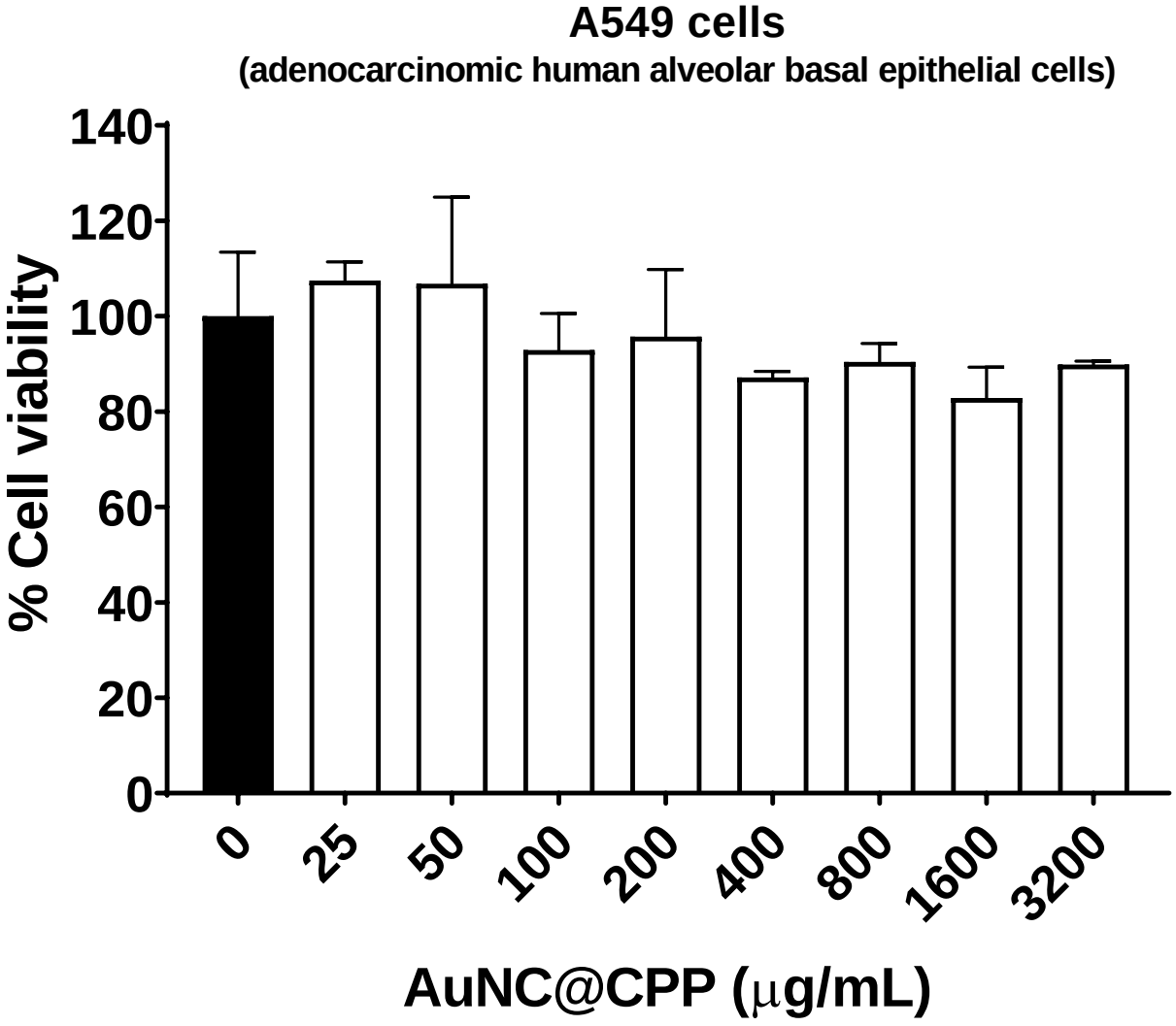


Fig. S8 Cytotoxic effects of AuNC@CPP. A549 cells were treated with AuNC@CPP for 24 h and the cell viability was determined by MTT assay. Cells exposed with AuNC@CPP at 3200 μg/mL shows more than 90% of viable cells. Data are presented as mean ± s.d. (n= 4).

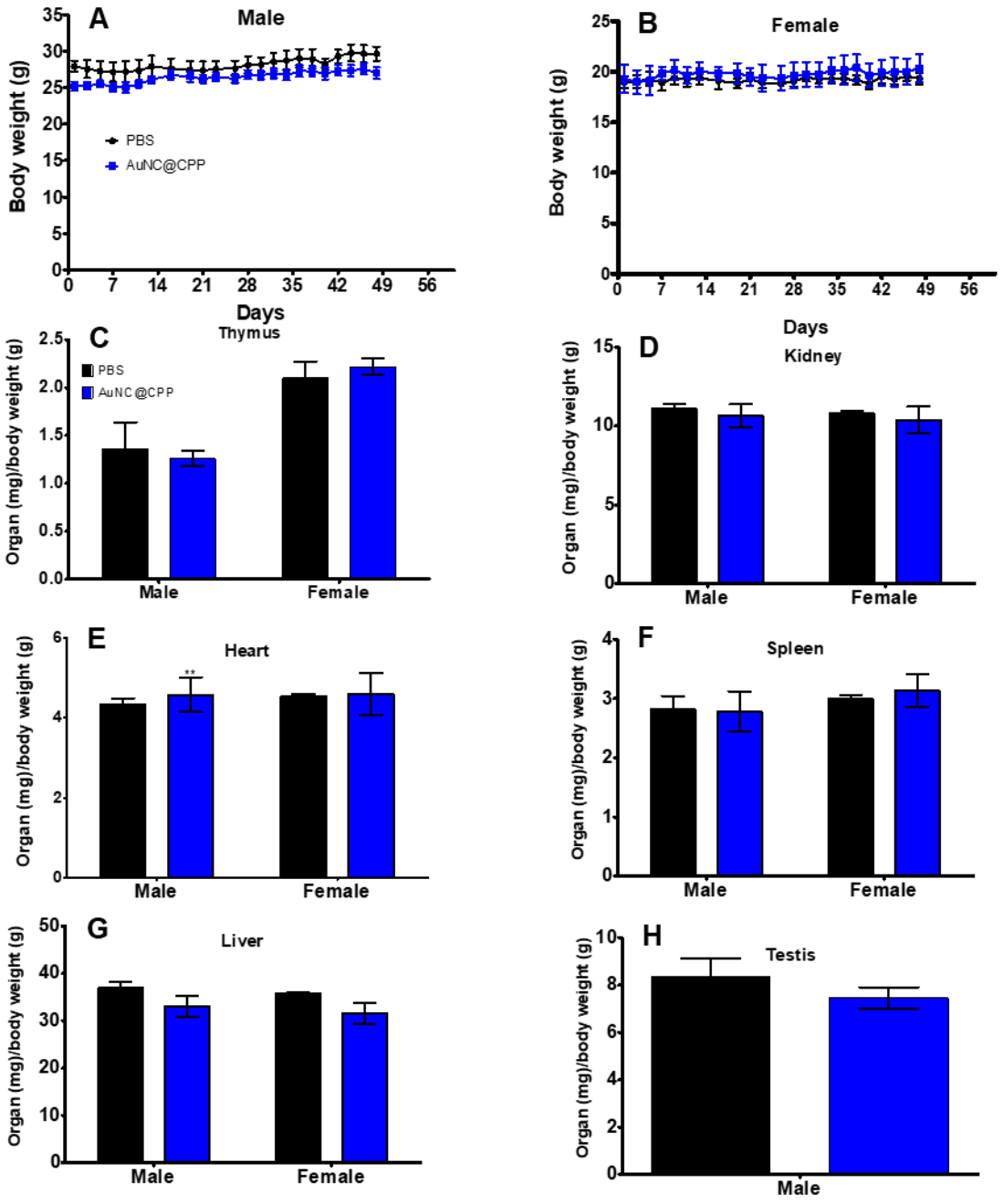


Fig. S9 A-B) Evolution of the body weight in healthy mice after oral gavage at a dose of 10 mg/kg for 14 days with AuNC@CPP and PBS. C-H) C57BL/6J mice were treated with 10 mg/kg every day for 14 days and the change of the body weight in healthy mice at 35 days was evaluated. * indicates statistically significant difference versus control (PBS) ($p < 0.05$). Data are presented as mean ± s.d.

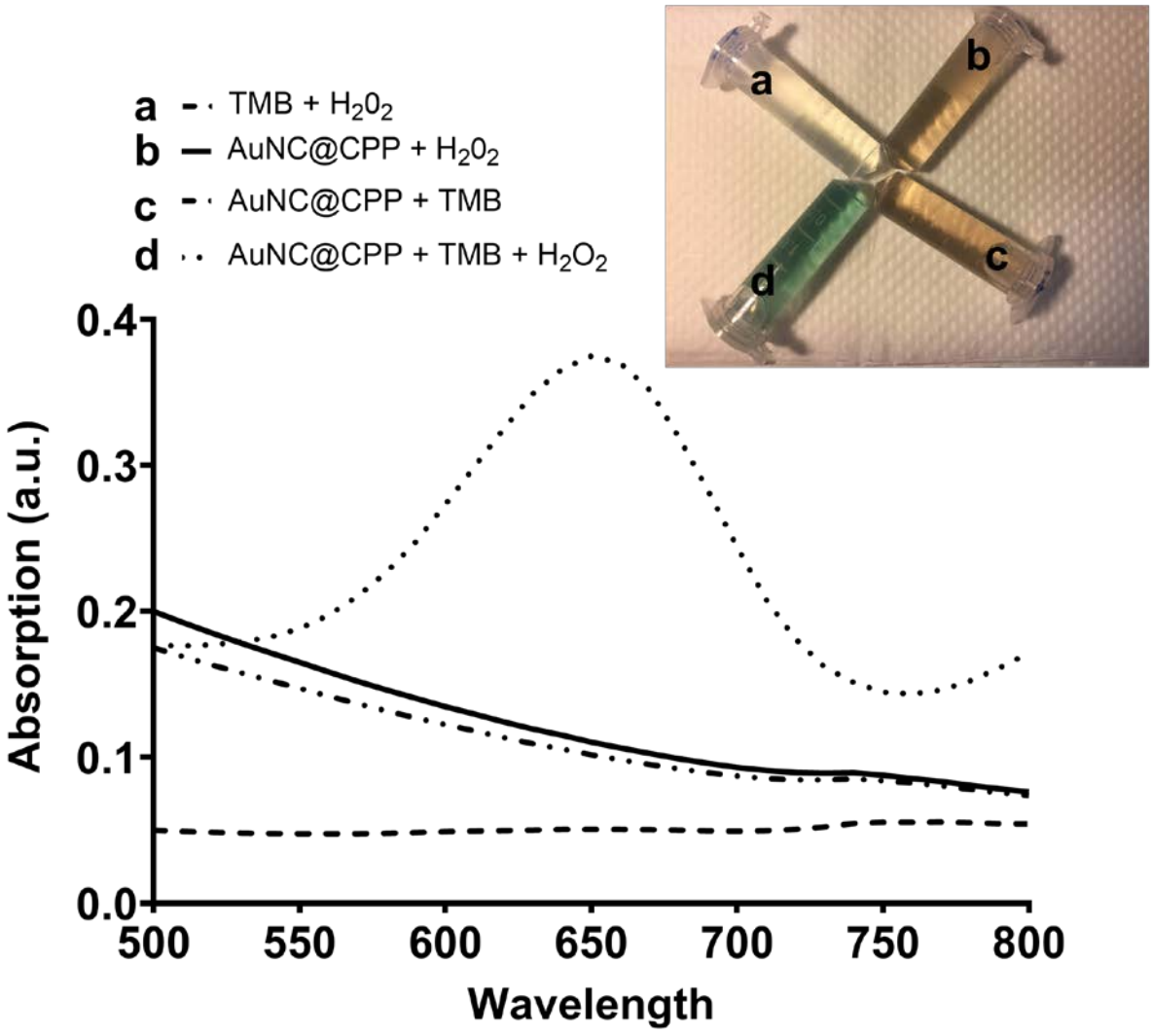


Fig. S10 Typical absorption spectra for monitoring the reaction of oxidizing TMB (3,3´,5,5´-tetramethylbenzidine) catalyzed by AuNC@CPP. Inset: the corresponding photographs of the mixtures containing (a) 500 µL of TMB and 2.45 M H_2O_2, (b) 400 µg/mL AuNC@CPP and 2.45 M H_2O_2, (c) 400 µg/mL AuNC@CPP and 500 µL of TMB, (d) 400 µg/mL AuNC@CPP, 500 µL of TMB and 2.45 M H_2O_2.

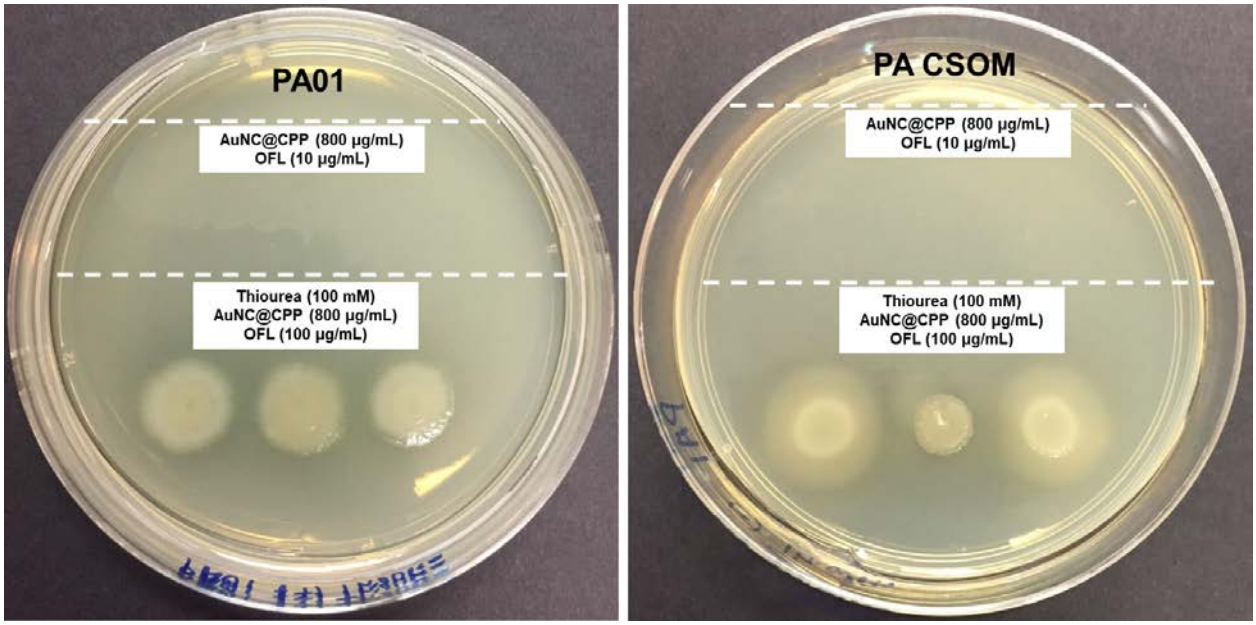


Fig. S11 The addition of thiourea, a powerful scavenger of hydroxyl radicals ($HO^{\bullet}$) preserved the viability of biofilms. Each spot represented an independent experiment (n = 3).

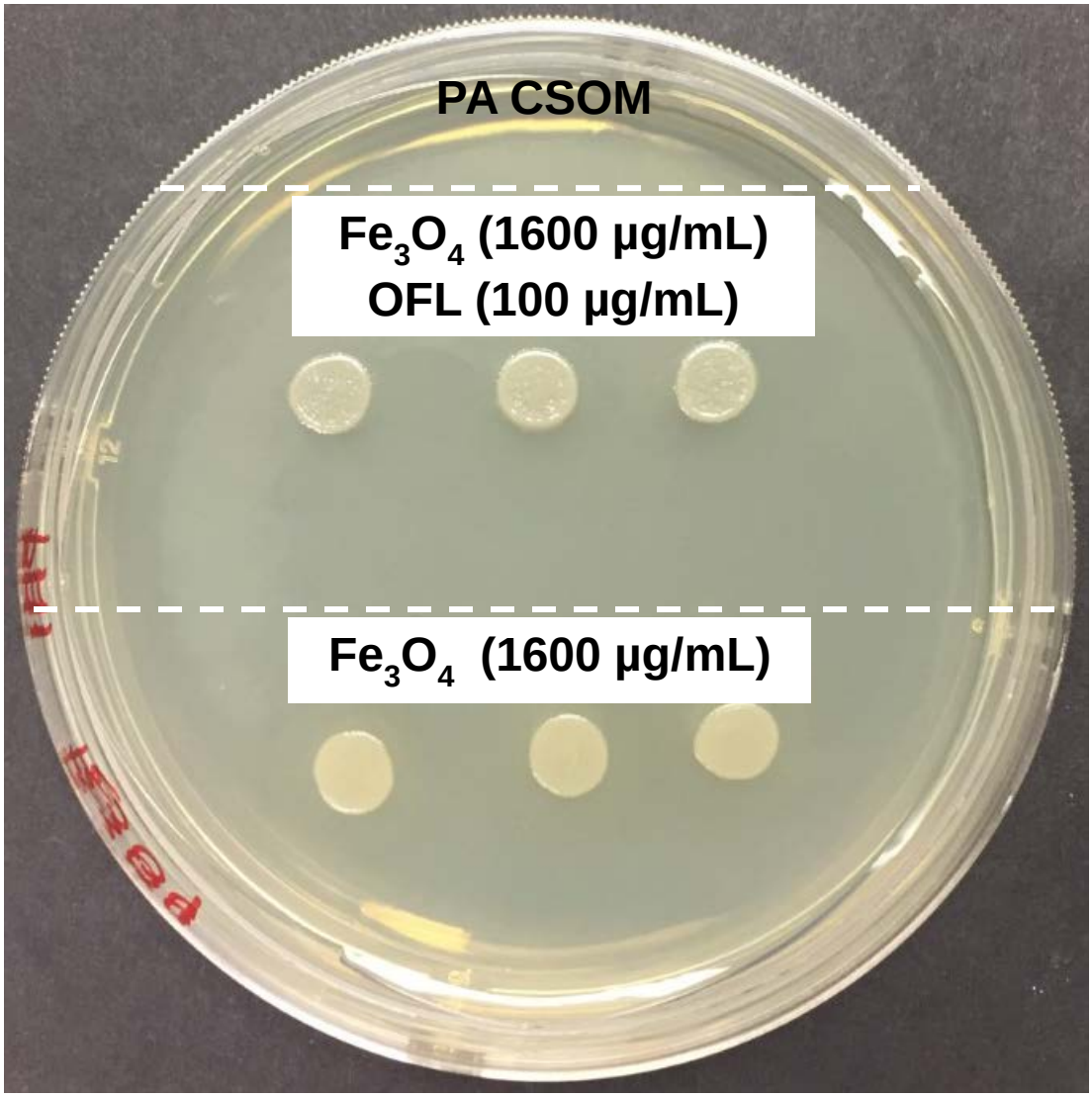


Fig. S12 Fe_3O_4 fails to enhance the lethal action of ofloxacin against biofilms. Representative petri dish showing persister cells resuscitation following 24 h treatment of 48h-old PA CSOM biofilms by carboxylic acid funtionalized iron oxide (Fe_3O_4; 797146 Sigma-Aldrich) and its combination with ofloxacin. The biofilm cells are left to recover (48h) in fresh media without drug and then 5 µL was plated on Luria-Bertani (LB) broth that contains agar. For each condition, the three spot on the petri dishe are the recovery media from three independent experiment (n= 3). Growth of PA CSOM is seen with Fe_3O_4 combined with ofloxacin (100 µg/mL).

Supplementary Table

Table S1 Erythrocytes and related parameters

	Mean Value ± SD					
	Male			Female		
Index	PBS	AuNC@CPP	P value	PBS	AuNC@CPP	P value
RBC (M/uLl)	8.50 ± 0.79	8.23 ± 1.05	P > 0.05	8.12 ± 1.64	7.10 ± 0.73	P > 0.05
HGB (gm/dL)	12.40 ± 0.80	12.47 ± 1.05	P > 0.05	11.65 ± 2.26	10.83 ± 0.68	P > 0.05
MCV (fL)	48.75 ± 2.27	48.60 ± 1.65	P > 0.05	46.65 ± 1.56	48.35 ± 1.70	P > 0.05
MCH (pg)	14.60 ± 0.51	15.20 ± 0.99	P > 0.05	14.38 ± 0.10	15.28 ± 0.83	P > 0.05
MCHC (g/dL)	30.00 ± 0.95	31.27 ± 1.36	P > 0.05	30.83 ± 0.80	31.68 ± 0.84	P > 0.05

RBC: Red blood cell; HGB: Hemoglobin; MCV: Mean corpuscular volume;
MCH: Mean cell haemoglobin; MCHC: Mean corpuscular hemoglobin concentration
P values versus PBS less than 0.05 were considered to be statistically significant.
Data are presented as mean ± s.d.

Table S2 Leucocytes and related parameters

	Mean Value ± SD					
	Male			Female		
Index	PBS	AuNC@CPP	P value	PBS	AuNC@CPP	P value
WBC (K/uL)	3.82 ± 1.13	5.24 ± 1.98	P > 0.05	2.70 ± 2.04	2.70 ± 1.51	P > 0.05
NEU (%)	9.50 ± 0.58	6.00 ± 1.00	P > 0.05	10.00 ± 2.58	9.25 ± 4.19	P > 0.05
MON (%)	3.50 ± 1.00	5.00 ± 0.00	P > 0.05	3.75 ± 1.71	3.00 ± 1.15	P > 0.05
LYM (%)	85.00 ± 2.00	87.33 ± 0.58	P > 0.05	85.5 ± 1.00	87.00 ± 3.74	P > 0.05
EOS (%)	2.00 ± 1.16	1.67 ± 0.58	P > 0.05	0.75 ± 0.50	0.75 ± 0.96	P > 0.05
Basophils	0	0	P > 0.05	0	0	P > 0.05
Platelet Count (K/uL)	1050 ± 377	934 ± 518.16	P > 0.05	649.5 ± 678.17	336.75 ± 174.88	P > 0.05
MPV (%)	6.20 ± 0.06	6.30 ± 0.30	P > 0.05	5.9 ± 0.14	6.35 ± 0.26	P > 0.05

WBC: White blood cells; NEU: Neutrophils; MON: Monocytes;
LYM: Lymphocytes; EOS: Eosinophils; MPV: Mean platelet volume
P values versus PBS less than 0.05 were considered to be statistically significant.
Data are presented as mean ± s.d

Table S3. Liver function related parameters

	Mean Value ± SD					
	Male			Female		
Index	PBS	AuNC@CPP	P value	PBS	AuNC@CPP	P value
AST（U/L）	40.33 ±	37.33 ± 2.89	P > 0.05	49.75 ±	61.25 ± 9.64	P > 0.05
ALT（U/L）	23.33 ±	26.00 ± 2.65	P > 0.05	23 ± 3.92	30.00 ± 4.69	P > 0.05
ALP（IU/L）	67 ± 10.4	68.33 ± 8.08	P > 0.05	125.5 ±	118.75 ±	P > 0.05
TBil (mg/dL)	0.2 ± 0	0.2 ± 0	P > 0.05	0.15 ± 0.06	0.23 ± 0.05	P > 0.05

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; Tbil: Toatal Bilirubin
P values versus PBS less than 0.05 were considered to be statistically significant.
Data are presented as mean ± s.d

Table S4. Kidney function related parameters

	Mean Value ± SD					
	Male			Female		
Index	PBS		P value	PBS	AuNC@CPP	P value
CR（mg/dL）	0.197 ±	0.22 ± 0.01	P > 0.05	0.20 ±	0.21 ± 0.03	P > 0.05
BUN	39.67 ±	88.5 ± 5.5	P > 0.05	41.5 ±	34.25 ± 5.32	P > 0.05

BUN: Blood urea nitrogen; CR: Creatinine; P values versus PBS less than 0.05 were considered to be statistically significant. Data are presented as mean ± s.d