Supplementary Information

Dendritic Polyamidoamine Supramolecular System Composed of Pillar[5]arene and Azobenzene for Targeting of Drug-Resistant Colon Cancer

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1. Materials and methods

PAMAM dendrimer (ethylenediamine core) G3, FITC, and DT Diaphorase (NQO1) were purchased from Sigma-Aldrich (Shanghai, China). 4-(Dimethylamino)azobenzene 4'-isothiocyanate (AZO) was purchased from TCI (Shanghai, China). 4-Methylmorpholine (NMM), NADPH, and $Na_2S_2O_4$ were purchased from HEOWNS (Tianjin, China). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Beijing, China). Blood agar plate and Brain Heart Infusion Broth were purchased from Qingdao Hope Bio-Technology Co., Ltd (Shandong, China). Anaerobic gas production bag (AnaeroPack-Anaero 2.5L) was purchased from Mitsubishi Gas Chemical Co., Inc (Qingdao, China). CP[5]A was synthesized according to the reported literature.[1]

UV-vis spectra and fluorescence spectra were recorded on a Nanophotometer NP80 Touch spectrophotometer (NP80, implen, Germany) and a fluorescence spectrophotometer (F-4500, Hitachi, Tokyo, Japan), respectively. Fourier transform infrared (FT-IR) spectra were obtained from a Bio Rad 6000 spectrophotometer (Thermo Electron, USA). Scanning electron microscope (FESEM, JEOL JSM-6700F, Japan) was employed to gain the morphology of the bacteria. Fluorescent images were obtained on a fluorescence microscope (Nikon DS-Ril/ECLIPSE, Japan). ^{1}H NMR spectra were recorded on a Bruker Avance-400 spectrometer (400 MHz, Bruker, Freemont, CA). Dynamic light scattering (DLS) was recorded by Zetasizer Nano ZS90 instrument (Malvern Instruments, Southborough, MA) at 298 K.

Synthesis of P-A: Polyamidoamine (PAMAM) (115.6 μL, 14.49 nmol), 4-methylmorpholine (NMM, 8 μL, 5.31 mmol), and 4-(dimethylamino)azobenzene 4'-isothiocyanate (AZO, 6.56 mg, 23.17 nmol) were added dropwise in a flask, and the mixture was stirred overnight at room temperature. The mixture was dialyzed with DMF and distilled water for 2 days to remove impurities, then filtered and lyophilized to achieve an orange powder of PAMAM-AZO (P-A) (44.8 mg, 48.4%). ^{1}H NMR (400 MHz, D_2O, δ): 7.42 (s, 1H), 6.98 – 6.54 (m, 1H), 3.37 (s, 1H), 3.24 (s, 2H), 2.97 (s, 1H), 2.76 (s, 2H), 2.56 (s, 1H), 2.38 (s, 3H) ppm.

Synthesis of Q-P-A: Excess of iodomethane (198 mg, 1.4 mmol) was added into 10 mL of H_2O/DMSO (1:1) solution containing 20 mg of P-A. After stirring at room temperature overnight, superfluous iodomethane was removed. The crude product was dialyzed with sodium chloride solution and distilled water for 2 days. After filtering out insoluble impurities, the Q-P-A was obtained by lyophilization as an orange powder (9.4 mg, 44.9%). ^{1}H NMR (400 MHz, D_2O, δ): 7.50 (s, 1H), 6.83 (s, 1H), 3.67 (s, 3H), 3.48 (s, 4H), 3.12 (s, 3H), 2.88 (s, 3H), 2.70 (s, 1H), 2.68 (s, 1H), 2.51 (s, 2H) ppm.

Preparation of Bacterial Stock Solutions: Gram-negative bacteria (*F. nucleatum*) were utilized to test the antibacterial performance of the as-prepared cationic antimicrobials. Typically, a single colony of *F. nucleatum* cultured on a blood agar plate was inoculated into 5 mL of brain heart infusion broth medium, and incubated in a shaking incubator under the anaerobic environment (37 °C, 170 rpm) overnight. After centrifugation (8000 rpm for 3 min) and washing with PBS (pH = 7.4) twice, the bacteria were dispersed in aquae sterilisata and diluted to a predetermined concentration as a stock solution. Finally, the concentration of bacteria were detected by a Nanophotometer NP80 Touch spectrophotometer at the wavelength of 600 nm.

Minimum Inhibitory Concentration (MIC) Determination: The spread plate method was employed to evaluate the antibacterial activity of the as-prepared antimicrobials. Briefly, the as-prepared antimicrobials were added into the diluted *F. nucleatum* solutions (~10^6 CFU mL^{-1}, 1 mL) and shaken at 37 °C for 3 h. After diluting the suspensions with an appropriate dilution

factor, 100 μL of the diluted suspensions were spread on solid blood agar plates and incubated in the anaerobic environment of anaerobic gas production bag at 37 °C for 18 h. The viabilities of *F. nucleatum* were confirmed according to the number of colony-forming units to calculate the MIC of Q-P-A and Q-P-A@CP[5]A.

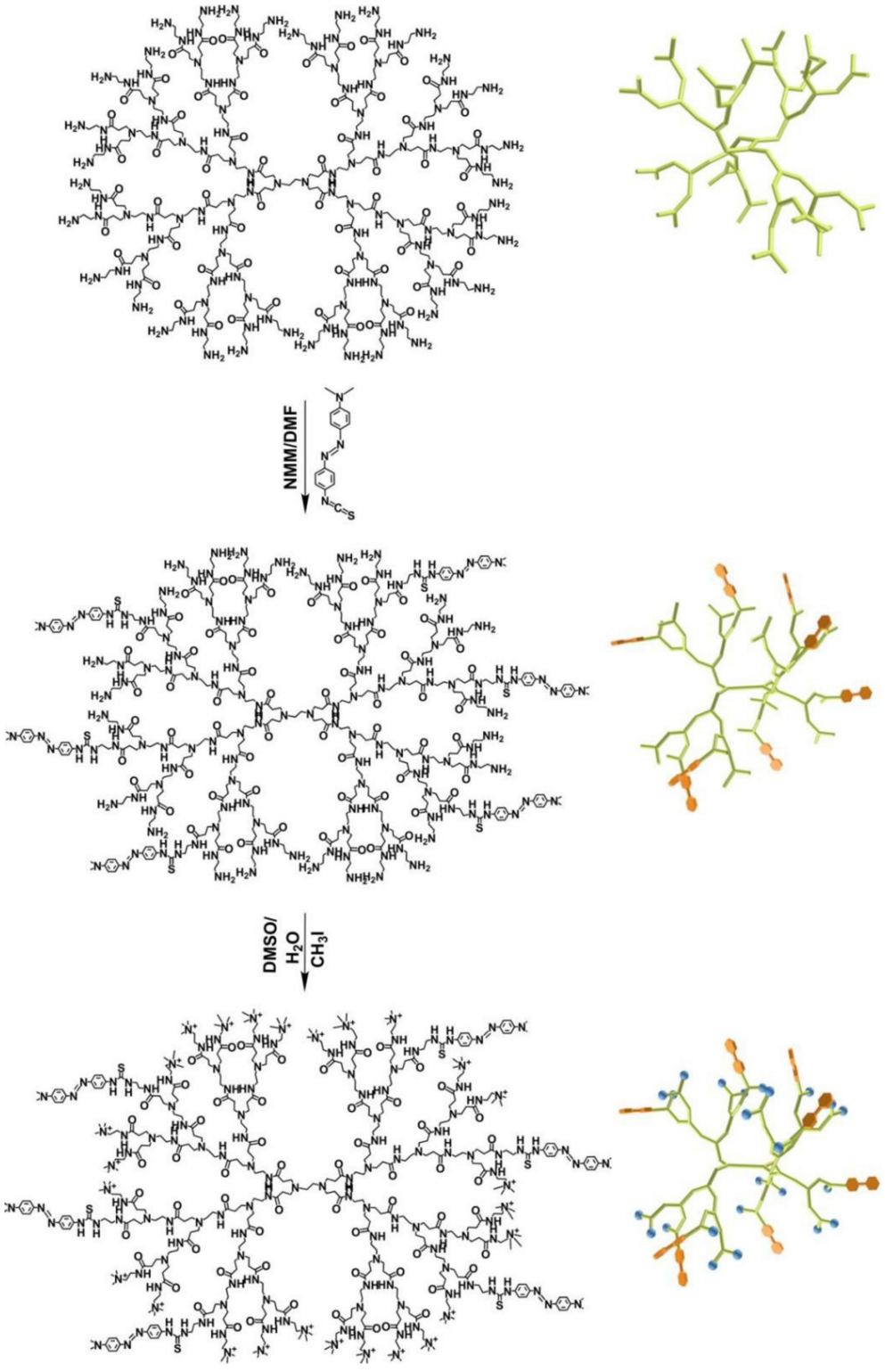
Scanning Electron Microscope Observation of F. nucleatum: To observe the influence of Q-P-A and Q-P-A@CP[5]A on the morphology of *F. nucleatum*, SEM observation was carried out. *F. nucleatum* was cultured at 37 °C in the anaerobic environment until the concentration reached 10^9 CFU mL^{-1}. The bacteria were harvested by centrifugation and resuspended in PBS (pH= 7.4). Then, Q-P-A or Q-P-A@CP[5]A was added into the bacterial suspension, shaken for 6 h (170 rpm, 37 °C) in the anaerobic environment of the anaerobic gas production bag, collected by centrifugation again (8000 rpm, 3 min), and washed 3 times with PBS. Subsequently, *F. nucleatum* was solidified with 2.5% glutaraldehyde for 4 h at room temperature, washed twice with PBS, dehydrated with ethanol in a graded series (30%, 50%, 70%, 80%, 95%, and 100%, respectively), and replaced with tertiary butanol. After drying, the samples were coated onto a silicon wafer and examined by SEM.

In Vitro Cell Viability Assay: CCK-8 assay was used to assess the cytotoxicity of as-prepared antimicrobials. Mouse fibroblast cells L929 and human colon adenocarcinoma cells HT29 were seeded in a 96-well plate with a density of ~5.5×10^3 cells per well and incubated at 37 °C and 5% CO_2 for 24 h. After removing the culture medium, the as-prepared antimicrobials with various concentrations were added into the well and incubated at 37 °C and 5% CO_2 for 24 h, followed by the addition of 10 μL of CCK-8 to culture another 30 min. The absorbance at 450 nm of each well was detected via a microplate reader to calculate the cell viability.

Live/Dead Cell Staining Assay: HT29 cells or L929 cells were seeded in 35 mm glass-bottom culture dishes, incubated at 37 °C and 5% CO_2 for 24 h, and then washed twice with PBS. After adding Q-P-A or Q-P-A@CP[5]A for 6 h, the cells were washed with PBS, stained with acridine orange (1 mg/mL) and ethidium bromide (1 mg/mL) for 30 min. Then, the cells were washed

with PBS again, and observed by Nikon LSM510 instrument using 488 nm and 514 nm excitation wavelength.

2. Characterizations



Scheme S1. Synthetic route to the quaternary ammonium PAMAM-AZO.

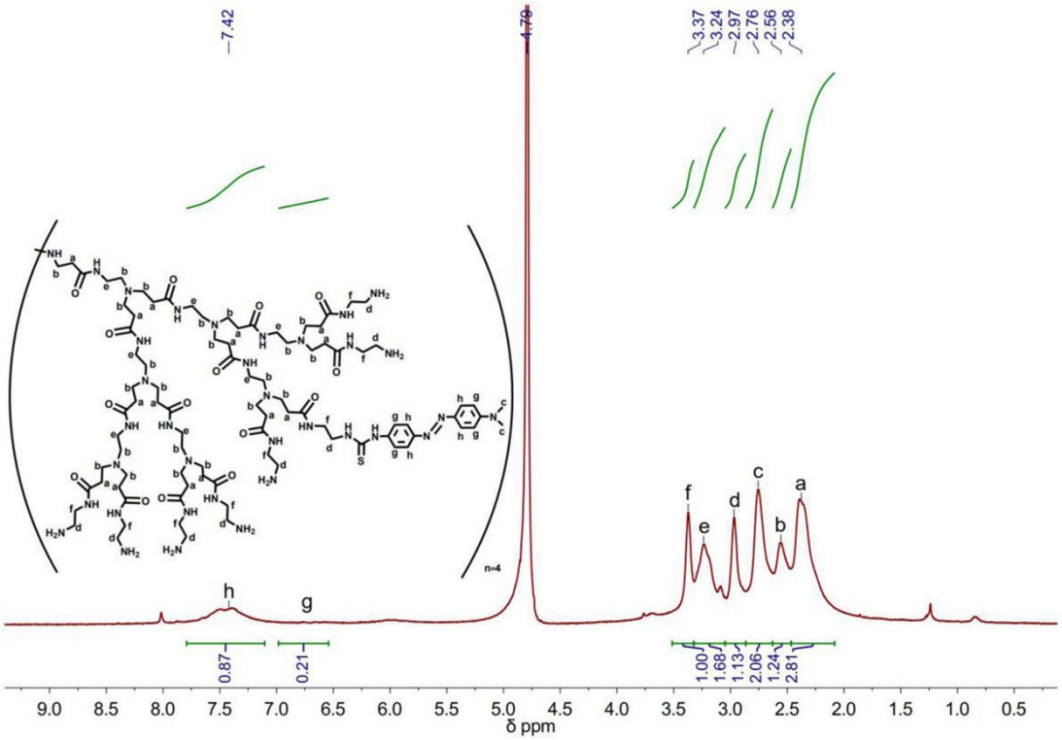


Fig. S1. ^{1}H NMR spectrum (400 MHz) of PAMAM-AZO in D_2O at 298 K.

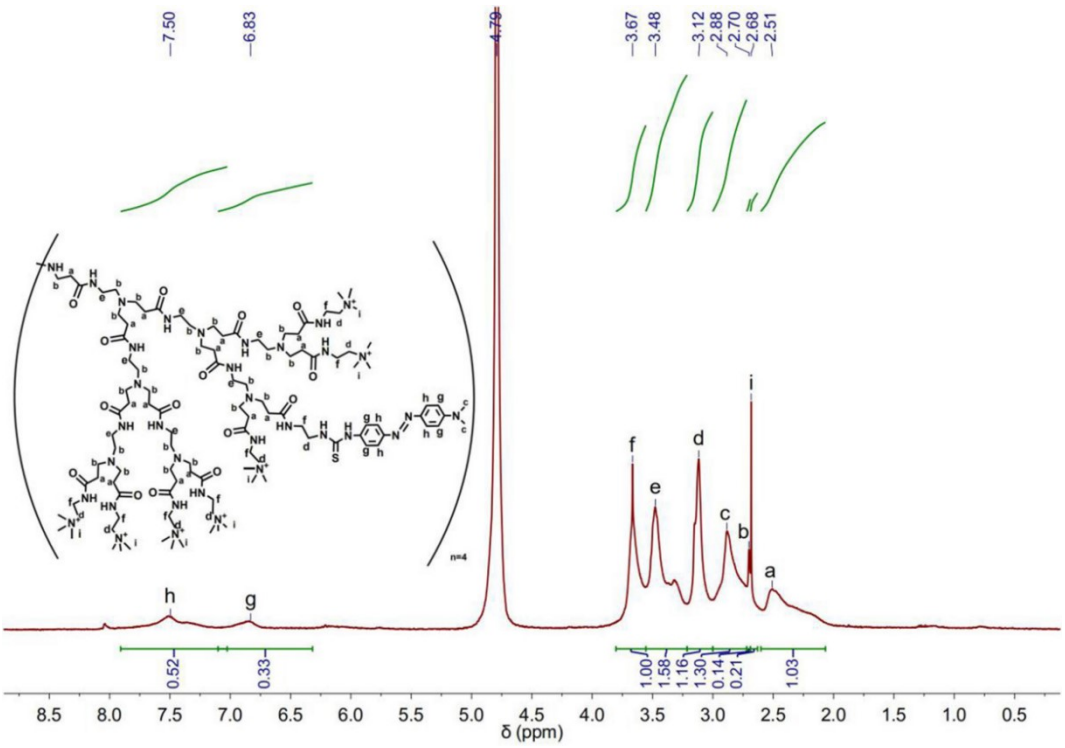


Fig. S2. ^{1}H NMR spectrum (400 MHz) of quaternary ammonium PAMAM-AZO (Q-P-A) in D_2O at 298 K.

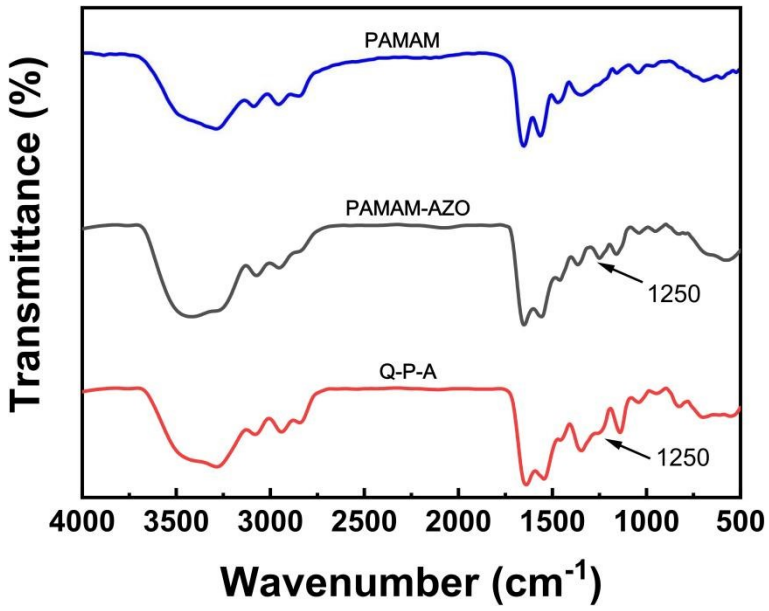


Fig. S3. FT-IR spectra of PAMAM, PAMAM-AZO and Q-P-A.

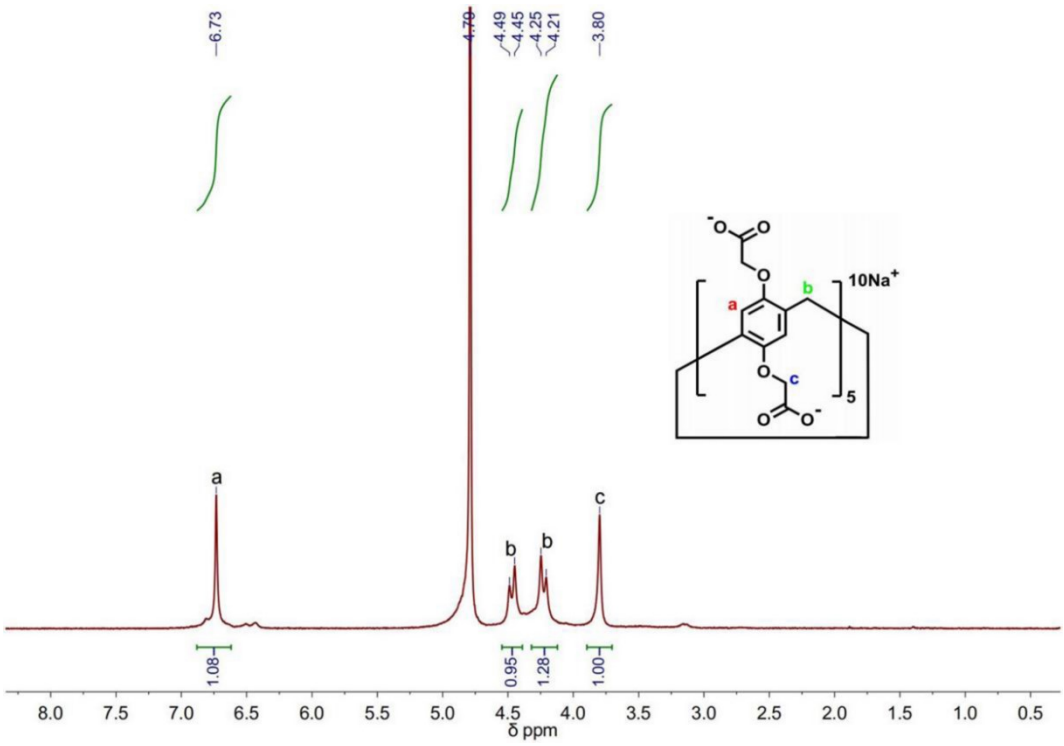


Fig. S4. ^{1}H NMR spectrum (400 MHz) of CP[5]A in D_2O at 298 K.

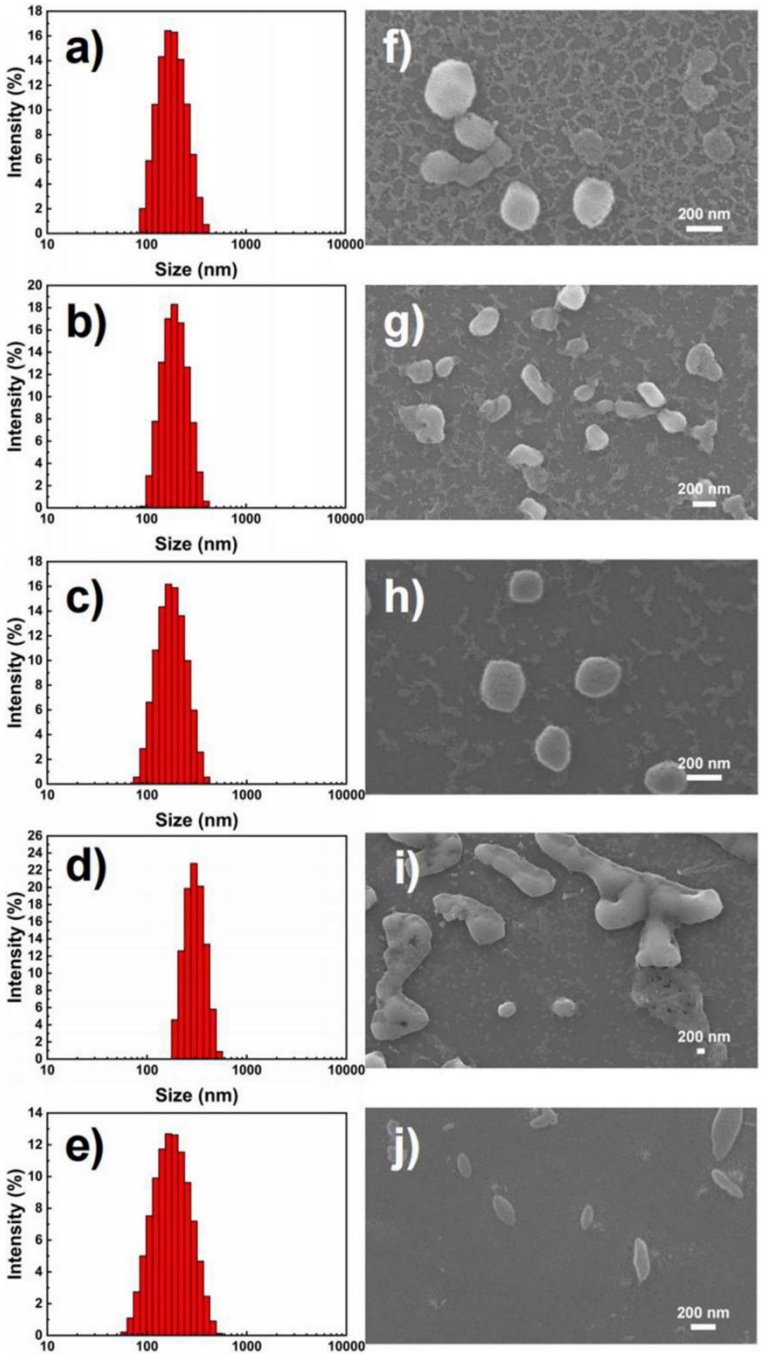


Fig. S5. Hydrodynamic diameter distributions and SEM images of Q-P-A@CP[5]A under different mole ratios ($–N^+CH_3$: CP[5]A) of (a) and (f) 3:1; (b) and (g) 3.75:1; (c) and (h) 5:1; (d) and (i) 7.5:1; (e) and (j) 15:1.

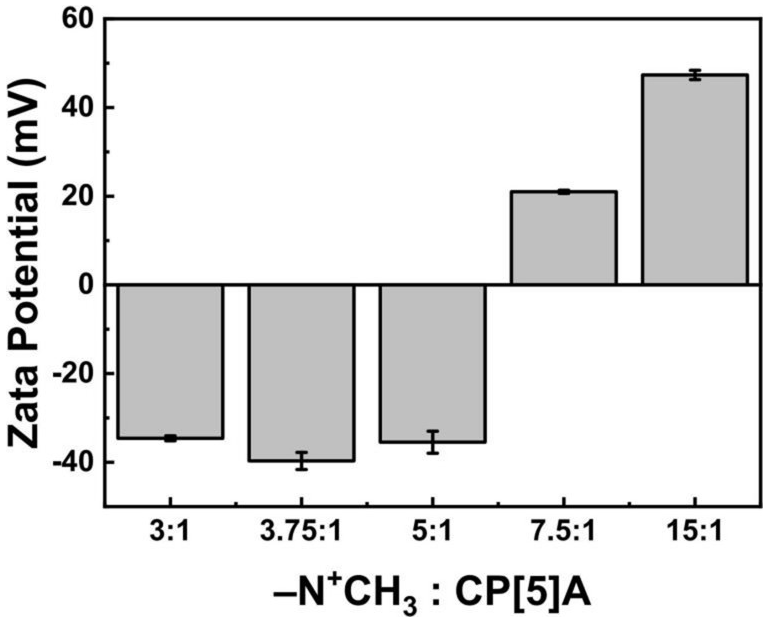


Fig. S6. ζ-potential values of Q-P-A@CP[5]A with different mole ratios ($–N^+CH_3$: CP[5]A).

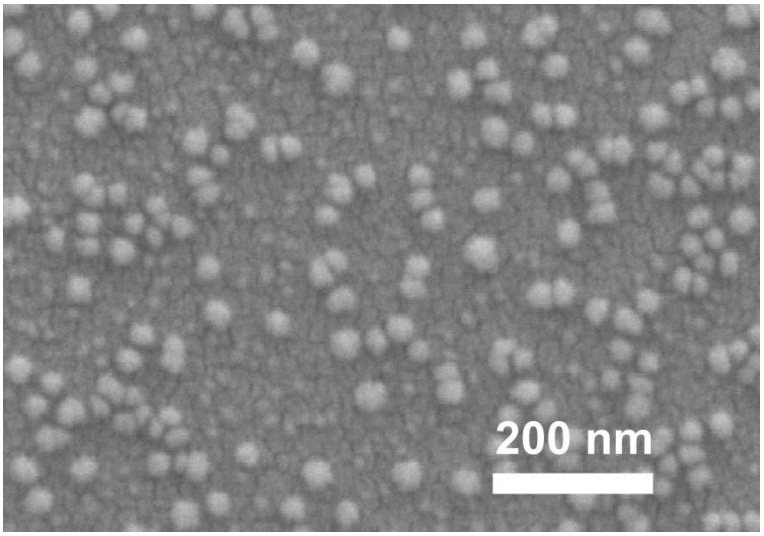


Fig. S7. Typical SEM image of Q-P-A@CP[5]A ($–N^+CH_3$: CP[5]A = 5 : 1) in the presence of $Na_2S_2O_4$.

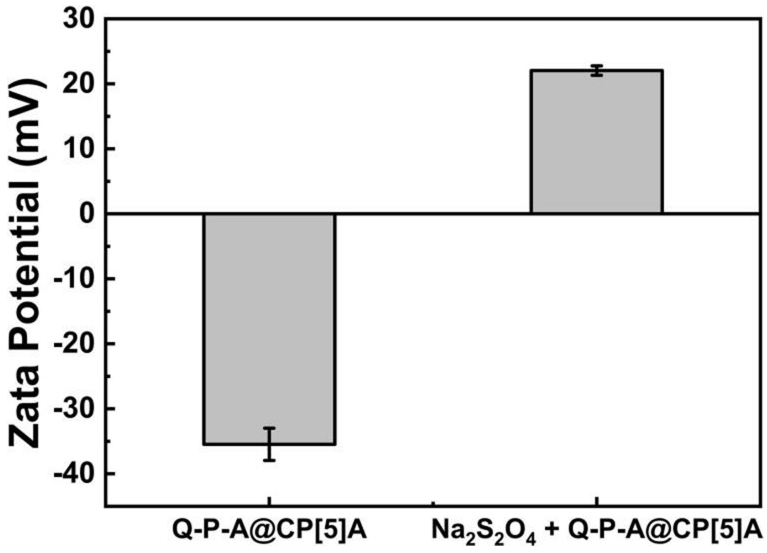


Fig. S8. ζ-potential values of Q-P-A@CP[5]A ($–N^+CH_3$: CP[5]A = 5 : 1) in the presence and absence of $Na_2S_2O_4$.

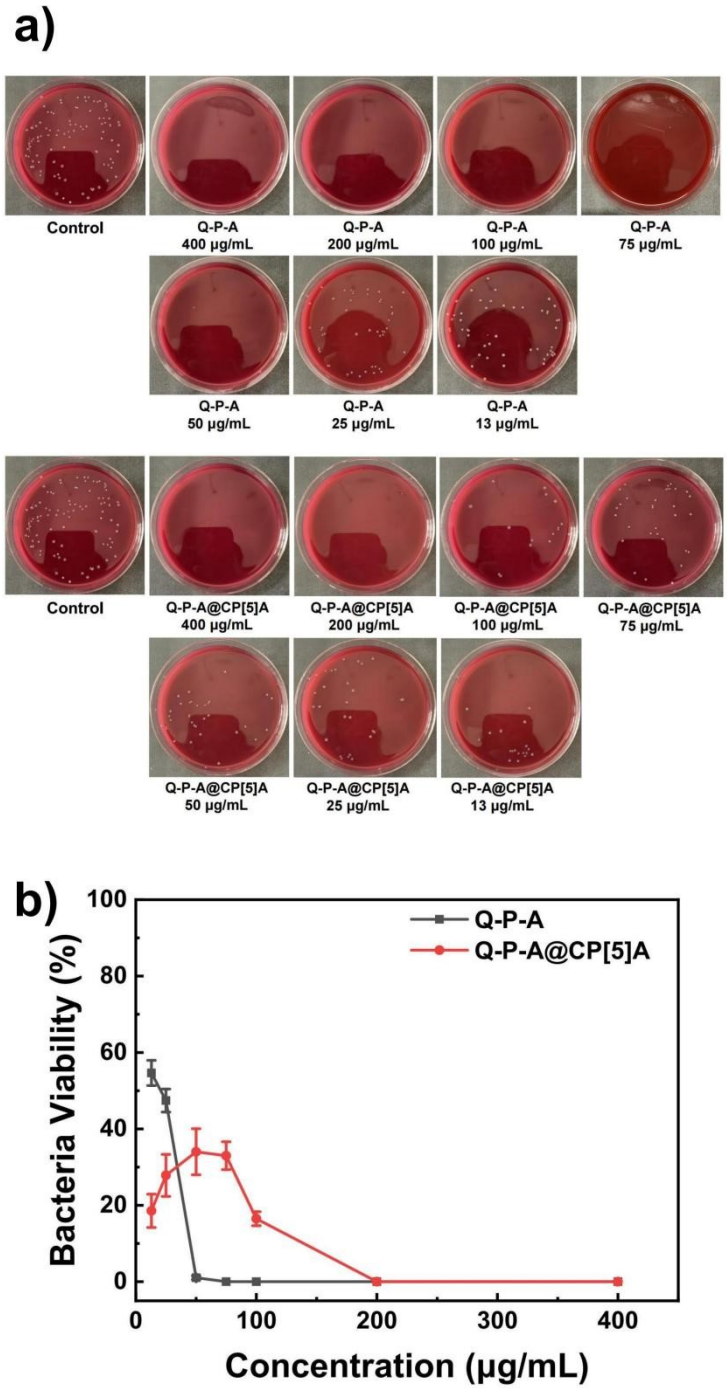


Fig. S9. (a) Images of *F. nucleatum* colonies treated with Q-P-A and Q-P-A@CP[5]A on blood agar plates. (b) Concentration-dependent antimicrobial activities of Q-P-A and Q-P-A@CP[5]A against *F. nucleatum*.

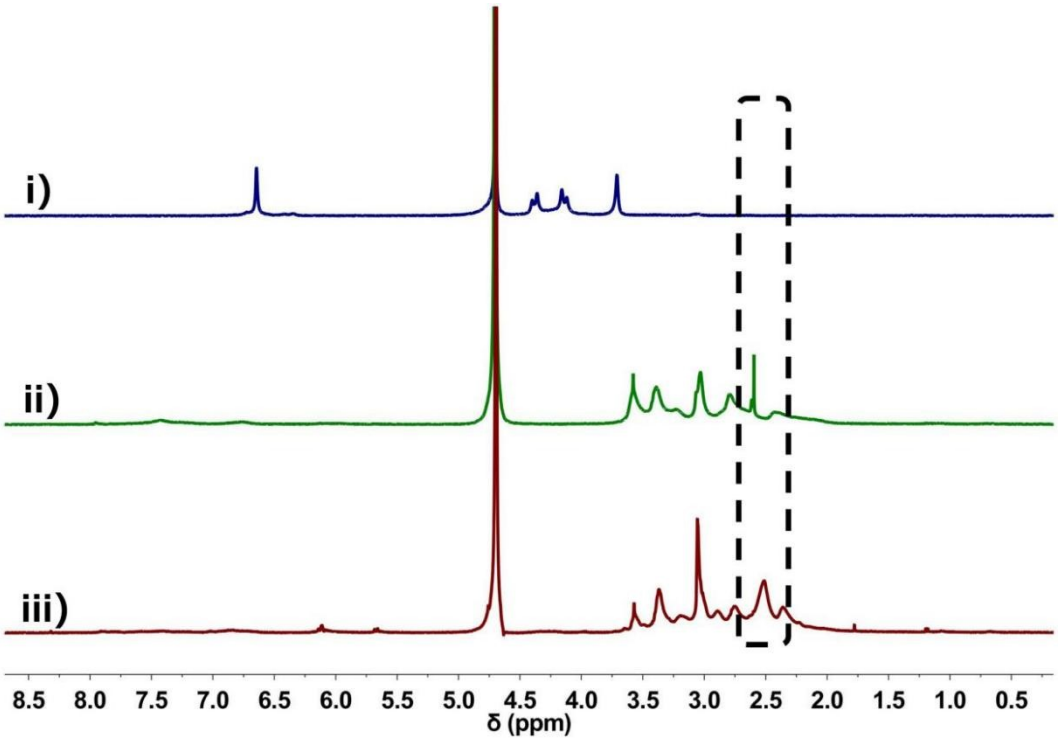


Fig. S10. ^{1}H NMR spectra (400 MHz, 298 K) of i) CP[5]A, ii) Q-P-A, and iii) Q-P-A@CP[5]A in D_2O at 298 K.

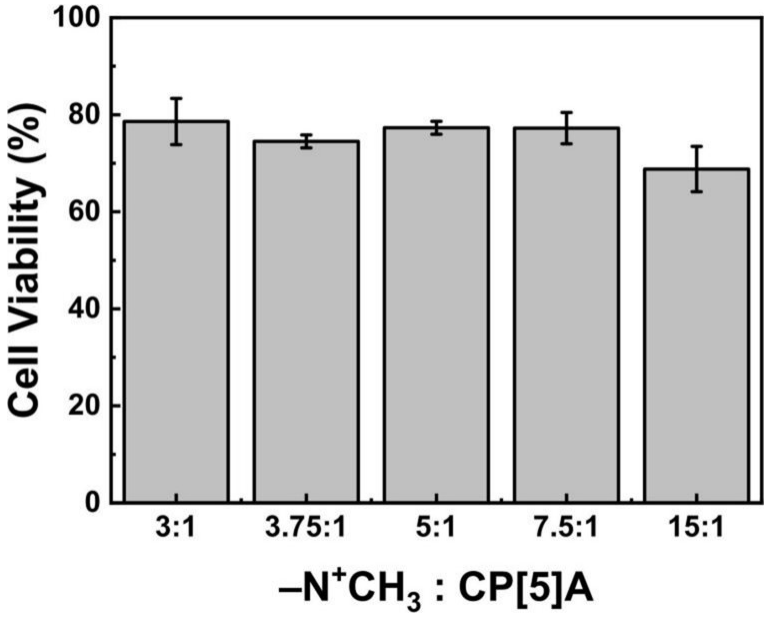


Fig. S11. Cell viabilities of L929 treated by Q-P-A@CP[5]A (Q-P-A concentration = 100 μg/mL) with different ratios of $–N^{+}CH_3$ and CP[5]A.

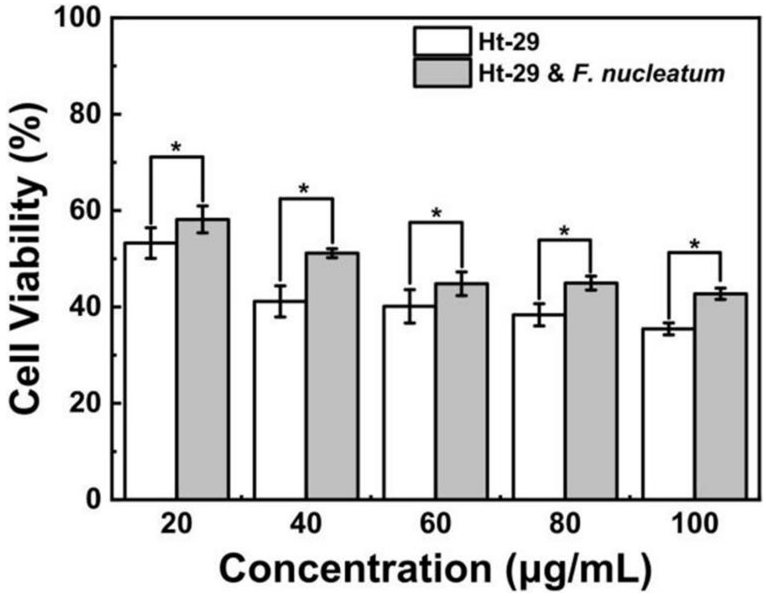


Fig. S12. Cell viabilities of HT29 cells and HT29 cells co-cultured with *F. nucleatum* after the treatment of oxaliplatin (*$p < 0.05$).

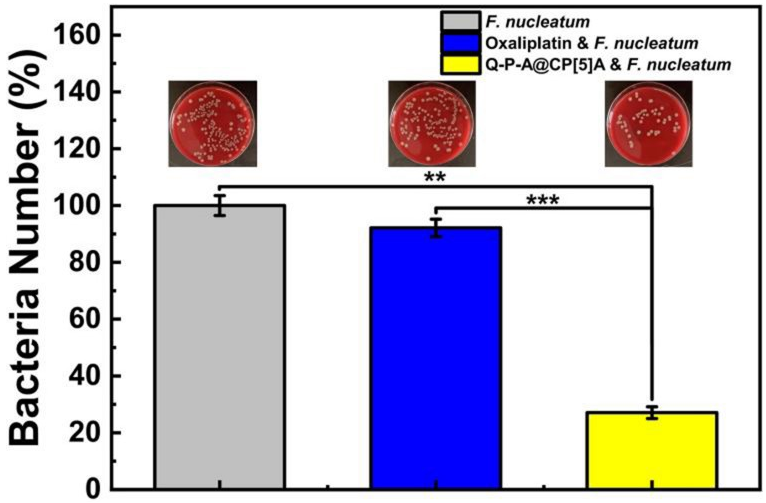


Fig. S13. The bacterial number of tumor tissues under various treatments. Inset picture: Corresponding colony counts in tumor tissues (**$p < 0.01$ and ***$p < 0.001$).

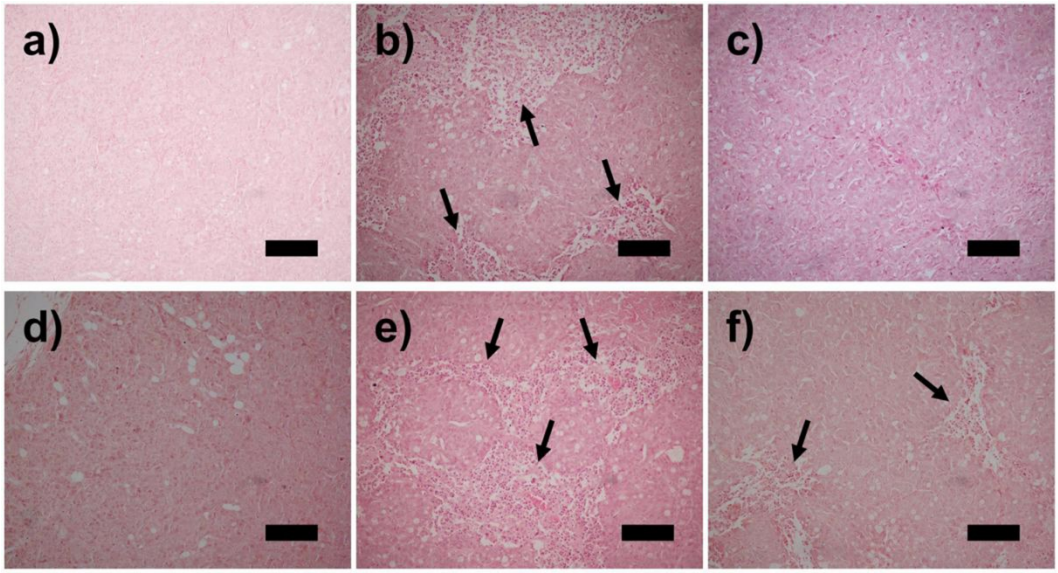


Fig. S14. H&E staining of the tumor sections treated with (a) PBS (control), (b) oxaliplatin, (c) *F. nucleatum*, (d) oxaliplatin & *F. nucleatum*, (e) Q-P-A@CP[5]A, and (f) Q-P-A@CP[5]A & *F. nucleatum* (scale bar = 50 μm).

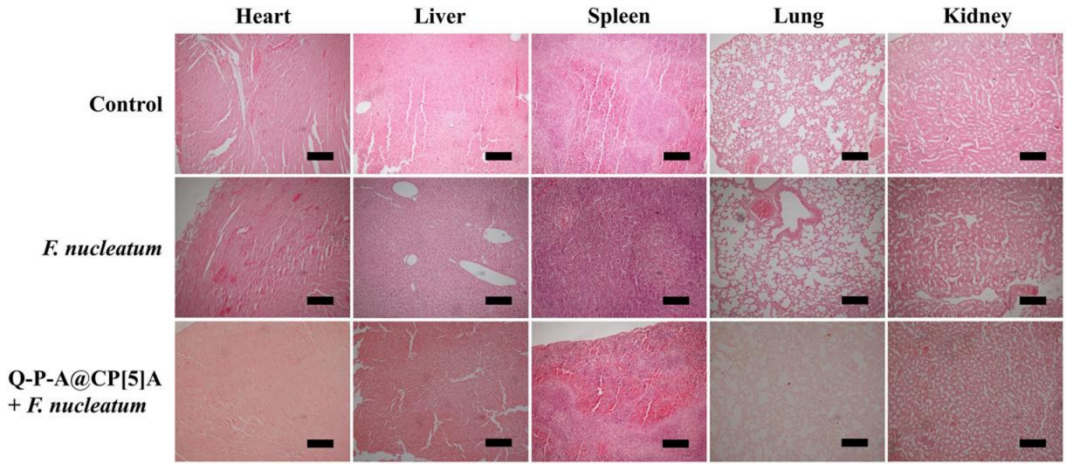


Fig. S15. Biosafety assay of H&E stained sections of major organs in various treatments (scale bar = 50 μm).

3. Reference

1. H. Li, D. X. Chen, Y. L. Sun, Y. B. Zheng, L. L. Tan, P. S. Weiss and Y. W. Yang, Viologen-Mediated Assembly of and Sensing with Carboxylatopillar[5]arene-Modified Gold Nanoparticles, *J. Am. Chem. Soc.*, 2013, **135**, 1570-1576.