Electronic Supplementary Information for

A Perylene-Based Membrane Intercalating Conjugated Oligoelectrolyte with Efficient Photodynamic Antimicrobial Activity

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Materials

The probe PC4 was synthesized via a simple procedure with high yield.[S1] Propidium iodide (PI) was bought from Life Technology (China), 1,3-diphenylisobenzofuran (DPBF) was purchased from Energy Chemicals (China). Lecithin was obtained from Aladdin (China). Calf thymus DNA was obtaiend from Yeason (China). A stock solution of PC4 (5 mM) was prepared in sterile water, stored at room temperature, and covered with aluminum foil to avoid light exposure. Other reagents were of high purity (≥ 95%) and used as received.

Physical measurements

UV-Vis absorption spectra were recorded with a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped with a xenon flash lamp. Fluorescence emission spectra were collected using Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA). Transmission electron microscopy (TEM) measurements were performed on a FEI TECNAI G2 high resolution transmission electron microscope (the Netherlands). Confocal laser scanning microscope (CLSM) images were obtained using Nikon Ti-E (Japan). Fluorescence microscope images were obtained using Nikon Ti-U (Japan).

Preparation of buffer and culture medium

150 mM PBS stock solution: 8 g NaCl, 0.2 g KCl, 3.62 g $Na_2HPO_4 \cdot 12H_2O$ and 0.24 g KH_2PO_4 were dissolved in 1 L ultrapure water, sterilized, and stored at 4 ºC. The 30, 60, and 90 mM PBS sample solutions were obtained via simple dilutions of the 150

mM PBS stock solution.

LB broth medium per liter: 10 g NaCl, 10 g tryptone, 5 g yeast extract.

LB agar medium per liter: 10 g NaCl, 10 g tryptone, 5 g yeast extract, 15 g agar.

Lipid vesicle preparation

The lipid vesicles containing PC4 were prepared following a literature report.[S2] First, 30 mg of lecithin and 1 mg of PC4 were dissolved in 3 mL methanol. The lecithin/PC4 solution was heated to 45 ºC, methanol was removed by argon flushing, and the solid was dried under vacuum. The resulting solid was dispersed in 30 mM PBS, sonicated for 5 min. For TEM and photophysical studies, the vesicles were filtered through a 0.45 μm syringe filter. For CLSM imaging, 10 μL of the obtained vesicles was put on a cover slide, and observed under microscope (488 nm laser light excitation).

Bacterial cell culture

Escherichia coli (*E. coli*, ATCC-25922) (Gram negative), Staphylococcus aureus (*S. aureus*, ATCC-25923) (Gram positive), Pseudomonas Aeruginosa (*P. aeruginosa,* ATCC-27853) (Gram negative) and Methicillin-resistant Staphylococcus aureus (MRSA, ATCC-43000) (Gram positive) were grown in LB broth medium at 37 ºC in a rotary incubator with shaking (180 rpm) overnight. The next day, bacteria were centrifuged at 3,000 rpm for 5 min, washed with PBS two times, and the amount of bacteria was determined using the UV-vis optical density at 600 nm (OD_{600}). Finally the bacteria were suspended in PBS to a final concentration of OD_{600} = 0.5 (ca. 5 x 10^8

cells/mL), and used in subsequent experiments.

Binding of PC4 to bacteria cells

The binding of PC4 to *E. coli* was measured using an indirect UV-vis absorption method. 1 mL *E. coli* suspension solution (OD_{600} = 0.5) was incubated with various amounts of PC4 in PBS. The final concentrations of PC4 were 2.5, 5, 10 and 20 μM. Control samples with the same concentration of PC4 but without bacteria were also prepared. After various time of incubation, the samples and controls were centrifuged at 3,000 rpm for 5 min, the supernatant was transferred and measured using a microplate reader at 470 nm. The binding affinity (BA) was calculated using the following formula:

$$BA = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100\%$$

For the stability of PC4 membrane-intercalating test, cells were incubated with PC4 (10 μM) for 15 min, centrifuged, and the supernatant was removed. The stained cells were washed several times (PBS for 10 min), centrifuged at 3,000 rpm for 5 min, and the supernatant was tested via UV-vis absorption. Control sample was the solution of PC4 (10 μM) in 30 mM PBS.

$^1O_2$ generation assay

The ROS generation of PC4 in micelle was studied with DPBF as indicator in SDS solution. The fresh sample solutions of SDS (50 mM) in water, DPBF (1 mg/mL) in DMF and PC4 (2 mM) in DMF were prepared separately. Then, the sample mixture

containing SDS (25 mM), DPBF (10 μg/mL), and PC4 (20 μM) was irradiated with 473 nm laser light for certain periods of time, and the UV-Vis absorption was recorded. In a control experiment, the same mixture of DPBF, PC4 and SDS was kept in the dark. In another control experiment, a sample solution of DPBF was irradiated with light, and the absorption changes were recorded. The plot of A/A_0 at 418 nm with time was calculated using the following equation:

$$\frac{A}{A_0} = \frac{A' - A_p}{A_0' - A_p}$$

A': the absorption of the sample mixture at 418 nm after irradiation, A_p: the absorption of PC4 at 418 nm, A_0': the absorption of the sample mixture before irradiation.

Antibacterial assay

1 mL of PC4 stained bacteria (15 min, 37 ºC) was placed in a 6-well plate and irradiated with light (450 nm) for 15 min. After irradiation, the cell suspension was serial diluted 5 x 10^5 times (100 x 100 x 50). 100 μL of the diluted sample solution was plated on the LB agar plates. The plates were incubated for 14 h at 37 ºC and the CFU/mL value was counted. For the dark control, the PC4 treated cells were kept in the dark and plated. For light irradiation control, cells without PC4 were exposed to light and plated.

Minimum inhibitory concentration (MIC) tests

The MIC values of PC4 towards bacteria were obtained following a literature report.[S3] PC4 (5 mM) was diluted with LB medium to 2.5 mM. LB solutions of PC4 of

concentrations 2.44 μM – 2.5 mM were obtained after series of dilutions. 200 μL of the PC4 solution was added to the 96-well plate. The bacteria were diluted in LB medium to the concentration of 10^6 CFU/mL, and 20 μL of the bacteria sample solution was mixed with 200 μL of PC4 solution. After 18 h incubation at 37 ºC, the MIC values were determined as the lowest concentrations of PC4 that prevented noticeable bacteria growth.

CLSM imaging

For cell staining experiments, 1 mL of the *E. coli* sample solution was incubated with 20 μM PC4 for 15 min at 37 ºC. The cells were centrifuged at 5,000 rpm for 5 min, rinsed with PBS twice and suspended in 1 mL PBS for further use.

For live cell imaging experiments, 100 μL of PC4 (10 μM) stained cells were placed on the agarose-treated glass slide for fluorescence imaging. For dead cell imaging experiments, 100 μL of PC4 (10 μM) and light (12.6 J/cm^2) treated cells were placed on the agarose-treated glass slide for fluorescence imaging.

For PI co-staining experiment, 1 mL of PC4 treated cells was co-stained with PI (1 μg/mL) in the dark for 15 min. The cells were centrifuged and washed with PBS twice. 100 μL of the cells were placed on the agarose-treated glass slide for fluorescence imaging.

For cell death marker study, 1 mL cells were irradiated with UV light for 2 h in PBS buffer. The cells were then stained with PC4 (5 μM, 15 min) and PI (1 μg/mL, 15 min), washed with PBS twice. Finally, 100 μL of the cells were placed on agarose-

treated glass slide for fluorescence imaging. Excitation wavelength: 488 nm for PC4, and 561 nm for PI.

Please note that the microscope imaging recording conditions were different for Fig. 1c and Fig. 4b.

Hemolysis assay

The hemolysis assay was performed according to the reported procedures.[S4] Whole blood was collected from C57BL6/J mice and stored in anticoagulant tube containing EDTA. To get the red blood cells (RBCs), 200 μL blood was diluted into 6 mL PBS. The sample solution was centrifuged at 1,000 rpm for 10 min, the supernatant was removed, and cells washed with PBS, and the same procedures were repeated twice. Then, 500 μL of the RBC solution was mixed with 500 μL of PC4 solution. The final PC4 concentrations were 5, 10, 20, 50 μM. And the RBS samples incubated with PBS and pure water were chosen as negative and positive controls. The sample solutions were incubated at 37 ºC for 1 h, and centrifuged at 1,000 rpm for 10 min. The UV-vis absorption of 200 μL supernatant at 540 nm was collected using a microplate reader. The percentage of hemolysis was calculated according to the following equation:

$$\text{Hemolysis } \% = \frac{A_s - A_n}{A_P - A_n} X100\%$$

A_s: the absorption of sample mixture at 540 nm; A_n: the absorption of negative control at 540 nm; A_p: the absorption of positive control at 540 nm.

Supporting figures

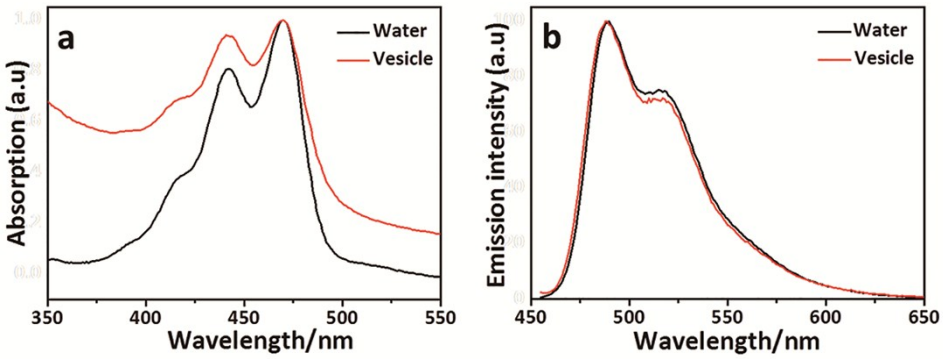

Fig. S1 Normalized UV-vis absorption (a) and fluorescence emission (b) spectra of PC4 in water or in lecithin vesicles, 30 mM PBS. Excitation wavelength: 450 nm.

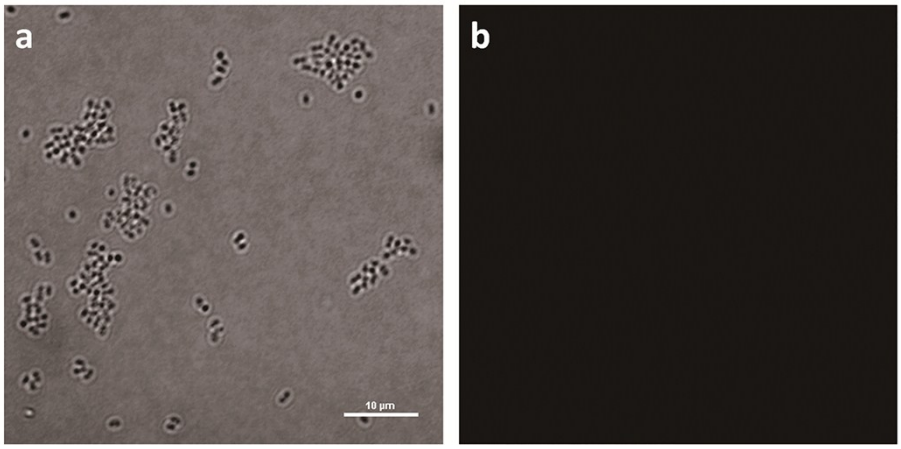

Fig. S2 CLSM images of *E. coli* cells without PC4 in the bright field (a) and fluorescence (b) channel. Scale bar: 10 µm.

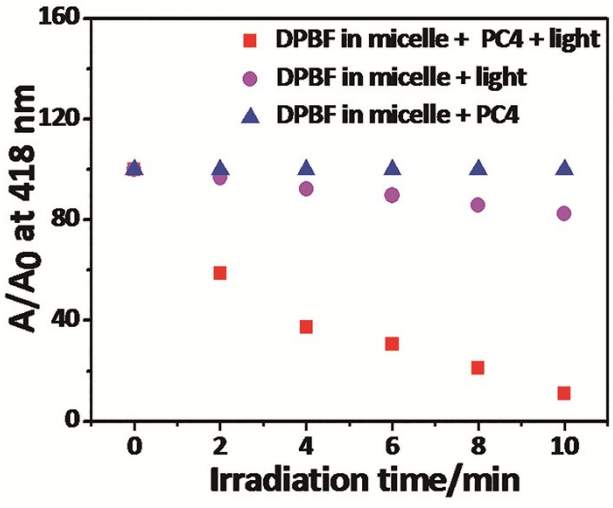

Fig. S3 (a) UV-vis absorption intensity changes of DPBF in micelle with sample incubation time. Conditions: PC4 + light, light or PC4.

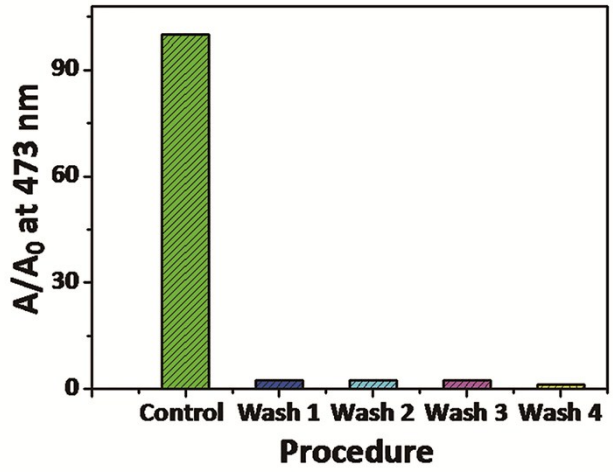

Fig. S4 Binding affinity assay. A_0 is the absorption of PC4 (10 μM), and A is the absorption of the washing medium after removal of the stained cells measured at 473 nm.

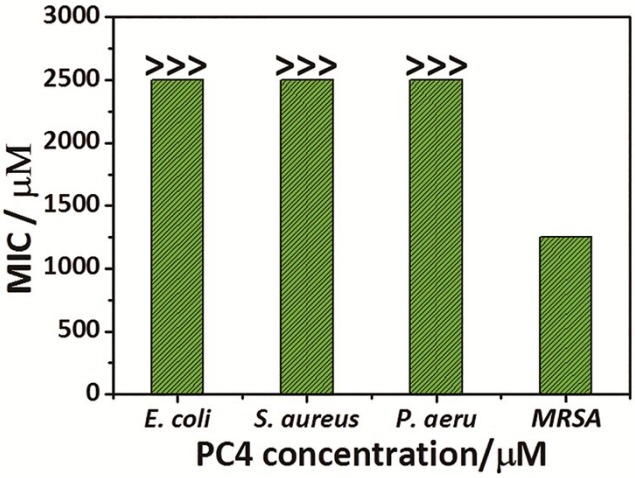

Fig. S5 MIC (minimum inhibitory concentration) of PC4 using different bacteria strains (">>>" refers to a MIC value larger than the highest concentration of PC4 tested).

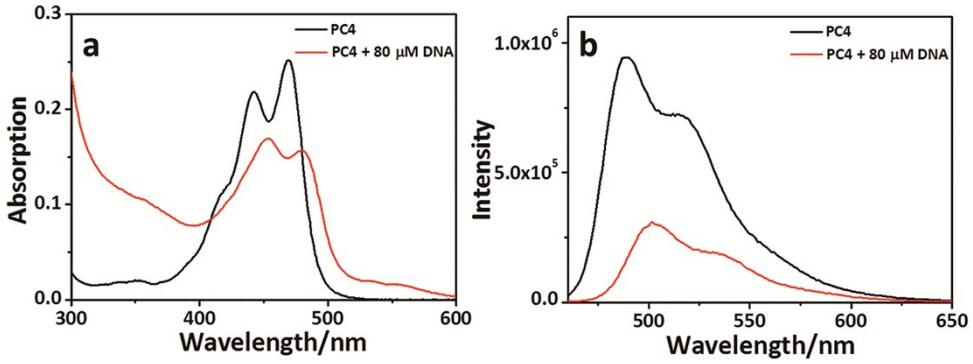

Fig. S6 UV-vis absorption (a) and fluorescence emission (b) changes of PC4 (10 μM) upon addition of calf thymus DNA (80 μM) in phosphate buffer (20 mM, pH 7.4).

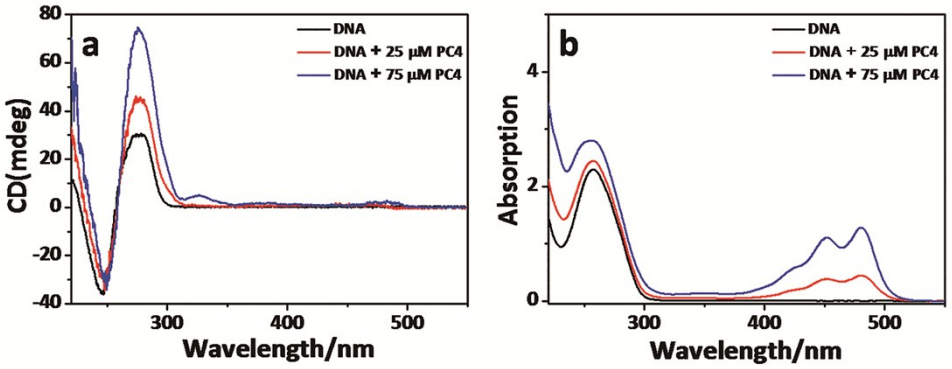

Fig. S7 CD spectra of calf thymus DNA (80 μM) in the absence and presence of PC4 (25 and 75 μM) in the phosphate buffer (20 mM, pH 7.4).

The CD spectra of DNA show a negative band at 247 nm and a positive band at 277 nm, which are characteristics of right-hand B form DNA.[S5] With the addition of PC4, the band intensity at 277 nm increased significantly, the band intensity at 247 nm increased slightly, and weak and broad positive CD signals were observed in the region of the absorption of PC4 (300 – 500 nm). The CD signal changes suggest that PC4 can efficiently bind to DNA.[S6,S7]

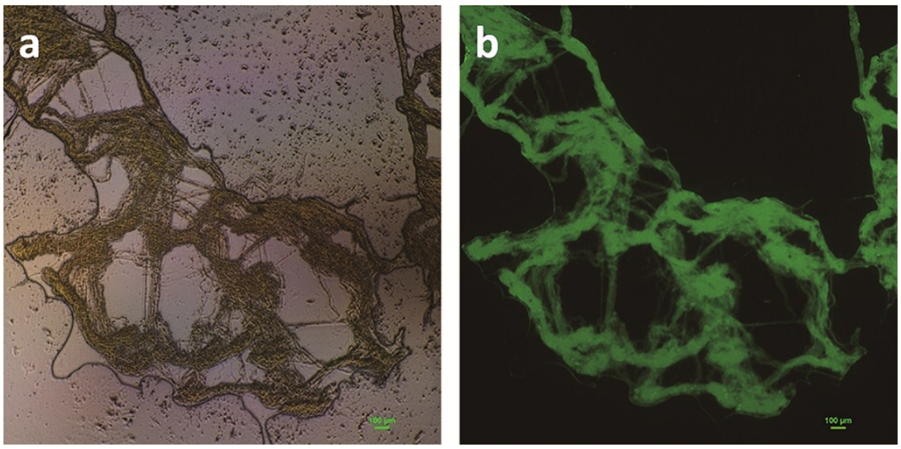

Fig. S8 Fluorescence microscope images of the DNA + PC4 complex under visible light (a) or blue light excitation (b). PC4, 10 μM; calf thymus DNA, 160 μM; scale bar: 100 μm.

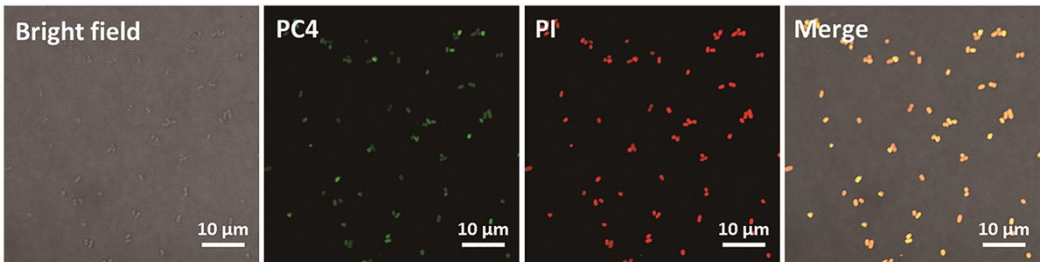

Fig. S9 CLSM images of *E. coli* cells killed after UV light exposure for 2 h, and stained with PC4 (5 μM) and PI (1 μg/mL). Scale bar: 10 μM.

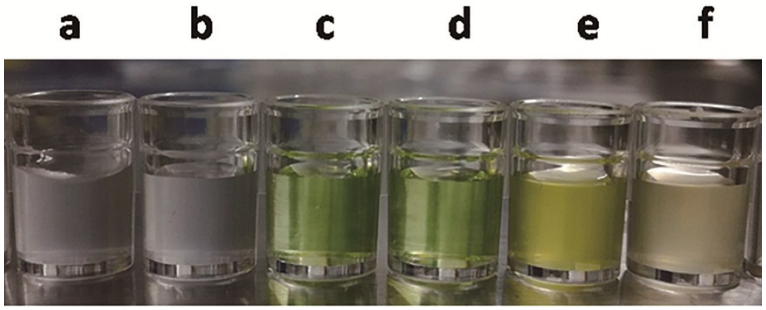

Fig. S10 Photograph of (a) *E. coli*, (b) *E. coli* + light, (c) PC4, (d) PC4 + light, (e) PC4 + *E. coli*, (f) PC4 + *E. coli* + light. Conditions: 30 mM PBS; *E. coli* OD_{600} = 0.5; light, 12.6 J/cm^2; PC4, 10 μM.

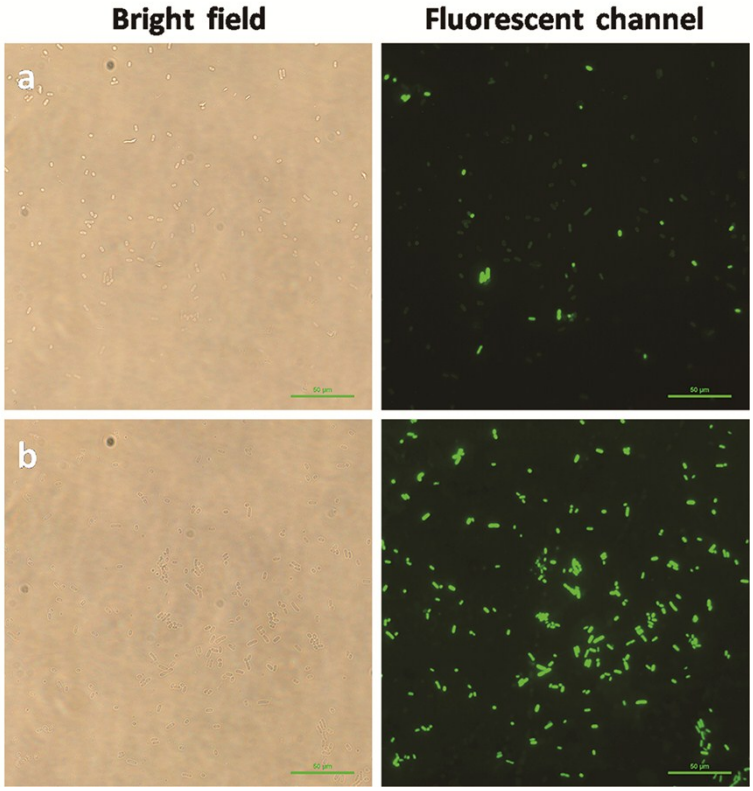

Fig. S11 Fluorescent microscope images of *E. coli* stained with PC4 (10 μM) (a) and killed with PC4 (10 μM+ light) (b). Scale bar: 50 μm.

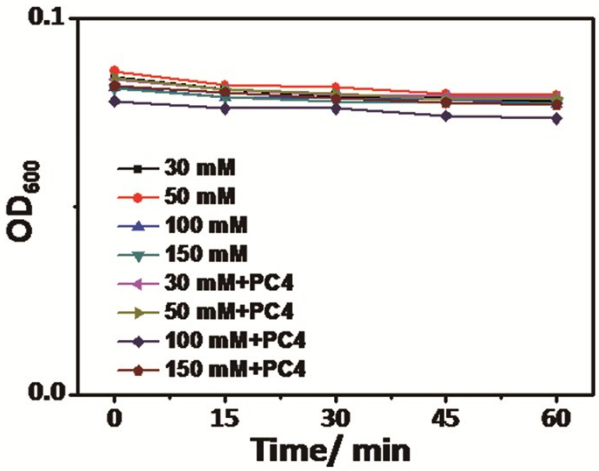

Fig. S12 Cell viability tests in different buffer systems without or with PC4 (10 μM).

References

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