

The Dark Antimicrobial Mechanism of Cationic Phenylene Ethynylene Polymers and Oligomers against *Escherichia coli*

I. Colony Forming Unit (CFU) Counting for *E. coli* cell

Figure S1. CFU counting for the antimicrobial activities of CPEs and OPEs against *E. coli* BL21(DE3)pLysS. Exponential growth phase *E. coli* cells ($\sim 10^8$ CFU/mL) were incubated with 10 μ g/mL CPE or OPE at 37 °C for one hour in the dark followed by 10^6 fold dilution. The diluted samples were loaded on Luria broth agar plates.

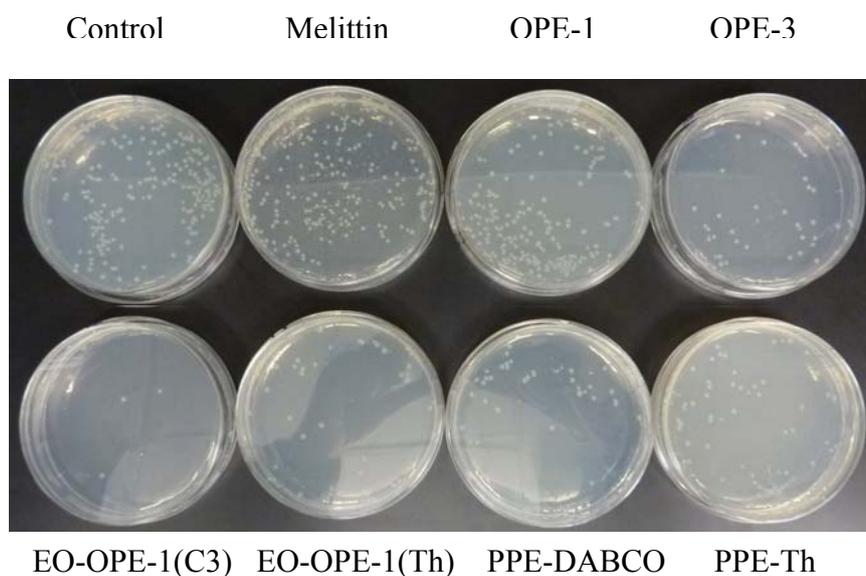
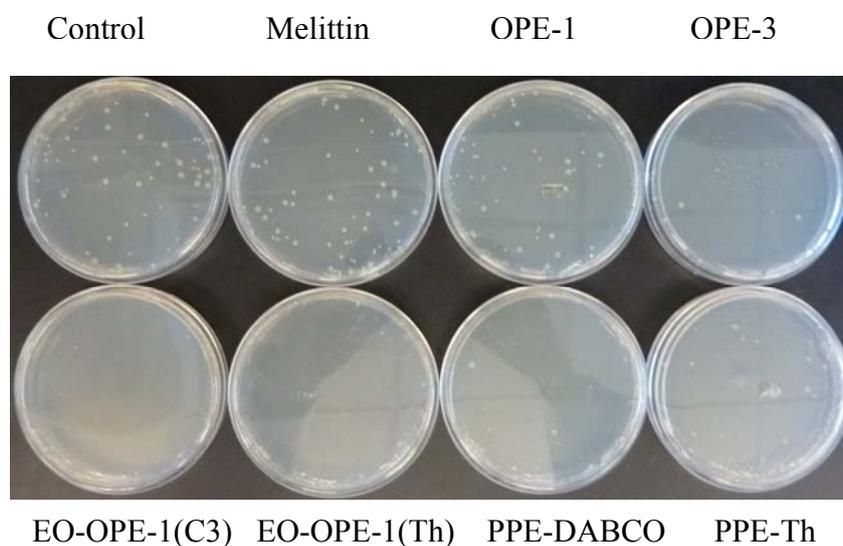


Figure S2. CFU counting for the antimicrobial activities of CPEs/OPEs against *E. coli* (ATCC 11303). Exponential growth phase *E. coli* cells ($\sim 10^8$ CFU/mL) were incubated with 10 μ g/mL CPEs/OPEs at 37 °C for one hour in the dark followed by 10^6 fold dilution. The diluted samples were loaded on Luria broth agar plates.



II. Cytoplasmic Membrane Permeability of CPEs

Experimental Method: Cytoplasmic membrane permeabilization caused by the addition of CPE compounds and melittin was determined by a modified protocol from literature [1]. The *E. coli* cells are first diluted to 10^7 CFU/mL with the HEPES buffer (5 mM HEPES and 5 mM glucose at pH 7.2) followed by the addition of the membrane potential-sensitive cyanine dye diSC3-5 [2] to a final concentration of 0.4 μ M. The mixture of *E. coli* cells and diSC3-5 was incubated in the dark for one hour. Then 100 mM KCl and various amounts of OPEs or melittin were added to the *E. coli* suspensions and incubated for another 30 minutes in the dark. Emission intensity of diSC3-5 was recorded by a spectrofluorometer at 674 nm (QuantaMaster 50 spectrofluorometer, Photon Technology International, Birmingham, NJ) with an excitation wavelength of 651 nm. A negative control sample was also prepared by incubating *E. coli* cells with the dye. The fluorescent intensity of this control was used as background. The membrane permeability assays were run in duplicates and the measurements were reproducible. Since the CPEs can strongly influence the fluorescence of diSC3-5, the effect of CPEs on membrane permeability was not determined.

Results and Discussion: The ability of OPEs and melittin to depolarize the cytoplasmic membrane is determined by using the cationic membrane potential-sensitive cyanine dye diSC3-5. The distribution of diSC3-5 between cell membrane and periphery medium is dependent on the cytoplasmic membrane potential gradient [1]. This cationic dye readily partitions into the bacterial cell membrane and aggregates within the membrane, causing self-quenching [2]. If the antimicrobial compounds perturb the cell membrane, it can lead to the loss of the membrane potential gradient, causing the dye to be released into the medium. As a result, the fluorescence intensity of the dye increases. Hancock *et al.* employed the mutant *E. coli* DC2 cell with increased outer membrane permeability for their cytoplasmic membrane permeability assay [1]. Herein, the laboratory strain *E. coli* BL21(DE3)pLysS cells growing with carbenicillin and chloramphenicol also possess modified loose outer membranes.

After incubating *E. coli* cells in the exponential growth phase with diSC3-5 for one hour, the fluorescence of this membrane potential dye decreased dramatically (Figure S3), which indicates that the dye has been taken up by the *E. coli* cells and that the cells have unperturbed membrane potential gradients. As shown in Figure S4, these antimicrobial compounds exhibit similar membrane permeability abilities against the two different strains of *E. coli* cells. Melittin strongly interacts with bacterial cytoplasmic membrane, causing dye release from the membrane and increasing the fluorescence of the dye in the sample. Melittin exhibits increased membrane perturbation ability with increased concentration. OPE-1 shows a similar concentration dependent trend, but fluorescence intensity is much lower than that caused by melittin, indicative of weaker interaction between OPE-1 and bacterial. Surprisingly, the addition of EO-OPE-1(C3) to both strains of bacteria caused increases in fluorescence at low oligomer concentrations, followed by decreases of diSC3-5's fluorescence at oligomer concentrations higher than 2 μ g/mL. One explanation for the observed trend is that EO-OPE-1(C3) exerts its biocidal activity by bacteriolysis. When the bacteria are disintegrated by EO-OPE-1(C3), the bacterial cytoplasm is released and may strongly interact with the cationic membrane potential dye and quench its fluorescence. Figure S5 shows that DNA is effective at quenching diSC3-5 fluorescence. Therefore the overall fluorescent intensity of diSC3-5 in Figure S4 is the result of two competing

processes. Upon the perturbation of bacterial cytoplasmic membrane by this antimicrobial agent, the released diSC3-5 initially increases the sample's fluorescent intensity. However, disruption of cell wall and membrane can subsequently release DNA and other contents of the cytoplasm, which effectively quench diSC3-5's fluorescence, resulting in overall decreases in the fluorescence intensity of diSC3-5. A similar trend was observed for OPE-3. Due to the loose outer membrane of *E. coli* BL21(DE3)pLysS, the decrease of diSC3-5's fluorescence occurred at a lower concentration of OPE-3 than that of *E. coli* (ATCC 11303). As concluded by Hancock *et al.*, since there is no correlation between the membrane permeability of antimicrobial compounds and their lethal effect against bacteria, other antimicrobial and inhibitory mechanisms may be involved [1]. The high membrane potential gradient perturbation ability of melittin only accounts for its efficient interaction with bacterial cytoplasmic membrane. In conclusion, the results of this assay verify that the four oligomeric antimicrobial compounds used herein can interact with bacterial cytoplasmic membrane and cause membrane depolarization.

Figure S3. Fluorescence change of diSC3-5 (0.4 μM) before and after mixing with fresh exponential growth phase *E. coli* BL21(DE3)pLysS cells (10^7 CFU/mL) in the HEPES buffer (5 mM HEPES, 5 mM glucose, pH 7.2). The same phenomenon was observed for *E. coli* (ATCC 11303) cells.

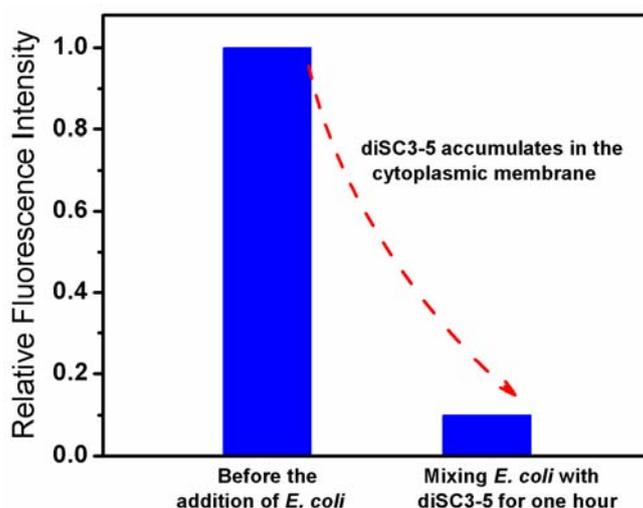


Figure S4. Cytoplasmic membrane permeability of *E. coli* cells induced by melittin and OPEs. The fluorescence changes of diSC3-5 as a function of antimicrobial compound concentration are plotted.

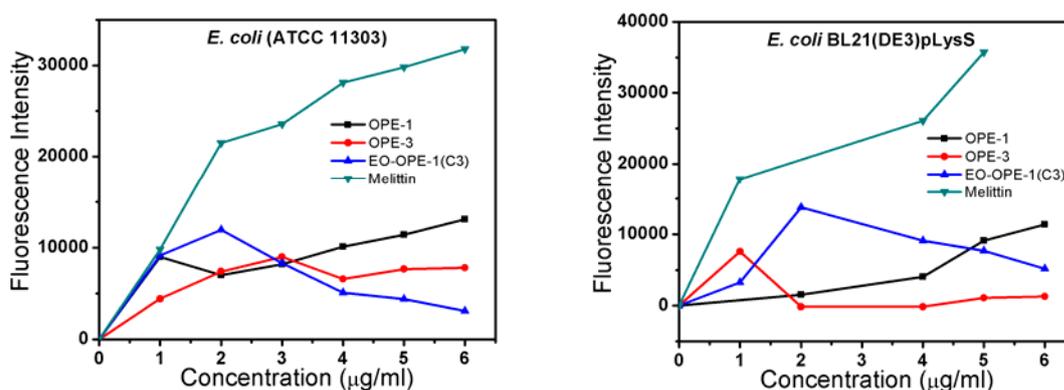
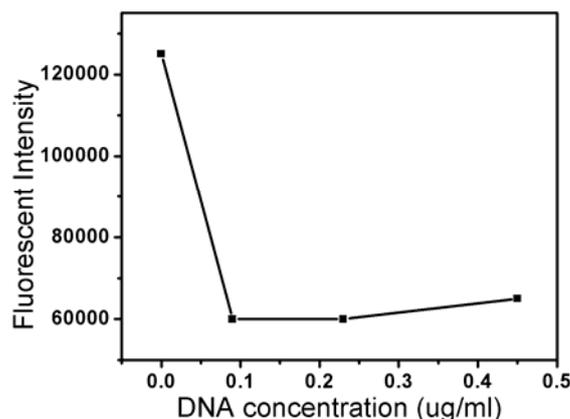
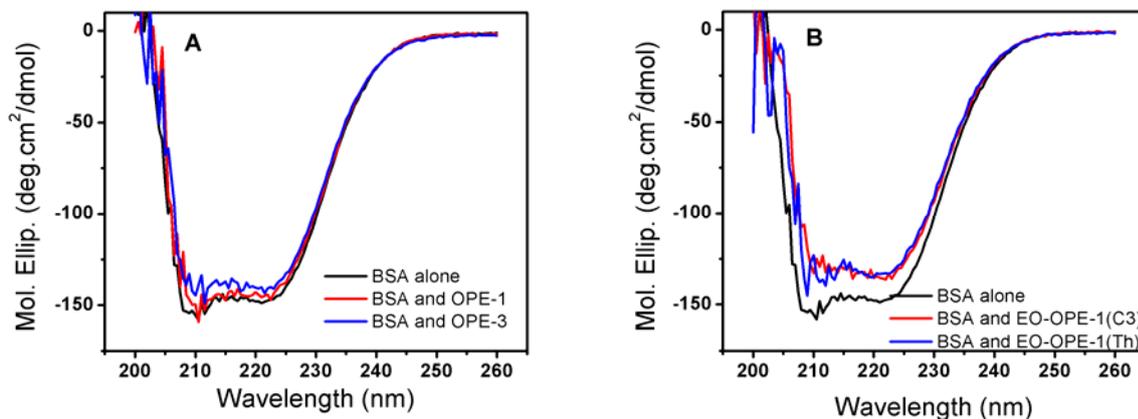


Figure S5. Effect of DNA (plasmid from *E. coli* BL21(DE3)pLysS) on diSC3-5's (0.4 μ M) fluorescence in the HEPES buffer (5 mM HEPES, 5 mM glucose, pH 7.2).



III. OPEs Induced BSA Conformational Changes

Figure S6. Circular dichroism spectra of BSA (0.1 mg/mL) along or in the presence of OPEs (10 μ g/mL) in phosphate buffer (2 mM NaH_2PO_4 , 8 mM Na_2HPO_4 , pH 7.4) at room temperature. OPEs alone do not have any circular dichroism signal.



References

1. Wu, M.H.; Maier, E.; Benz, R.; Hancock, R.E.W. Mechanism of interaction of different classes of cationic antimicrobial peptides with planar bilayers and with the cytoplasmic membrane of *Escherichia coli*. *Biochemistry* **1999**, *38*, 7235-7242.
2. Sims, P.J.; Waggoner, A.S.; Wang, C.H.; Hoffman, J.F. Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. *Biochemistry* **1974**, *13*, 3315-3330.