Supporting Information

Guanidinium-Pendant Oligofluorene for Rapid and Specific Identification of Antibiotics with Membrane-Disrupting Ability

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Experimental Section

Materials: Compound **1** was synthesized according to the procedure in the literature.[1] The ampicillin-resistant *Escherichia coli* (PBV-GFP/DH5α) was constructed by our group. Streptomycin sulfate (STR), Kanamycin sulfate (KAN) and Polymyxin B (PLB) were purchased from Xinjingke Biotechnology Co., Ltd (Beijing, China). Piperacillin sodium salt (TZP) and Levofloxacin (LEV) were purchased from Tokyo chemical industry Co., Ltd. Cefoxitin sodium (FOX) and Mafenide acetate (MAF) were purchased from J&K Scientific Ltd. Polymyxin E (PLE) was purchased from yijishiye Co., Ltd (Shanghai, China). Norfloxacin (NOR) was purchased from Aladdin Industrial Corporation (Shanghai, China). Sulfamethizol (SMT) was purchased from Dr.Ehrenstorfer GmbH (Germany). BugBuster protein extraction reagent and benzonase nuclease were purchased from Merck KGaA, (Darmstadt, Germany). Lysozyme was purchased from

TIANGEN Biotechnology Co., Ltd (Beijing, China). Other chemicals were purchased from Acros, Aldrich Chemical Company or Alfa-Aesar and used as received. All organic solvents were purchased from Beijing Chemical Works and used as received. Water was purified by a Millipore filtration system. Phosphate buffer saline (PBS) was purchased from Hyclone.

Measurements: The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Mass spectra were recorded on a Bruker Apex IV FTMS for high resolution mass spectra (HRMS). UV-Vis absorption spectra were taken on a JASCO V-550 spectrophotometer. Fluorescence measurements were obtained in a 3 mL polystyrene cuvette at room temperature using a Hitachi F-4500 fluorimeter equipped with a Xenon lamp excitation source. The slit width and PMT voltage of the measurements were 2.5 nm and 700 V, respectively. SEM images were performed by Hitachi S-4800 scanning electron microscope. Zeta potentials were measured on a Nano ZS90 (Malvern, UK). Confocal laser scanning microscopy (CLSM) characterization was conducted by a confocal laser scanning biological microscope (FV1200-IX61, Olympus, Japan). The absorbance for IC_{50} measurement was performed on a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 630 nm.

Synthesis of compound 2: To a solution of compound **1** (203.6 mg, 83 μmol) in DMF (20 mL) was added NaN_3 (820 mg, 12.6 mmol) at room temperature, and the mixture was stirred for 5 h at 100°C. After cooling down to room temperature, water (20 mL) was added and the mixture was extracted with dichloromethane (2 × 30 mL). The combined organic layer was washed with water (20 mL) for twice, and then dried over anhydrous $MgSO_4$ for 30 min. After removing the solvent, the residue

was dissolved with tetrahydrofuran (18.5 mL) and water (2.5 mL), and then triphenylphosphine (2.2 g, 8.4 mmol) was added. After the mixture was stirred for 24 h at room temperature, Di-tert-butyl dicarbonate (2.3 g, 10.6 mmol) was added, and the resulting solution was stirred for another 24 h at room temperature. The solvent was removed and the residue was purified by silica gel chromatography using dichloromethane/ethyl acetate (v/v = 3:1) as eluent to give a faint yellow solid (134.7 mg, 57%). ^{1}H-NMR(400 MHz, $CDCl_3$): δ7.68 (m, 26H), 7.36 (m, 6H), 4.44 (br, 10H, NH), 2.98 (s, 20H), 2.04 (d, 20H), 1.40 (d, 90H), 1.28 (s, 20H), 1.10 (s, 40H), 0.70 (d, 20H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ156.0, 151.6, 151.3, 150.8, 140.8, 140.4, 140.1, 140.0, 132.2, 132.1, 132.0, 128.6, 128.5, 127.1, 127.0, 126.2, 126.1, 122.9, 121.3, 121.2, 120.1, 120.0, 128.5, 126.2, 120.1, 78.9, 55.3, 55.1, 40.5, 40.4, 30.0, 29.8, 29.7, 28.4, 27.8, 26.6, 26.5, 24.0 23.8. HRMS (ESI) m/z: $[M+2H]^{2+}$ calcd 1407.9583, found 1407.9598.

Synthesis of OF: Hydrochloride was bubbled into a solution of compound **2** (95.5 mg, 33.9μmol) in 10 mL of trichloromethane for 5 h at room temperature. After turbidity was observed in the solution, 10 mL of methanol was added to dissolve the precipitation and hydrochloride was bubbled into the solution for another 5 h at room temperature. After removing the solvent and excessive hydrochloride, the residue was dried under vacuum to afford compound **3** (73.5 mg, 99.6%). To a solution of compound **3** (44.4 mg, 20.4 μmol) in methanol (2 mL) and tetrahydrofuran (1 mL), diisopropylethylamine (150 μL, 861.2 μmol) was added and the reaction mixture was stirred for 1 h at room temperature. 1H-pyrazole-1-carboxamidine hydrochloride (130 mg, 887 μmol) and diisopropylethylamine (150 μL, 861.2 μmol) were then added into the mixture, and the resulting solution was stirred for 24 h at room temperature. After removing the solvent, the residue was

dissolved with DMSO (1 mL), and then doubly distilled water (10 mL) was added in the solution. The resulting solution was dialyzed using a membrane with a 500 cut-off for one week. Water was removed under vacuum to yield a faint yellow solid (43.6 mg, 95%). ^{1}H-NMR(400 MHz, DMSO-d_6): δ7.85 (m, 26H), 7.36 (br, 46H), 2.97 (br, 20H), 2.21 (br, 20H), 1.26 (br, 20H), 1.10 (br, 40H), 0.65 (br, 20H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 157.0, 151.4, 151.0, 150.4, 140.2, 139.9, 139.6, 139.3, 126.9, 125.9, 122.9, 120.9, 120.5, 55.1, 54.8, 40.6, 40.1, 38.8, 28.8, 28.3, 25.7, 23.5. HRMS (ESI) m/z: $[M+5H]^{5+}$ calcd 447.7268, found 447.7261.

The expression of GFP in *E. coli* and the extraction and purification of GFP

A single colony of *E. coli* on a solid LB/Amp agar plate was transferred to 10 mL of liquid LB/Amp culture medium and grown at 37°C and 180 rpm overnight. 2 mL of the overnight cultures were used to inoculate 75 mL cultures, which were incubated at 30°C and 180 rpm. After 3 hours ($OD_{600} \approx 0.4$), the cultures were induced by adding 75 mL 65°C preheated cultures. After culturing another 6 hours at 40°C and 180 rpm, the expression of GFP in *E. coli* was terminated. GFP was extracted and purified according to the procedure in the literature.[2]

The interactions between OF and GFP

The fluorescence spectra of OF, GFP and OF/GFP ([OF] = 3×10^{-6} M, [GFP] = 8.3×10^{-7} M) in 1 mL of Tris-HCl buffer solution (50 mM, pH7.5) were measured. The solutions of OF, GFP and OF/GFP aforementioned were utilized in CLSM experiment. Solutions (10 μL) of OF, GFP and OF/GFP were added to three CLSM dishes respectively followed by slightly covering coverslips for immobilization. Fluorescence images were then taken by CLSM.

Preparation of antibiotics solutions

The stock solution of NOR were prepared in 0.04 M NaOH solution (the presence of NaOH solution had little effect on interaction *E. coli* and the antibiotics) and that of TZP, LEV and SMT were prepared in DMSO. Other antibiotics stock solutions were prepared in water.

Membrane-disrupting antibiotics screening through the FRET between OF and GFP

1) Preparation of bacteria solution

E. coli were harvested by centrifuging (8000 rpm for 3 min). The supernatant was discarded and *E. coli* was resuspended in liquid LB culture medium , and diluted to an optical density of 2.0 at 600 nm (OD_{600} = 2.0).

2) The interactions between *E. coli* and PLB

After 9 mL of *E. coli* expressing GFP (OD_{600} = 2.0) was centrifuged at 8000 rpm for 3 min, the supernatant was discarded. *E. coli* incubated with PLB of which final concentration is 0.1 mg/mL, 1 mg/mL and 10 mg/mL in 1 mL PBS at 37° C and 180 rpm for 12 h, respectively. For the control group, *E. coli* was resuspended in 1 mL PBS and incubated at 37°C and 180 rpm for 12 h. The fluorescence emission spectrum of OF ([OF] = 3×10^{-6} M) after addition of the *E. coli* (30 μL) and PLB-treated *E. coli* (30 μL) in 1 mL Tris-HCl buffer solution (50 mM, pH 7.5) was measured upon excitation at 380 nm, respectively.

3) The incubation time of *E. coli* and PLB

After 9 mL of *E. coli* expressing GFP (OD_{600} = 2.0) was centrifuged at 8000 rpm for 3 min, the supernatant was discarded. *E. coli* was incubated with 1 mg/mL PLB (final concentration) in 1 mL

PBS at 37°C and 180 rpm. Every two hours, 30 μL of the above *E. coli* solution was added to 1 mL of OF Tris-HCl buffer solution (50 mM, pH 7.5, [OF] = 3×10^{-6} M) and the corresponding fluorescence emission spectrum was measured upon excitation at 380 nm.

4) The interactions between *E. coli* and the antibiotics

After 9 mL of *E. coli* expressing GFP (OD_{600} = 2.0) was centrifuged at 8000 rpm for 3 min, the supernatant was discarded. *E. coli* was incubated with 1 mg/mL (final concentration) of each antibiotics listed in table 1 in 1 mL PBS at 37°C and 180 rpm for 12 h. 30 μL of the above *E. coli* solution was added respectively to 1 mL of OF Tris-HCl buffer solution (50 mM, pH 7.5, [OF] = 3×10^{-6} M) and the corresponding fluorescence emission spectrum was measured. For the control group, *E. coli* was resuspended in 1 mL PBS and incubated at 37°C and 180 rpm for 12 h. The following operations were identical to the experiment group.

SEM characterization

After *E. coli* was incubated with antibiotics, the following operations were conducted totally according to the procedures in the literature.[3]

The measurement of IC_{50} for the antibiotics.

Bacteria were seeded in 96-well plates at a density of 2×10^6 cfu/well in all the experiments. The bacteria used here were *E. coli* (PBV-GFP / DH5α) without expressing GFP. The bacteria were incubated with different concentrations of antibiotics at 37°C for 20 h. After shaking the plate for 2 min, the absorbance at 630 nm of each well was read by a microplate reader. The results were plotted as "inhibition" versus antibiotics concentrations to generate the IC_{50} value, which was

defined as the concentration of antibiotics that cause 50% inhibition of the bacteria. The inhibition (%) was calculated according to the following equation:

$$I\ (\%) = \{[(A-B) - (C-B)]/ (A-B)\} \times 100\%$$

where A is the absorbance of the bacteria control (without adding antibiotics, other operations were identical to the experiment group), B is the absorbance of the culture medium control (only added equivalent culture medium used in experiment group, other operations were identical to the experiment group) and C is the absorbance of the experiment group.

The drafting of standard curve

After 9 mL of *E. coli* expressing GFP (OD_{600} = 1.0) was centrifuged at 8000 rpm for 3 min, the supernatant was discarded. *E. coli* was incubated with PLB of which the final concentration is 2.5 mg/mL in 1 mL PBS at 37°C and 180 rpm for 12 h. For the control group, 9 mL of *E. coli* expressing GFP (OD_{600} = 1.0) was stored at 4°C for 12 h and then centrifuged at 8000 rpm for 3 min. The supernatant was discarded and *E. coli* was resuspended in 1 mL PBS. The *E. coli* incubated with PLB for 12 h was considered as totally disrupted the membrane and the corresponding membrane disrupting degree was defined as 100%. For the control group, the membrane of *E. coli* was not disrupted at all and the corresponding membrane disrupting degree was defined as 0. To plot the standard curve applied for determining the membrane disrupting degree of antibiotics, the PLB-treated *E. coli* and *E. coli* in control group were proportionally mixed. The membrane disrupting degree of the mixture was equal to the mixed proportion. For example, the membrane disrupting degree of the mixture of 5 μL PLB-treated *E. coli* and 45 μL *E. coli* in control group was 10%. Different mixtures (50 μL) were respectively added to 950 μL of OF Tris-

HCl buffer solution (50 mM, pH 7.5, the final concentration of OF was 3×10^{-6} M) and the corresponding fluorescence emission spectrum was measured upon excitation at 380 nm. Finally, the standard curve applied for determining the membrane disrupting degree of antibiotics was plotted.

Detection of membrane-disrupting ability of antibiotics: 9 mL of *E. coli* expressing His6-GFP (OD_{600} = 1.0) was centrifuged at 8000 rpm for 3 min, and then the supernatant was discarded. *E. coli* was incubated with different concentrations of antibiotics in 1 mL PBS at 37°C and 180 rpm for 12 h. To 950 μL of **OF** solution in Tris-HCl buffer solution (50 mM, pH 7.5) was added 50 μL above *E. coli* solution (the final concentration of OF was 3×10^{-6} M) and the corresponding fluorescence emission spectra were measured upon excitation at 380 nm. The FRET ratio ($I_{515\ nm}/I_{428\ nm}$) of **OF**/His6-GFP pair was calculated based on the emission intensities of **OF** and His6-GFP.

a

	OF	His6-GFP	OF/His6-GFP
Zeta potential (mV)	24.0±3.3	-15.2±1.4	16.7±1.3

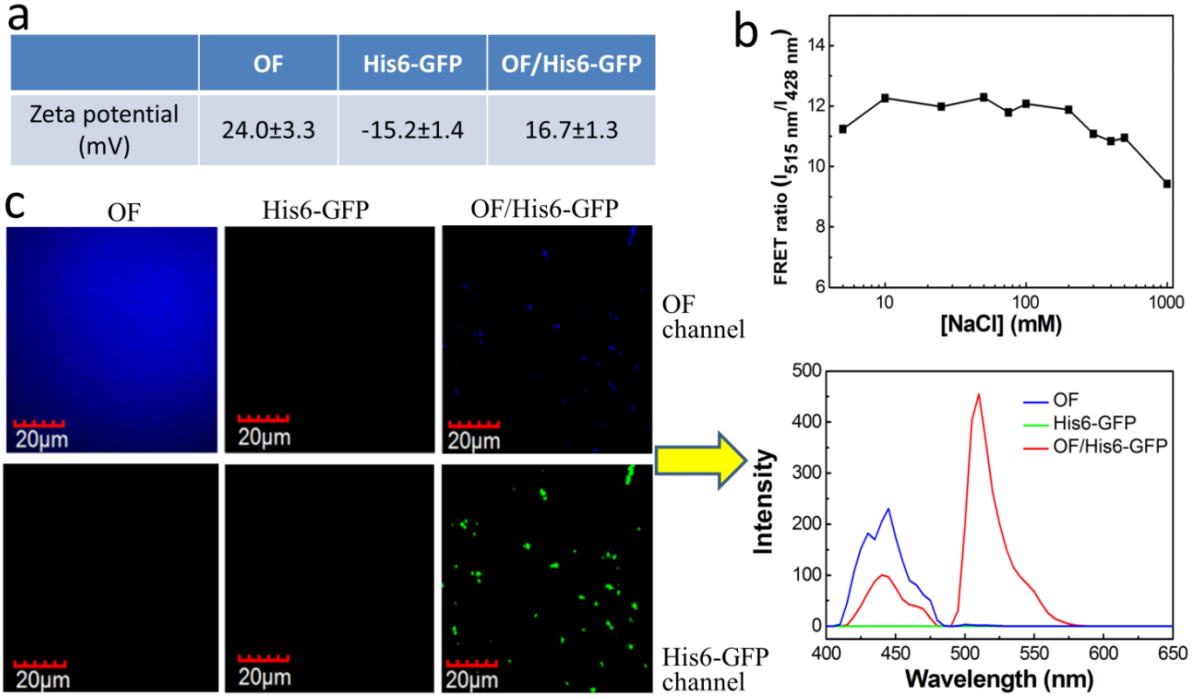

Figure S1. a) Zeta potentials of OF, His6-GFP and OF/His6-GFP complex in Tris-HCl buffer solution (50 mM, pH = 7.5) at room temperature. [OF] = 3×10^{-6} M, [His6-GFP] = 8.3×10^{-7} M. Each value was an average of three measurements. b) The FRET ratio ($I_{515\ nm}/I_{428\ nm}$) of OF/His6-GFP pair versus ionic strength. [OF] = 3×10^{-6} M, [His6-GFP] = 8.3×10^{-7} M. All measurements were performed in Tris-HCl buffer solution (50 mM, pH = 7.5) with an excitation at 380 nm. c) Confocal laser scanning microscopy images of OF, His6-GFP, and OF/His6-GFP complex in Tris-HCl buffer solution and their corresponding spectra.

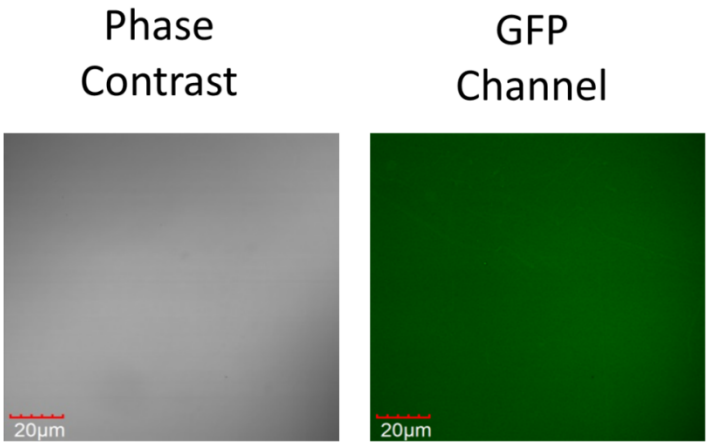

Figure S2. Fluorescence images of GFP obtained from CLSM in Tris-HCl buffer solution (50 mM, pH7.5) upon excitation at 480 nm ([GFP] = 8.3 $\times 10^{-7}$ M) .

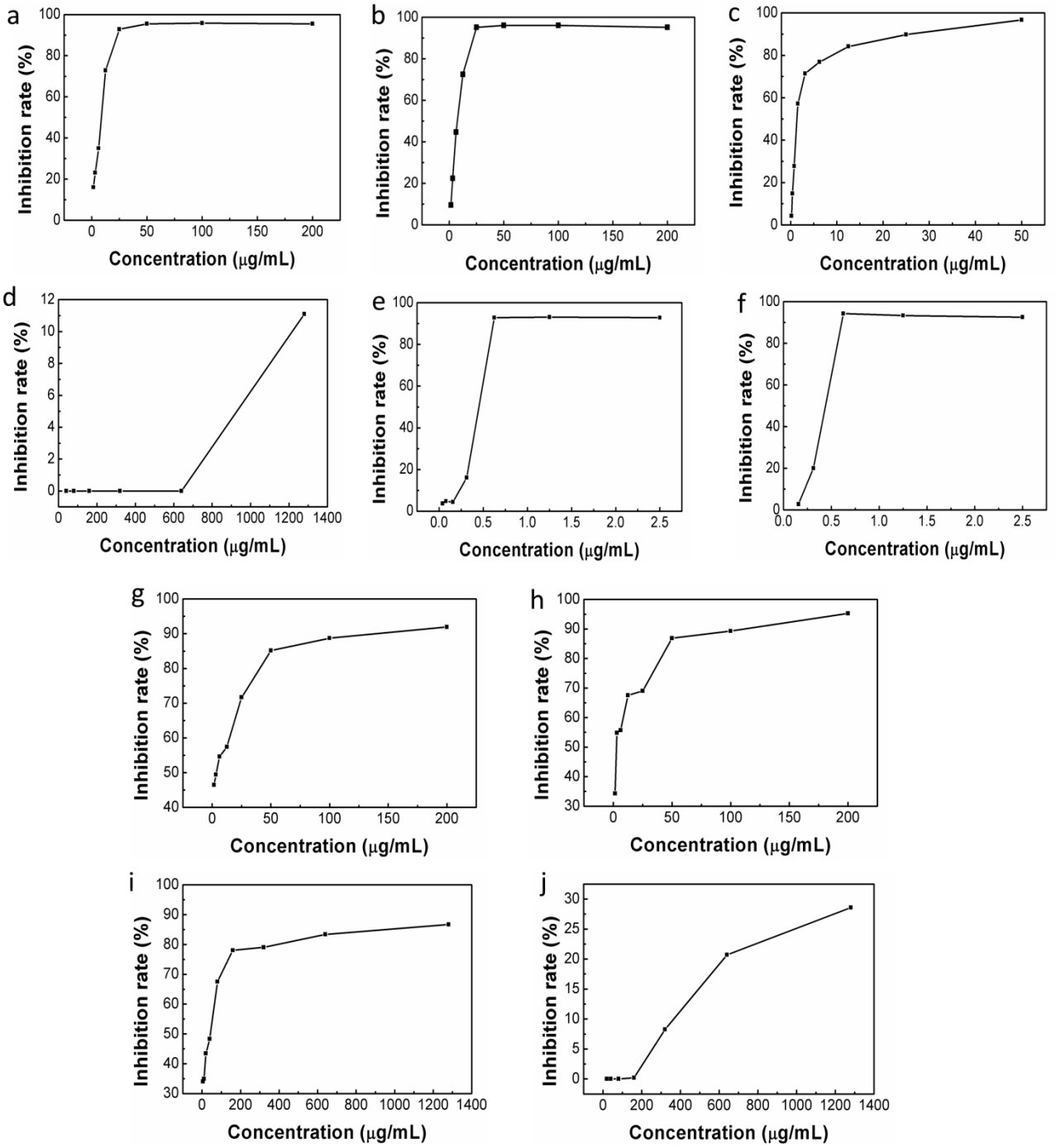

Figure S3. The inhibition rate toward *E. Coli* of STR (a), KAN (b), FOX (c), TZP (d), PLB(e), PLE (f), LEV (g), NOR (h), SMT (i) and MAF (j).

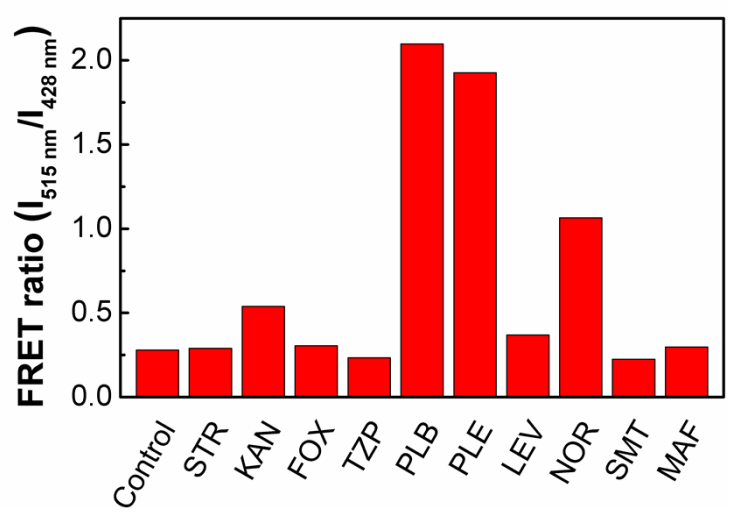

Figure S4. The FRET ratio ($I_{515\ nm}/I_{428\ nm}$) of OF/GFP pair versus different antibiotics at 4 h.

Table S1. The antibiotics used in the experiment, their antibacterial activities (IC_{50}) and mechanisms.

Classification	**Antibiotics**	**IC_{50} (μg/mL)**	**Mechanism of action**
Aminoglycosides	Streptomycin sulfate (STR)	< 12.5	Inhibition of protein synthesis[4-7]
	Kanamycin sulfate (KAN)	< 12.5	
β-Lactams	Cefoxitin sodium (FOX)	< 1.56	Inhibition of cell-wall turnover[4,7]
	Piperacillin sodium salt (TZP)	> 1280	
Polypeptides	Polymyxin B (PLB)	< 0.625	Disruption of the outer membrane[8]
	Polymyxin E (PLE)	< 0.625	
Quinolones	Levofloxacin (LEV)	< 6.25	Inhibition of DNA replication and repair[4,9]
	Norfloxacin (NOR)	< 3.13	
Sulfonamides	Sulfamethizol (SMT)	< 80	Inhibition of dihydropteroate synthase[10-13]
	Mafenide acetate (MAF)	> 1280	

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