ACS APPLIED MATERIALS & INTERFACES

Letter

www.acsami.org

Conjugated Polythiophene for Rapid, Simple, and High-Throughput Screening of Antimicrobial Photosensitizers

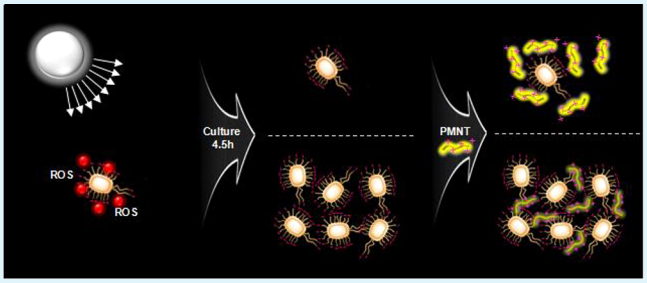
Ruihua Li,[†,‡] Ruimin Niu,[§,‡] Junjie Qi,[§] Hongbo Yuan,[§] Yibing Fan,[§] Hailong An,[§] Wenmin Yan,[†] Huanrong Li,[†] Yong Zhan,*[,§] and Chengfen Xing*[,†,§]

[†]School of Chemical Engineering and Technology, Hebei University of Technology, Tianjin 300130, P. R. China

[§]Key Laboratory of Hebei Province for Molecular Biophysics, Institute of Biophysics, Hebei University of Technology, Tianjin 300401, P. R. China

Supporting Information

ABSTRACT: The cationic conjugated poly[3-(3′-*N*,*N*,*N*-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride] (PMNT) has been developed for high-throughput screening of photodynamic antimicrobial chemotherapy photosensitizers (PSs). The bacterial number can be detected quantitatively by PMNT via various fluorescence quenching efficiencies. The photosensitized inactivation of bacteria is not efficient with ineffective PSs, and thus the bacteria grow exponentially and can be coated tightly by PMNT through electrostatic and hydrophobic interactions, resulting in aggregates and fluorescence quenching of PMNT, whereas, conversely, effective PSs lead to original and strong fluorescence of PMNT. This new platform of high-throughput screening is promising for discovering new PSs.



KEYWORDS: *biosensor, conjugated polymers, fluorescence, high-throughput screening, photosensitizer*

With the overuse of antibiotics in the past decades, the antibiotic resistance of microorganisms is increasing and has become a serious therapeutic problem.[1,2] Thus, new antimicrobial drugs and efficient therapy strategies for the treatment of pathogenic infections are becoming extremely important.[3,4] Photodynamic antimicrobial chemotherapy (PACT) can be effective in killing microbial cells with resistance to antibiotics.[5,6] Additionally, PACT can be designed to target localized infections and offers little possibility to produce photoresistant species.[7,8] Because the behavior of photosensitizers (PSs) is crucial for enhancing the efficiency of PACT, accessing and finding suitable and efficient PSs have been extensively explored.[9] However, traditional methods for photocytotoxicity screening of PS including agar well dilution assay, agar spot assay, and a disk diffusion test require long incubation time, which is usually over 20 h, and depend on the stability of PSs,[10−12] which is time-consuming and insensitive. To improve the techniques of antimicrobial activity testing, several sensitive and rapid strategies such as real-time principal-component regression,[13] flow cytometry,[14] and adenosine triphosphatase based measurements[15] have been developed. However, either expensive equipment and materials or complicated operations limit their extensive application.[16] High-throughput screening techniques exhibit high efficiency and improved functionality relative to the traditional methods.[17] For example, a droplet-based microfluidic system was reported recently to perform high-throughput screening of PS activity by multiparameter assessment.[18] Currently, more simple and rapid high-throughput screening strategies of PS activity in PACT are still in urgent need.[9]

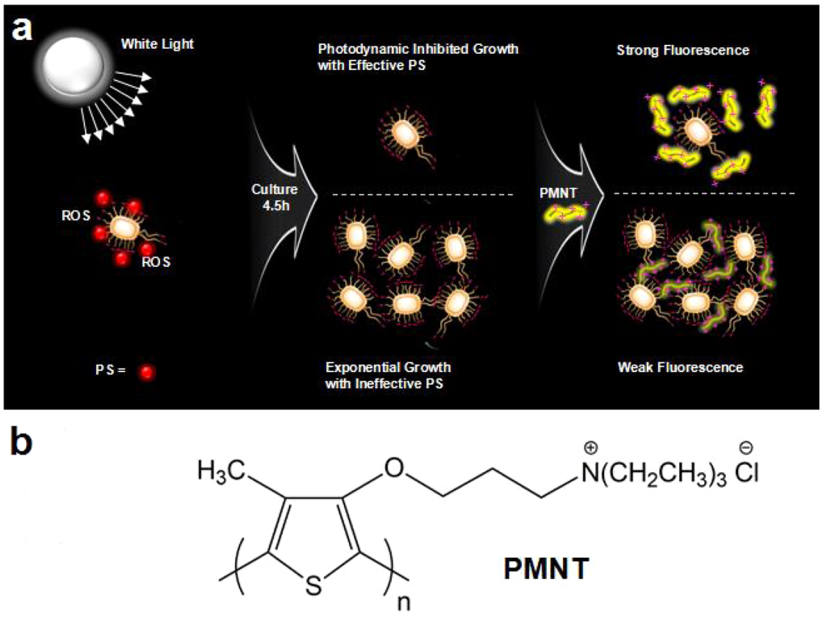
Conjugated polymers (CPs) have been extensively explored for chemical sensors and biosensors based on their excellent light-harvesting and optical amplification.[19−23] Water-soluble CPs containing multivalent binding sites with microorganisms have been applied for microbial pathogen detection.[24−29] Recently, Wang and co-workers developed a fluorescence resonance energy transfer based strategy for the rapid and high-throughput screening of antibiotics by using cationic conjugated polyfluorene (donor) and fluorescein (acceptor).[30] Inspired by these systems, we describe here a new, rapid, simple, and high-throughput method with only a single cationic conjugated polythiophene derivative, poly[3-(3′-*N*,*N*,*N*-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride] (PMNT), to realize the screening of PS activity.

As illustrated in Scheme 1, the antimicrobial activity assessment for PSs in PACT is based on the fluorescence intensity changes of PMNT. In the presence of ineffective PSs, the photosensitized inactivation of bacteria under irradiation is not efficient. Therefore, the bacteria grow exponentially and can be coated tightly by PMNT through electrostatic and hydrophobic interactions between PMNT and negatively

Received: May 25, 2015
Accepted: July 2, 2015
Published: July 2, 2015

DOI: 10.1021/acsami.5b04552
ACS Appl. Mater. Interfaces 2015, 7, 14569−14572

Scheme 1. (a) Schematic Representation of a PMNT-Based Platform for Screening of PACT PSs and (b) Chemical Structure of PMNT



charged bacteria, resulting in the formation of aggregates and the fluorescence quenching of PMNT. In the presence of effective PSs, the bacteria can be efficiently killed in PACT and the fluorescence of PMNT is not obviously affected, leading to original and strong fluorescence of PMNT in the system. By detection of the fluorescence intensity of PMNT, it is possible to evaluate the antimicrobial activity and find new effective PSs in PACT.

Figure 1a shows the fluorescence emission spectra of PMNT in a 40% ethanol aqueous solution with the successive addition of an *Escherichia coli* suspension. The emission of PMNT ([PMNT] = 100.0 μM in repeat units, RUs) was gradually quenched with increasing colony forming units (Cfu) of *E. coli*. The fluorescence quenching efficiencies $(1 - I/I_0)$ of PMNT in the presence of various amounts of *E. coli* were evaluated as well. As shown in Figure 1b, the plot of the emission quenching efficiency versus the amount of *E. coli* can be applied as the standard curve to quantitatively determine the *E. coli* amount. The detection limit of *E. coli* is 3.0×10^5 Cfu, demonstrating that water-soluble PMNT offers a fluorescence platform for sensitive quantitative testing of the *E. coli* amount.

To directly visualize formation of the PMNT/*E. coli* complex, the coating of PMNT to the surface of *E. coli* was checked by fluorescence microscopy. As illustrated in Figure 1c,d, phase-contrast and fluorescence microscopy images of the PMNT/*E. coli* complex were studied; the *E. coli* cells were fluorescent with incubation with PMNT, indicating the binding of PMNT with the surface of *E. coli*. Furthermore, the ζ potentials provide further evidence for interactions between *E. coli* and PMNT (Table 1). The ζ potential of *E. coli* became more positive upon incubation with PMNT at a concentration of 100.0 μM in RUs, and overcompensation in the *E. coli* surface charge was observed, demonstrating the tight coating and aggregation of PMNT to the *E. coli* surface. These observations prove that formation of the PMNT/*E. coli* complex through strong electrostatic and hydrophobic interactions between PMNT and *E. coli* lead to the aggregation and fluorescence quenching of PMNT.

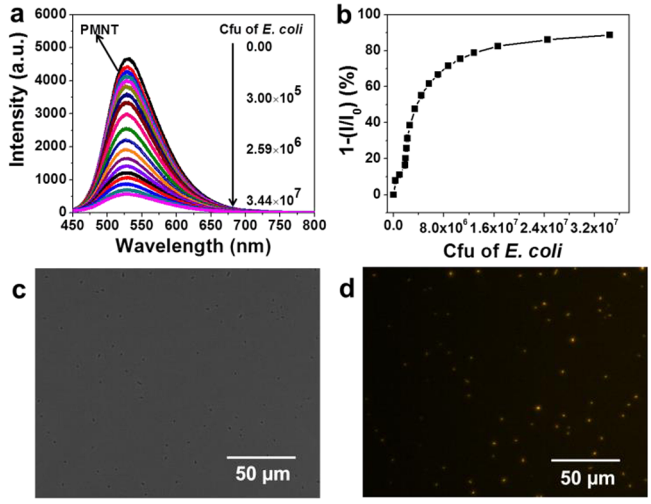


Figure 1. Fluorescence emission spectra and images of the PMNT/*E. coli* complex. (a) Fluorescence emission spectra of PMNT in a 40% ethanol aqueous solution with the successive addition of an *E. coli* suspension. (b) Fluorescence quenching efficiencies of PMNT with different amounts of *E. coli*. I_0 and I represent the emission intensity of PMNT before and after the addition of *E. coli*. The excitation wavelength is 428 nm. The error bars show the standard deviations of three separate experiments. (c) Phase-contrast image and (d) fluorescence microscopy image of the PMNT/*E. coli* complex in aqueous solution. The phase-contrast image was taken at 1 ms exposure time, and the fluorescence microscopy image was taken at 1000 ms exposure time. The pseudocolor is yellow, and the types of light filters are D470/20 nm exciter, 400 nm beamsplitter, and D605/70 nm emitter. The magnification of the objective lens is 40×.

The PACT efficacy of six different PSs toward the antibiotic-resistant *E. coli* was examined in a high-throughput fashion for PS screening. A 96-well microplate was applied for the high-throughput screening measured by the automatic microplate reader. Figure 2a shows the schematic representation of the high-throughput screening of PS activity in the fluorescence pattern on a microplate corresponding to various PSs with

DOI: 10.1021/acsami.5b04552
ACS Appl. Mater. Interfaces 2015, 7, 14569−14572

Table 1. ζ Potentials and Average Hydrodynamic Diameter of PMNT, *E. coli*, and PMNT/*E. coli* in a 40% Ethanol Aqueous Solution Checked by Dynamic Light Scattering Measurements ([PMNT] = 100.0 μM in RUs; [*E. coli*] = 3.42 × 10^6 Cfu mL^{-1})

	ζ potential (mV)	diameter (nm)
PMNT	18.5 ± 3.4	138.0 ± 5.9
E. coli	−20.1 ± 6.2	523.4 ± 10.4
PMNT/*E. coli*	40.7 ± 2.8	780.3 ± 70.4

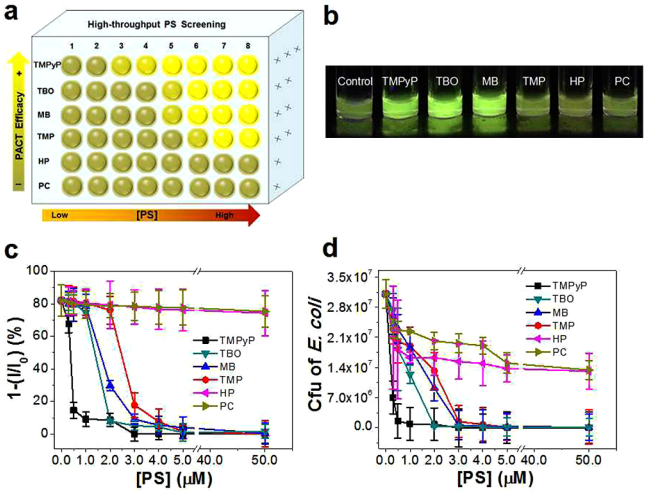


Figure 2. (a) Scheme of the high-throughput screening of PS activity in the fluorescence pattern on a microplate corresponding to various PSs with different PACT efficacies. 1−8 represent PS with increasing concentration, and the vertical axis represents different PSs with increasing PACT efficacy. Light- and dark-yellow wells represent efficient and inefficient photoinactivation of bacteria. (b) Photographs of *E. coli* after PACT treatments with different PSs in the presence of PMNT under UV light (λ_{max} = 365 nm). [PMNT] = 100 μM in RUs; [PS] = 5.0 μM. (c) Plot of the fluorescence quenching efficiency of PMNT as a function of the PS concentration. [PMNT] = 100 μM in RUs. The excitation wavelength is 428 nm. The measurements were performed in a 40% ethanol aqueous solution. The error bars show the standard deviations of three separate experiments. (d) Plot of the *E. coli* amount versus the PS concentration. See the Supporting Information for definitions of PS abbreviations. The error bars show the standard deviations of three separate experiments.

different PACT efficacies. For the PS with high PACT efficacy, the light-yellow fluorescent signal obtained from the direct readout of the microplate reader is maintained. However, the PS with low PACT efficacy causes a change from light yellow to dark yellow. The photographs of *E. coli* after PACT treatments with different PSs with one concentration of 5.0 μM in the presence of PMNT ([PMNT] = 100 μM in RUs) under UV light (λ_{max} = 365 nm) were also checked. As shown in Figure 2b, an emission color of light yellow indicates efficient photoinactivation of bacteria by PS, while a dark-yellow color illustrates inefficient photoinactivation. For high-throughput screening, an *E. coli* suspension was incubated with various PSs in different concentrations in a microplate and then exposed to an optical fiber of 90 mW cm^{-2} white light for 5 min (27 J cm^{-2}). Subsequently, *E. coli* was cultured at 37 °C for 4 h, and then the bacterial pellet obtained by centrifugation was suspended and added into the 40% ethanol aqueous solution of PMNT ([PMNT] = 100 μM in RUs) in a 96-well microplate for fluorescence measurement by an automatic microplate reader. It is worth noting that ethanol is only present in the detection of fluorescence, and the "mix-and-detect" approach to assay bacteria leads to rapid detection, without an ethanol effect on the growth of bacteria. Figure 2c shows a plot of the fluorescence quenching efficiency of PMNT ([PMNT] = 100 μM in RUs) as a function of the PS concentration, and a curve of the *E. coli* amount versus the PS concentration (Figure 2d) can be obtained accordingly based on the standard curve for quantitatively determining the *E. coli* amount, as shown in Figure 1b. The six PSs can be divided into three types: highly efficient (TMPyP, TBO, and MB), efficient (TMP), and inefficient (HP and PC). TMPyP is superefficient in killing *E. coli* cells with white-light irradiation. The *E. coli* suspension after treatment with TMPyP ([TMPyP] > 3.0 μM) in PACT caused no fluorescence quenching (quenching efficiency = 0.0%) when added into the solution of PMNT for fluorescence measurement, illustrating the complete killing of *E. coli* cells. However, negatively charged PSs of HP and PC cannot lead to efficient reduction of the cell viability in PACT, demonstrating that electrostatic repulsion between anionic PS and the surface of *E. coli* leads to less adsorption of PS to *E. coli*.

In summary, we have developed a CP-based platform for the high-throughput screening of PACT PSs. This new strategy has three significant characteristics. First, the single self-luminous PMNT molecules can simply be used to quantitatively measure the amounts of bacteria in high sensitivity only via various fluorescence quenching efficiencies in the presence of bacteria. Second, no expensive materials and instruments were required, leading to the low cost of our method (~$1.0 for one high-throughput screening of six PSs). Third, the high-throughput screening of PACT efficacy only takes 4.5 h to complete the assay including incubation, irradiation, culture, and detection. This new platform is efficient and promising for discovering new kinds of PACT PSs.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, characterization, device fabrication, and measurements. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b04552.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: zhan_yong2014@163.com.
*E-mail: xingc@hebut.edu.cn.

Author Contributions

‡These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Prof. Libing Liu (Institute of Chemistry, Chinese Academy of Sciences) for valuable suggestions. We are grateful for financial support of the "100 Talents" Program of Hebei Province, China (Grant E2014100004), the Program for Excellent Innovative Talents in Universities of Hebei Province, China (Grant BJ2014011), the Natural Science Foundation of Hebei Province (Grants B2015202330 and E2015202073), and the Tianjin Natural Science Foundation (Grant 15JCYBJC17500).

DOI: 10.1021/acsami.5b04552
ACS Appl. Mater. Interfaces 2015, 7, 14569−14572

REFERENCES

(1) Khan, R.; Islam, B.; Akram, M.; Shakil, S.; Ahmad, A.; Ali, S. M.; Siddiqui, M.; Khan, A. U. Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *Molecules* **2009**, *14*, 586−597.

(2) Morens, D. M.; Folkers, G. K.; Fauci, A. S. The Challenge of Emerging and Re-emerging Infectious Diseases. *Nature* **2004**, *430*, 242−249.

(3) Liu, F.; Soh Yan Ni, A.; Lim, Y.; Mohanram, H.; Bhattacharjya, S.; Xing, B. Lipopolysaccharide Neutralizing Peptide−Porphyrin Conjugates for Effective Photoinactivation and Intracellular Imaging of Gram-Negative Bacteria Strains. *Bioconjugate Chem.* **2012**, *23*, 1639−1647.

(4) Smith, P. A.; Romesberg, F. E. Combating Bacteria and Drug Resistance by Inhibiting Mechanisms of Persistence and Adaptation. *Nat. Chem. Biol.* **2007**, *3*, 549−556.

(5) Ohulchanskyy, T. Y.; Gannon, M. K.; Ye, M.; Skripchenko, A.; Wagner, S. J.; Prasad, P. N.; Detty, M. R. "Switched-On" Flexible Chalcogenopyrylium Photosensitizers. Changes in Photophysical Properties upon Binding to DNA. *J. Phys. Chem. B* **2007**, *111*, 9686−9692.

(6) Donnelly, R. F.; McCarron, P. A.; Tunney, M. M. Antifungal Photodynamic Therapy. *Microbiol. Res.* **2008**, *163*, 1−12.

(7) Li, K.; Lei, W.; Jiang, G.; Hou, Y.; Zhang, B.; Zhou, Q.; Wang, X. Selective Photodynamic Inactivation of Bacterial Cells over Mammalian Cells by New Triarylmethanes. *Langmuir* **2014**, *30*, 14573−14580.

(8) Cassidy, C. M.; Tunney, M. M.; McCarron, P. A.; Donnelly, R. F. Drug Delivery Strategies for Photodynamic Antimicrobial Chemotherapy: from Benchtop to Clinical Practice. *J. Photochem. Photobiol., B* **2009**, *95*, 71−80.

(9) Banfi, S.; Caruso, E.; Buccafurni, L.; Battini, V.; Zazzaron, S.; Barbieri, P.; Orlandi, V. Antibacterial Activity of Tetraaryl-Porphyrin Photosensitizers: An in Vitro Study on Gram Negative and Gram Positive Bacteria. *J. Photochem. Photobiol., B* **2006**, *85*, 28−38.

(10) Jorgensen, J. H. Selection Criteria for an Antimicrobial Susceptibility Testing System. *J. Clin. Microbiol.* **1993**, *31*, 2841−2844.

(11) Rahman, M.; Kuhn, I.; Rahman, M.; Olsson-Liljequist, B.; Mollby, R. Evaluation of a Scanner-Assisted Colorimetric MIC Method for Susceptibility Testing of Gram-Negative Fermentative Bacteria. *Appl. Environ. Microbiol.* **2004**, *70*, 2398−2403.

(12) Pereira, E.; Santos, A.; Reis, F.; Tavares, R. M.; Baptista, P.; Lino-Neto, T.; Almeida-Aguiar, C. A New Effective Assay to Detect Antimicrobial Activity of Filamentous Fungi. *Microbiol. Res.* **2013**, *168*, 1−5.

(13) Rolain, J. M.; Mallet, M. N.; Fournier, P. E.; Raoult, D. Real-Time PCR for Universal Antibiotic Susceptibility Testing. *J. Antimicrob. Chemother.* **2004**, *54*, 538−541.

(14) Budde, B. B.; Rasch, M. A Comparative Study on the Use of Flow Cytometry and Colony Forming Units for Assessment of the Antibacterial Effect of Bacteriocins. *Int. J. Food Microbiol.* **2001**, *63*, 65−72.

(15) Finger, S.; Wiegand, C.; Buschmann, H. J.; Hipler, U. C. Antibacterial Properties of Cyclodextrin-Antiseptics-Complexes Determined by Microplate Laser Nephelometry and ATP Bioluminescence Assay. *Int. J. Pharm.* **2013**, *452*, 188−193.

(16) Wainwright, M. Phenothiazinium Photosensitisers: V. Photobactericidal Activities of Chromophore-Methylated Phenothiazinium Salts. *Dyes Pigm.* **2007**, *73*, 7−12.

(17) Segers, K.; Klaassen, H.; Economou, A.; Chaltin, P.; Anne, J. Development of a High-Throughput Screening Assay for the Discovery of Small-Molecule SecA Inhibitors. *Anal. Biochem.* **2011**, *413*, 90−96.

(18) Cho, S.; Kang, D. K.; Sim, S.; Geier, F.; Kim, J. Y.; Niu, X.; Edel, J. B.; Chang, S. I.; Wootton, R. C.; Elvira, K. S.; deMello, A. J. Droplet-Based Microfluidic Platform for High-Throughput, Multi-Parameter Screening of Photosensitizer Activity. *Anal. Chem.* **2013**, *85*, 8866−8872.

(19) Zhu, C.; Liu, L.; Yang, Q.; Lv, F.; Wang, S. Water-Soluble Conjugated Polymers for Imaging, Diagnosis, and Therapy. *Chem. Rev.* **2012**, *112*, 4687−4735.

(20) Thomas, S. W.; Joly, G. D.; Swager, T. M. Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers. *Chem. Rev.* **2007**, *107*, 1339−1386.

(21) Usmani, S. M.; Zirafi, O.; Muller, J. A.; Sandi-Monroy, N. L.; Yadav, J. K.; Meier, C.; Weil, T.; Roan, N. R.; Greene, W. C.; Walther, P.; Nilsson, K. P. R.; Hammarstrom, P.; Wetzel, R.; Pilcher, C. D.; Gagsteiger, F.; Faendrich, M.; Kirchhoff, F.; Munch, J. Direct Visualization of HIV-Enhancing Endogenous Amyloid Fibrils in Human Semen. *Nat. Commun.* **2014**, *5*, 3508−3515.

(22) Ho, H. A.; Najari, A.; Leclerc, M. Optical Detection of DNA and Proteins with Cationic Polythiophenes. *Acc. Chem. Res.* **2008**, *41*, 168−178.

(23) Xing, C. F.; Yuan, H. B.; Xu, S. C.; An, H. L.; Niu, R. M.; Zhan, Y. Nucleobase-Functionalized Conjugated Polymer for Detection of Copper (II). *ACS Appl. Mater. Interfaces* **2014**, *6*, 9601−9607.

(24) Xing, C.; Xu, Q.; Tang, H.; Liu, L.; Wang, S. Conjugated Polymer/Porphyrin Complexes for Efficient Energy Transfer and Improving Light-Activated Antibacterial Activity. *J. Am. Chem. Soc.* **2009**, *131*, 13117−13124.

(25) Zhu, C.; Yang, Q.; Liu, L.; Wang, S. Visual Optical Discrimination and Detection of Microbial Pathogens Based on Diverse Interactions of Conjugated Polyelectrolytes with Cells. *J. Mater. Chem.* **2011**, *21*, 7905−7912.

(26) Wang, L. H.; Pu, K. Y.; Li, J.; Qi, X. Y.; Li, H.; Zhang, H.; Fan, C. H.; Liu, B. A Graphene-Conjugated Oligomer Hybrid Probe for Light-Up Sensing of Lectin and Escherichia Coli. *Adv. Mater.* **2011**, *23*, 4386−4391.

(27) Phillips, R. L.; Miranda, O. R.; You, C. C.; Rotello, V. M.; Bunz, U. H. Rapid and Efficient Identification of Bacteria Using Gold-Nanoparticle−Poly(para-phenyleneethynylene) Constructs. *Angew. Chem., Int. Ed.* **2008**, *47*, 2590−2594.

(28) Yuan, H. X.; Liu, Z.; Liu, L. B.; Lv, F. T.; Wang, Y. L.; Wang, S. Cationic Conjugated Polymers for Discrimination of Microbial Pathogens. *Adv. Mater.* **2014**, *26*, 4333−4338.

(29) Bunz, U. H.; Rotello, V. M. Gold Nanoparticle−Fluorophore Complexes: Sensitive and Discerning "Noses" for Biosystems Sensing. *Angew. Chem., Int. Ed.* **2010**, *49*, 3268−3279.

(30) Zhu, C.; Yang, Q.; Liu, L.; Wang, S. Rapid, Simple, and High-Throughput Antimicrobial Susceptibility Testing and Antibiotics Screening. *Angew. Chem., Int. Ed.* **2011**, *50*, 9607−9610.

DOI: 10.1021/acsami.5b04552
ACS Appl. Mater. Interfaces 2015, 7, 14569−14572