

Journal of Materials Chemistry B

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ARTICLE

Multifunctional Semiconducting Polymer Dots for Imaging, Detection, and Photo-Killing of Bacteria

Cite this: DOI: 10.1039/x0xx00000x

Yi Wan¹, Laibao Zheng², Yan Sun¹, and Dun Zhang^{†1}, and Wei Liu³

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

The diagnosis and kill of bacterial infections and medical implants remain a key challenge in medicine and environment safety. Motivated by these properties and by present clinical requirement, numerous diagnostic and therapeutic nanomaterials have emerged. Here, a family of agents, termed antibiotic-based multifunctional semiconducting polymer dots (Pdots), which can detect bacteria with high sensitivity and selectively damage gram-positive bacteria or gram-negative bacteria, are reported. The multifunctional Pdots that exhibit good flexibility and stability at room temperature are usually smaller, and can be easily modified to biomolecules. The highlights of this work lie in that the developed bioconjugated Pdots system simultaneously possess the abilities of detection, imaging, and pathogen photo-killing. This study not only demonstrates a facile approach to fabricate a bioconjugated probe for bacteria detection, but also provides a powerful and reliable platform to photo-killing of pathogens, thus rendering potential broad antibacterial applications, bacteria diagnosis, and live-image assays.

ARTICLE

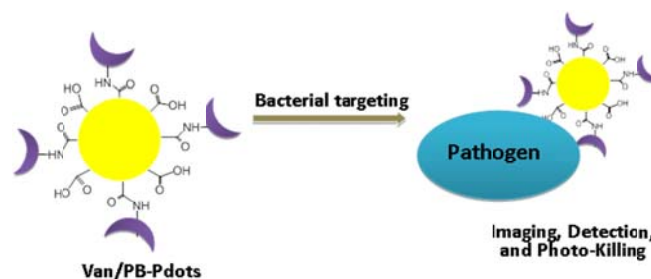
Introduction

In U.S.A., a total of 19,531 infections and hospitalizations associated with diseases were reported in 2012.¹ The identification of pathogen for clinical diagnosis and food safety are crucial for public health protection. Various sensitive, reliable, and rapid methods based on nanomaterials as signal amplifiers combined with electrochemical,^{2,3} fluorescence⁴⁻⁶ and other techniques^{7, 8} have been reported for detection of bacteria and other biomolecules.

Semiconducting polymer dots (Pdots) coupled with biomolecules have emerged as a new type of nanoprobe that has been used for flow cytometry, fluorescence microscopy, and biosensing, for example, distribution of biomolecules *in vivo*, conformation dynamics, target analyte and biomolecule interaction.⁹ Because conventional organic dyes, such as fluorescein, rhodamine, alexa fluor and cyanine derivatives, limited brightness, poor photostability and poor thermal stability in aqueous solution, various strategies for designing brighter fluorescent nanoprobe have been advanced. Despite these improvements, the intrinsic limitations of conventional dyes can still pose great difficulties, requiring high excitation light intensities in UV-visible light region or long-term imaging.¹⁰ There is also a great deal of interest in the development of quantum dots (QDs). QDs have been facilitating the development of DNA array technology, immunofluorescence assays, and animal biology over the past two decades due to their high quantum yield, narrow and tunable emission spectrum, and good photostability.¹¹⁻¹⁵ To date, the preparation of monodisperse, luminescent, high brightness, nontoxic, and bioconjugated QDs, has not been successful in high ionic buffer or in biological matrices. Compared with above-mentioned fluorescence labels using organic dyes and quantum dots, green fluorescent protein (GFP) is unique among light-emitting proteins in that it does not require a presence of cofactors or substrates for generation of its green light.¹⁶ However, the GFP signal cannot be amplified in a controlled manner, preventing detection of low expression levels. Increasing worldwide demand requires simple, stable, and sensitive labels for biomolecules and cellular assay evaluation and monitoring. The Pdots as fluorescent nanoprobe offer several useful advantages, such as good biocompatibility for living organisms or biological cells,¹⁷ easy functionalization with antibodies or other biomolecules, excellent photostability and thermal stability ability to be tracked with nanometer accuracy.⁹ Chiu and his colleagues designed a series of fluorescent semiconducting polymers, such as multicolor narrow emissive Pdots based on different boron dipyrromethene units,¹⁸ semiconducting polymer poly(9-vinylcarbazole) doped with europium complexes,¹⁹ highly emissive polymer dot bioconjugates,²⁰ highly luminescent, fluorinated semiconducting polymer dots,²¹ hybrid semiconducting Pdots-quantum dot,²² for cellular imaging and analysis in a variety of *in vitro* and *in vivo* applications. Our group also investigated

poly[9,9-dioctylfluorenyl-2,7-diyl-co-1,4-benzo-{2,10-3}-thiadiazole] (PFBT) fluorescent polymer systems to identify and quantify bacteria. Bacterial cell membranes disrupt the fluorescent polymer system, generating a unique fluorescence response array.²³

Semiconducting polymers, key features in photodynamic therapy, are compounds that can produce reactive oxygen species under light irradiation in the presence of oxygen to kill microbial cells. Whitten and coworkers reported a cationic conjugated polyelectrolyte with quaternary ammonium groups that exhibited a highly biocidal activity against bacteria.²⁴⁻²⁶ Wang and coworkers also reported that a cationic poly(*p*-phenylene vinylene) derivative with polyethylene glycol side chains is used for selective recognition, imaging, and killing of bacteria.^{27, 28} However, none of a combination on bacteria detection and antibacterial property simultaneously is reported.



Scheme 1. Schematic illustration of covalently functionalized semiconducting polymer and antibiotic bioconjugation for specific bacterial targeting.

Here semiconducting polymers containing antibiotic units for specific bacterial cell labelling by fluorescence imaging and selective photo-killing for bacteria are performed (Scheme 1). The developed bioconjugated Pdots simultaneously possess an ability to selectively recognize, image, and photo-kill gram-positive and gram-negative pathogens.

Experimental

Materials and Measurements

Unless otherwise noted, all reagents for syntheses of semiconducting polymer were purchased from Sigma-Aldrich. ¹H NMR spectra were recorded on a Bruker AV500 spectrometer. IR spectra were collected on Nicolet iS10 FT-IR. The particle size and morphology of Pdots was investigated using transmission electron microscopy (TEM, Hitachi-H600). The hydrodynamic size and zeta potential of the Pdots was also measured in aqueous solution using a dynamic light scattering instrument (DLS, Malvern Zetasizer Nano ZS 90). Fluorescence spectra of Pdots were taken with a Hitachi 3500

fluorespectrometer with a xenon lamp excitation source. UV-vis absorption spectra of Pdots were taken in water with a Hitachi U-2900 spectrophotometer. The white light source (400-800 nm) was provided by a metal halogen lamp (500 W). Scanning electron microscopy (SEM) images were recorded on a JEOL JSM-6700F. The photo-killing assay for bacteria was performed in photochemical work station (XuJiang Electromechanical Plant XPA-7).

Preparation of Semiconducting Polymer Dots

A polymer functionalized with side-chain carboxylic acid group (PFBT) was synthesized using a method reported with modification.²⁹ The Pdots were obtained from PFBT using the reprecipitation method. 0.2 mL PFBT (1 mg mL⁻¹) in THF stock solution was injected into 10 mL H₂O under sonication for 20 min. The tetrahydrofuran (THF) was then removed by purging with Ar on a 90 °C hotplate for 1 hour. The resulting Pdot solution was filtered through a 0.2 µm membrane filter to remove any aggregates formed during preparation.

Antibiotics bioconjugation

Bioconjugation was prepared by using the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimidehydrochloride (EDC)-catalyzed reaction between carboxyl Pdots.²⁹ In detail, 0.1 mL of polyethylene glycol (5%) and 0.1 mL of 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (pH7.2, 1 mol L⁻¹) were added to 4 mL of Pdots solution. Then, 0.5 mL of vancomycin or polymyxin B (5 mg mL⁻¹) was added to the solution and mixed on a vortex. After that, 0.1 mL of freshly-prepared EDC solution (5 mg mL⁻¹) was added to the solution, and the mixture was stirred for 2 hours at room temperature. Vancomycin-Pdots (Van-Pdots) and Polymyxin B-Pdots (PB-Pdots) mixture were dialyzed for 2 days to remove excess antibiotics using phosphate buffered saline (PBS, pH7.4, 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄) water under stirring. To the Van-Pdots or PB-Pdots mixture was added 50 µL of Triton X-100 in MilliQ water (2.0 wt%) and subsequently transferred to a centrifugal ultrafiltration tube and then concentrated to 0.5 mL by centrifugation.

Bacterial Culture

All bacteria were offered from the Key Laboratory of Experimental Marine Biology. Bacteria were seeded and cultured in suspension using the following media: *S. aureus*, *P. aeruginosa* and *E. coli* in Luria-Bertani media; *V. fischeri*, *B. cereus* and *B. firmus* in yeast extract medium; *E. tarda* and *V. alginolyticus* in yeast extract peptone dextrose medium. For these bacteria, a single colony was inoculated in bacterial medium at 30 °C overnight, shaking at 200 rpm. After centrifugation (4,000 rpm) for 10 min and PBS washing, the bacterial cells were diluted to the desired concentration or optical density in phosphate buffer. Bacterial cell numbers were estimated by plating onto standard agar plates.

Bacterial Labeling, Detection and Imaging

Bacterial cells were first washed with PBS containing 2 mg mL⁻¹ bovine serum albumin. For direct labelling with Van-Pdots or PB-Pdots, bacterial cells were incubated with 5 µg mL⁻¹ conjugate Pdots in PBS for 30 min at room temperature. Excess unbound Pdots were removed by washing the bacterial cells in three times. For competition assays, 0-20 µmol L⁻¹ vancomycin or PB was added to 5 µg mL⁻¹ conjugate Pdots. Increased relative fluorescence (RFU%) values are estimated as $RFU\% = (F_{\text{sample}} - F_{\text{control}}) / F_{\text{control}} \times 100$, where F_{sample} and F_{control} are intensity values of bacterial sample and PBS sample, respectively. All labeling experiments were carried out at three times to obtain the reproducibility of our approach. For fluorescence microscopy, bacterial cells-conjugated Pdots complex were fixed in 10% PFA for 30 min.

SEM characterization for bioconjugated Pdots and bacteria cells

1 mL (1.2 × 10⁷ cfu mL⁻¹) of each pathogen (*S. aureus* and *P. aeruginosa*) were incubated with 1 mL Van-Pdots (5 µg mL⁻¹) and PB-Pdots for 10 min at room temperature, respectively, then immediately fixed with glutaraldehyde (2.5%, 5 mL) solution for 1 hour. The films were dehydrated by adding ethanol in a graded series (30%-100%) and then dried. The microbes were observed by FE-SEM for morphology changes.

Antibacterial Experiments

The photokilling properties of Van-Pdots and PB-Pdots were measured by incubation with bacterial cell suspensions at room temperature for 20 min in the dark. After being exposed to white light (400-800 nm) for 20 min, the bacterial cells were washed with PBS two times. Bacterial pellets were diluted with PBS. A series of tenfold dilution were prepared, and plated out on Luria Bertani agar. The plates were incubated for 48 h at 30 °C, and colony-forming units were counted. A bacterial sample without Pdots was used as a control. The results are expressed as $Kill\% = (N_{\text{control}} - N_{\text{survivor}}) / N_{\text{control}} \times 100$, where N_{survivor} is bacterial counts of a specimen incubated with conjugated Pdots and N_{control} is the number of control in absence of conjugated Pdots.

Results and discussion

Synthesis and Characterization of polymers and Bioconjugated Pdots

The synthesis of carboxylic acid-functionalized semiconducting polymer was carried out in three steps (Figure S1), including the synthesis of 2,7-dibromo-9,9-bis(3-(tert-butyl propanoate))fluorene, copolymerization of three monomers, and removal of the protecting tert-butyl groups using trifluoroacetic acid.^{23, 29} The purity and identity of product were confirmed via ¹H NMR (Figure S2). The presence of carboxylic acid groups in the polymer was further characterized by FT-IR spectra (Figure S3). FT-IR analysis of carboxylic acid-functionalized polymer shows O-H stretching (a broad band at 3250-3750 cm⁻¹ centered around 3650 cm⁻¹), surface carboxylic groups O=C-O (1320 cm⁻¹) and epoxy C-O (1250 cm⁻¹).

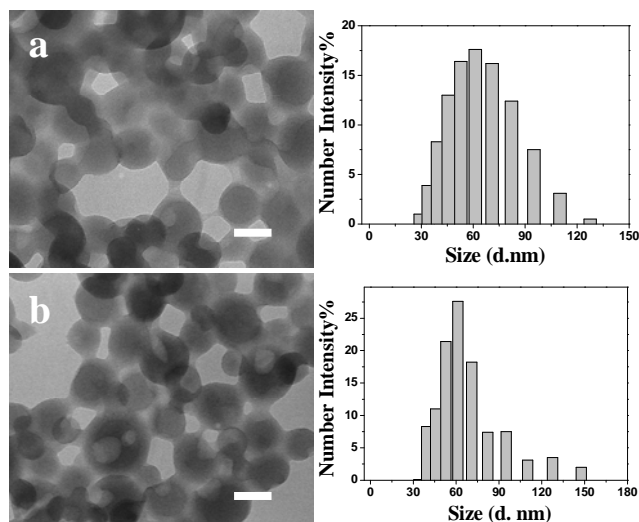


Figure 1 Transmission electron microscopy (left) and dynamic light scattering (right) measurements of Van-Pdots (a) or polymyxin B sulfate (b). Scale bars is 50 nm.

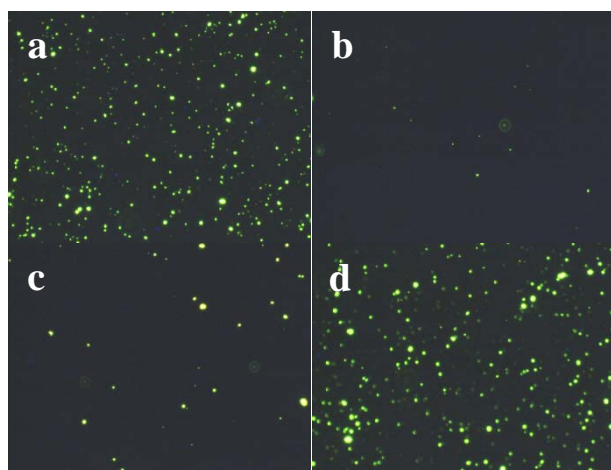


Figure 2 Fluorescence microscopy images of *S. aureus* (a, b) and *P. aeruginosa* (c, d) labeled with Van-Pdots (a, c) or PB-Pdots (b, d), respectively. The bacterial count is 1.2×10^7 cfu mL⁻¹.

A reprecipitation method is used to dissolve the hydrophobic polymer in an organic solvent, such as THF. Pdots were prepared by injection of a polymer suspended in a THF solution into water under ultrasonication. After removing the organic solvents by evaporation, water-dispersible Pdots can be obtained. Bioconjugations were prepared using the EDC-catalyzed reaction between carboxyl Pdots and vancomycin or polymyxin B. Absorption and fluorescence spectra of the Pdots recorded in water are shown in Figure S5 and their spectroscopic properties are given in Table S1. All spectra had a maximum absorption around 475 nm and a maximum fluorescence emission around 535 nm. They showed a quantum yield around 14. The size of Pdots was characterized by TEM (Figure S4) and DLS (Figure S6). The sizes depend on the precursor polymer concentration and injection THF volume. Up to now, there are two methods, miniemulsion and reprecipitation, to fabricate water-dispersible Pdots.³⁰ As for the miniemulsion method, amphiphilic surfactant molecules are used to prepare water-miscible micelles that contains the hydrophobic polymer. Unlike the miniemulsion strategy,

Pdots prepared by reprecipitation are usually smaller (5-150 nm), and can be easily modified to biomolecules.³¹

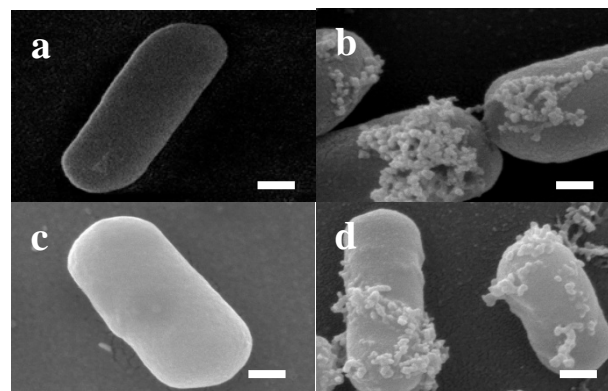


Figure 3 SEM images of *S. aureus* (a) or *P. aeruginosa* (c) labeled with Van-Pdots (b) or PB-Pdots (d), respectively. Scale bar is 200 nm.

Selective Recognition and Imaging using Bioconjugated Pdots

To demonstrate recognition and bio-imaging capabilities of the particles, *S. aureus* and *P. aeruginosa* were chosen as the target cell incubated with bioconjugated Pdots. The proposed principle of selective recognition and imaging is illustrated in Scheme 1. For gram-positive bacteria, *S. aureus*, the binding of vancomycin-Pdots (Van-Pdots) to bacteria was obtained via a five point hydrogen bond interaction with the terminal D-Ala-D-Ala moieties of the NAM/NAG-peptides.³⁰ For gram-negative bacteria, *P. aeruginosa*, the binding of polymyxin B-Pdots (PB-Pdots) to bacteria achieved an electrostatic attraction for the positively charged amino groups in the cyclic peptide portion.³¹ Figure 2 shows fluorescence microscopic images of *S. aureus* and *P. aeruginosa* incubated with Van-Pdots or PB-Pdots, respectively. These results suggest that the bioconjugated Pdots could selectively recognize and image *S. aureus* and *P. aeruginosa*. Scanning electron microscopy (SEM) was further able to show the presence of bioconjugated Pdots, coated across the surface of bacterial cells (Figure 3). On the basis of these results we formed a hypothesis that bioconjugated Pdots have the potential to selectively image different bacteria.

Over the past few years, many research groups have developed novel approaches to fabricate the nanoprobe for bacterial imaging. For example, Ning et al., presented a family of maltodextrin-based imaging probes to detect bacteria *in vivo* with high sensitivity and specificity.⁴ Oosten et al., explored the use of fluorescently labelled vancomycin for real-time *in vivo* imaging of bacterial infections and a human post-mortem implant model.³² There is a disadvantage for previous examples, which significantly impedes their broad application due to poor photostability. Recently, Wang et al., report a cationic poly(p-phenylene vinylene) derivative with polyethylene glycol side chains which is used for selective recognition, imaging, and killing of bacteria over mammalian cells.²⁷ Unfortunately, there is no specificity for different bacteria. Bioconjugated Pdots appear to be a good alternative one to replace the small probe, due to their excellent photostability and thermal stability.

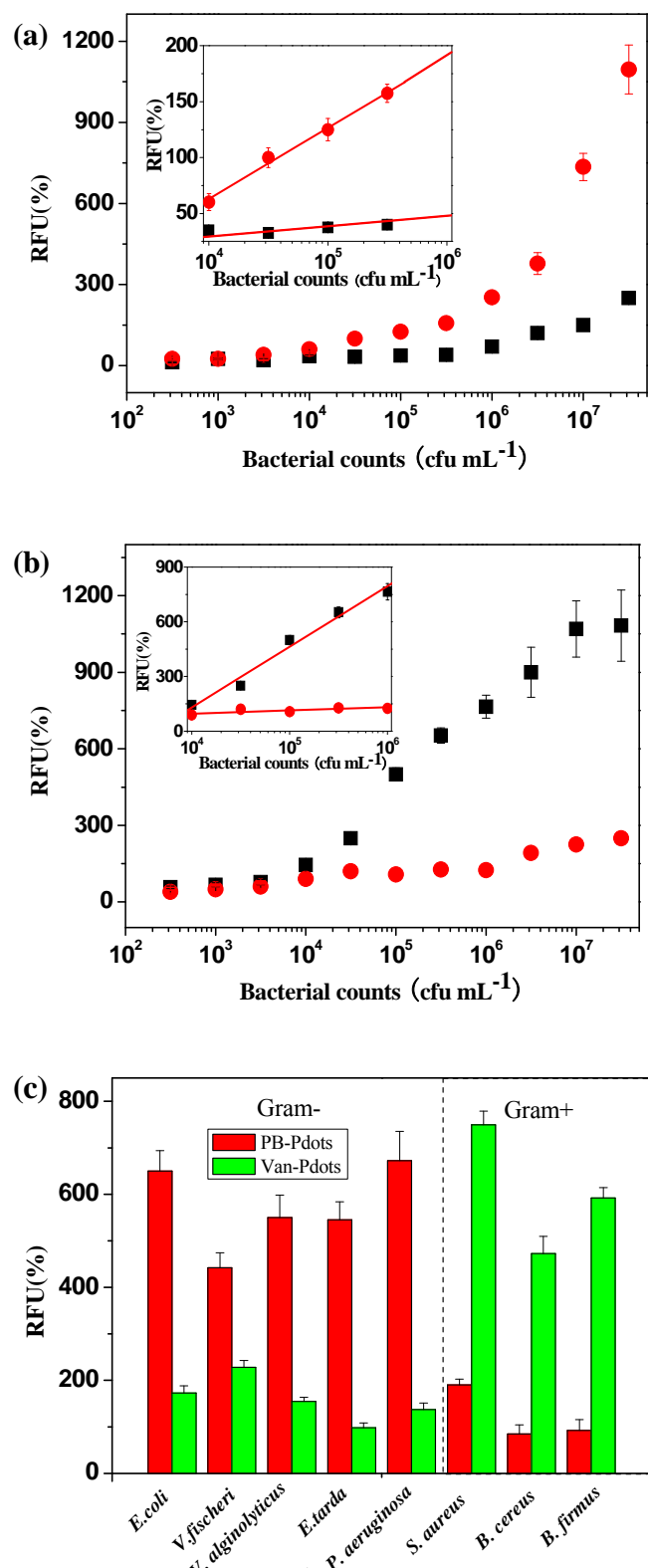


Figure 4 Detection sensitivity using PB-Pdots (a) and Van-Pdots (b) for *S. aureus* (■) and *P. aeruginosa* (●) as a function of bacteria concentration. Fluorescence response patterns (c) conjugated Pdots complex in the presence of bacteria (1.2×10^7 cfu mL⁻¹).

Bacterial Detection and Determination via Bioconjugated Pdots

Bioconjugated Pdots have been mainly used for detection of bacterial strains from other insensitive cells.³³⁻³⁶ The bacteria concentrations from 3.1×10^2 to 5.0×10^7 cfu mL⁻¹ were prepared by serial dilution in PBS. Figure 4 shows fluorescence response obtained for Van-Pdots and PB-Pdots incubated with different bacterial concentrations. The fluorescence response increased regularly with the increase of the bacteria concentrations, indicating that Van-Pdots or PB-Pdots were absorbed onto the surface of bacterial cells to produce a bioprobe-bacterial cells complex. The results for bacteria detection showed that the concentration of bacteria and the response were highly correlated (Figure 4a and 4b). A linear relationship between the response and the logarithm of the *S. aureus* concentration using Van-Pdots was obtained for concentrations ranging from 1.0×10^4 to 1.0×10^6 cfu mL⁻¹, with a slope of 60.9 and a correlation coefficient of 0.994 (Figure 4a). Figure 4b shows a linear relationship between the response and the logarithm of the *P. aeruginosa* concentration using PB-Pdots, with a slope of 342.8 and a correlation coefficient of 0.997. The different of slope for bacterial detection using Van-Pdots and PB-Pdots, respectively, may due to that the PB-Pdots shows more binding site for bacterial cells. Polymyxin B alters bacterial outer membrane permeability by binding to a negatively charged site in the lipopolysaccharide layer.³⁷ However, the hydrophilic molecule of vancomycin only can form hydrogen bond interactions with D-alanyl-D-alanine moieties.³⁸ The minimum numbers of bacterial detection by bioconjugated Pdots were also performed. To evaluate the limit of detection and broad applicability of the bioconjugated Pdots platform in biologically relevant systems, we analyzed various concentrations of multiple species spiked in PBS. Figure 4a demonstrates that the Van-Pdots is capable of detecting as few as 3.1×10^4 cfu mL⁻¹ for *S. aureus*. Figure 4b demonstrates that the PB-Pdots is capable of detecting as few as 1.0×10^4 cfu mL⁻¹ for *P. aeruginosa*.

The specificity of bioconjugated Pdots for bacteria was further performed. Figure 4a indicates that there is a minor change in the fluorescence response of the Van-Pdots immersed in *P. aeruginosa* for 30 min. Van-Pdots immersed in *S. aureus* have a twofold increase in fluorescence intensity compared with control sample. PB-Pdots immersed in *P. aeruginosa* have a sixfold increase in the fluorescence intensity (Figure 4b). We next examined how effectively different bacteria strains could be detected. The following eight bacteria were tested: *S. aureus*, *P. aeruginosa*, *E. coli*, *V. fischeri*, *B. cereus*, *B. firmus*, *E. tarda* and *V. alginolyticus*. Figure 4c shows that the nanoprobe has a high selectivity for gram-positive bacteria or gram-negative bacteria. The obtained variations for eight species in the fluorescence intensities were due to different D-Ala-D-Ala content across bacterial strains³⁹.

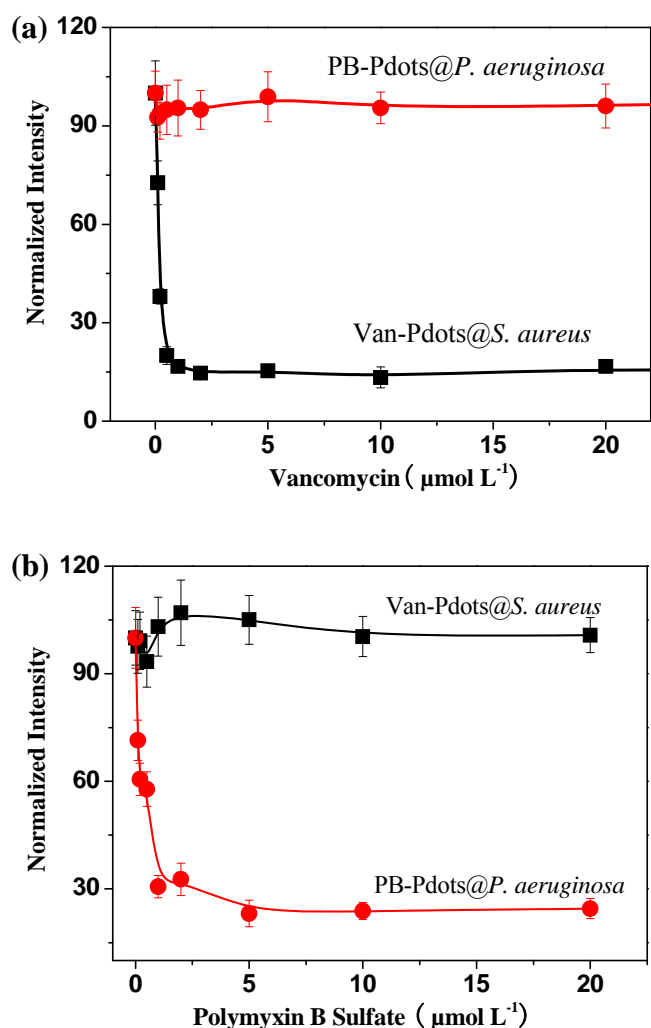


Figure 5 Competition assays between antibiotic and antibiotic-functionalized Pdots using fluorescence measurements. For comparison, Normalized intensity = $\text{RFU}_{\text{sample}}/\text{RFU}_0$, where $\text{RFU}_{\text{sample}}$ is response intensity values of bacterial sample (1.2×10^7 cfu mL⁻¹) and bioconjugated Pdots incubated with some concentration of antibiotic, and without antibiotic (RFU_0) respectively.

A competitive inhibition assay to examine the influence of binding properties of antibiotic and antibiotic-functionalized Pdots were estimated (Figure 5). As the concentration of antibiotic increased, the binding efficacy of target bacteria sharply decreased. However, the control system did not show any effect with the nanoprobe during a short treatment period. For a system of Van-Pdots and gram-positive bacteria (Figure 5a), the binding of vancomycin to D-Ala-D-Ala via an extensive first principles investigation based on accurate Hartree–Fock and density functional theory simulations was found to be stronger by about 3–5 kcal mol⁻¹.⁴⁰ The inhibition binding constant was c.a. 6 μmol L⁻¹ for vancomycin-*trans*-cyclooctene and gram-positive bacteria.³⁹ Our results show that the inhibition binding constant for Van-Pdots and *S. aureus* is c.a. 0.4 μmol L⁻¹, which is far less than that of Van-TCO and *S. aureus*. For the system of PB-Pdots and gram-negative bacteria (Figure 5b), the inhibition binding constant for PB-Pdots and *P. aeruginosa* is c.a. 1.1 μmol L⁻¹. The

pull-down assay based on agarose-immobilized polymyxin B demonstrate that the binding of bacterial lipopolysaccharides by polymyxin B is approximately 1–3 μmol L⁻¹,⁴¹ which was similar to that of PB-Pdots.

Though antibiotics, such as vancomycin and polymyxin B, show less specificity/selectivity than monoclonal antibodies, it is very attractive as a ligand allowing affinity capture of a wide range of microbial cells with antibiotic functionalized nanoparticle for bacteria detection. Previous work in our group suggested that vancomycin-functionalised nanoparticles for selective detection of bacteria can significantly improve biosensing limit of detection under an external magnetic field.⁴² Vancomycin-resistant *enterococci* and other Gram-positive bacteria can be captured and detected using biofunctional magnetic nanoparticles or dendrimer-Based multivalent nanoplatform at concentrations of 10 cfu mL⁻¹ within an hour.^{43–45} Weissleder and coworkers describe a bioorthogonal modification of small molecule antibiotics (vancomycin and daptomycin), which bind to the cell wall of gram-positive bacteria. Compared to abovementioned covalent nanoparticle conjugates, the bioorthogonal method demonstrated 1–2 orders of magnitude greater sensitivity for bacteria detection.³⁹

Photo-killing Properties of Bioconjugated Pdots

Traditional methods for bacterial inactivation, such as chlorination, ultraviolet (UV) sterilisation, and ozone, effectively inhibit and kill pathogenic bacteria. During the past decade, the application of nanomaterials in medicine distinctly increased, which resulted in raising hopes for using nanoparticles as alternative antimicrobial agents.^{46–52} Our group previously reported the synthesis of vancomycin-functionalised nanoparticles and their enhanced bactericidal activities.⁵³ The improvement of bactericidal activity under UV irradiation is due to the targeting capacity of Van-functionalised nanoparticles for the bacteria.

Whether bioconjugated Pdots could fulfill the purpose of selective photo-killing? Fig. 6a and 6b further verified that Van-Pdots and PB-Pdots showed a selective photo-killing for Van-sensitive bacteria, *S. aureus* and PB-sensitive bacteria, *P. aeruginosa*, respectively. It was almost completely killed within 1 h in Van-Pdots or PB-Pdots under white light irradiation. The partial inactivation of Van-insistent bacteria or PB-insistent bacteria occurred under the same condition. These results indicated that bioconjugated Pdots were capable of selective phototoxicity to specific bacteria. The antibacterial activity of bioconjugated Pdots can be due to two mechanisms, including the singlet oxygen and other reactive oxygen species in the presence of photosensitizer and oxygen under white light irradiation and the binding of antibiotic and D-Ala-D-Ala of bacterial cell's surface.²⁷ Figure S7 showed that the nanoprobe for eight bacteria have a relative selective photo-killing for gram-positive bacteria or gram-negative bacteria. The obtained variations for eight species in relative antibacterial intensities were due to the discrimination of bacterial strains and the direct interaction for Pdot and bacterial cells. Figure S8 illustrated that in the bioconjugated Pdots concentration range of 0–0.12 μmol L⁻¹, the killing efficiency is less than 10%,

even with white light illumination. In other word, bioconjugated Pdots itself show a good biocompatibility in low concentration.

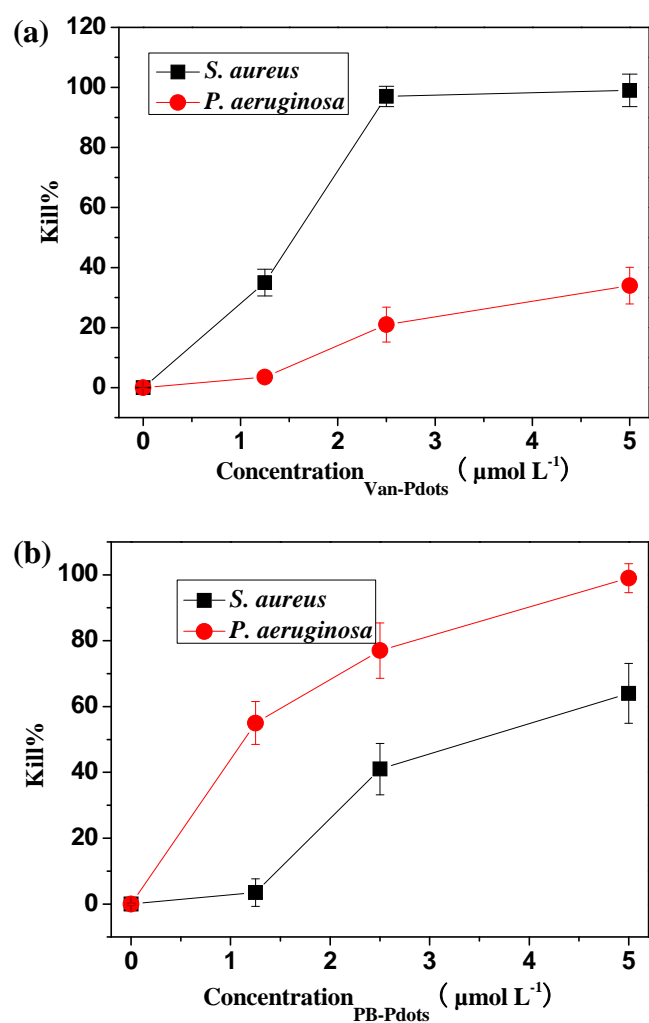


Figure 6 Photo-killing properties of *S. aureus* (1.2×10^5 cfu mL^{-1}) and *P. aeruginosa* (1.2×10^5 cfu mL^{-1}) after incubation with Van-Pdots (a) and PB-Pdots (b) under white light illumination for 1 hour.

Functionalised molecules modified onto surface of nanoparticles to enhance antimicrobial activities have recently gained academic interest. A synthesis of vancomycin-capped Au nanoparticles and their enhanced *in vitro* antibacterial activities that act as a rigid polyvalent inhibitor of vancomycin-resistant *enterococci* have been reported.⁵⁴ Qi et al, demonstrated that vancomycin-modified mesoporous silica nanoparticles can efficiently target and kill gram-positive bacteria preferentially over macrophage-like cells.⁵⁵ Park et al., investigate the antimicrobial activity of conjugates of the peptide antibiotic polymyxin B to Au nanoparticles and CdTe quantum dots.⁵⁶ These results show that it is possible to create antimicrobial agents using concentrations of functionalized quantum dots that do not harm mammalian cells.

Conclusions

In summary, the Pdots combined with vancomycin or polymyxin B is specific and sensitive enough for the trace image and detection of bacteria. Because the experimental setup requires only bioprobe, the developed approach is simple and cost-efficient, which means that it can be employed in the on-site detection of gram-positive bacteria and gram-negative bacteria at hospitals, and even homes for food safety monitoring. Multifunctional Pdots dispersions also exhibited selective antibacterial activity toward gram-positive bacteria and gram-negative bacteria. As far as we know, this is the first developed multifunctional Pdots that combines recognition, imaging, detection, and antimicrobial properties in a single system to offer an effect for the selective analysis of bacteria and photo-killing function.

Acknowledgements

We gratefully acknowledge support from the National Natural Science Foundation of China (No. 41306072), Science & Technology Basic Research Program of Qingdao (No. 13-1-4-181-jch) and the Shandong Provincial Natural Science Foundation, China (No. BS2013HZ005).

Notes and references

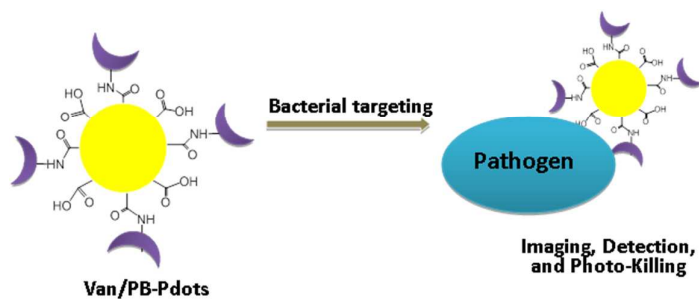
- ¹Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China.
- ²Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China Qingdao, China
- ³Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China.
- [†]Corresponding Author's Email: zhangdun@qdio.ac.cn; Fax/Tel:+86 532 82898960.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1. U. S. S. Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food -Foodborne Diseases Active Surveillance Network, 1996-2012, in *Morbidity and Mortality Weekly Report* 2013, pp. 283-287.
2. G. A. Zelada-Guillén, J. Riu, A. Düzgün and F. X. Rius, *Angew. Chem. Int. Ed.*, 2009, **48**, 7334-7337.
3. Y. Wan, Y. Wang, J. Wu and D. Zhang, *Anal. Chem.*, 2011, **83**, 648-653.
4. X. Ning, S. Lee, Z. Wang, D. Kim, B. Stubblefield, E. Gilbert and N. Murthy, *Nat. Mater.*, 2011, **10**, 602-607.
5. R. L. Phillips, O. R. Miranda, C.-C. You, V. M. Rotello and U. H. F. Bunz, *Angew. Chem. Int. Ed.*, 2008, **47**, 2590-2594.
6. J. Gao, L. Li, P. L. Ho, G. C. Mak, H. Gu and B. Xu, *Adv. Mater.*, 2006, **18**, 3145-3148.
7. H. J. Chung, C. M. Castro, H. Im, H. Lee and R. Weissleder, *Nat. Nano.*, 2013, **8**, 369-375.
8. G. Budin, H. J. Chung, H. Lee and R. Weissleder, *Angew. Chem. Int. Ed.*, 2012, **51**, 7752-7755.
9. C. Wu and D. T. Chiu, *Angew. Chem. Int. Ed.*, 2013, **52**, 3086-3109.

10. U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke and T. Nann, *Nat. Meth.*, 2008, **5**, 763-775.
11. M. Bruchez, M. Moronne, P. Gin, S. Weiss and A. P. Alivisatos, *Science*, 1998, **281**, 2013-2016.
12. W. C. W. Chan and S. N. Nie, *Science*, 1998, **281**, 2016-2018.
13. I. L. Medintz, H. T. Uyeda, E. R. Goldman and H. Mattoussi, *Nat. Mater.*, 2005, **4**, 435-446.
14. X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir and S. Weiss, *Science*, 2005, **307**, 538-544.
15. H. N. Abdelhamid and H.-F. Wu, *J. Mater. Chem. B*, 2013, **1**, 6094-6106.
16. M. Chalfie, Y. Tu, G. Euskirchen, W. Ward and D. Prasher, *Science*, 1994, **263**, 802-805.
17. L. P. Fernando, P. K. Kandel, J. Yu, J. McNeill, P. C. Ackroyd and K. A. Christensen, *Biomacromolecules*, 2010, **11**, 2675-2682.
18. Y. Rong, C. Wu, J. Yu, X. Zhang, F. Ye, M. Zeigler, M. E. Gallina, I. C. Wu, Y. Zhang, Y.-H. Chan, W. Sun, K. Uvdal and D. T. Chiu, *ACS Nano*, 2013, **7**, 376-384.
19. W. Sun, J. Yu, R. Deng, Y. Rong, B. Fujimoto, C. Wu, H. Zhang and D. T. Chiu, *Angew. Chem. Int. Ed.*, 2013, **52**, 11294-11297.
20. C. Wu, S. J. Hansen, Q. Hou, J. Yu, M. Zeigler, Y. Jin, D. R. Burnham, J. D. McNeill, J. M. Olson and D. T. Chiu, *Angew. Chem. Int. Ed.*, 2011, **50**, 3430-3434.
21. Y. Zhang, J. B. Yu, M. E. Gallina, W. Sun, Y. Rong and D. T. Chiu, *Chem. Commun.*, 2013, **49**, 8256-8258.
22. Y.-H. Chan, F. Ye, M. E. Gallina, X. Zhang, Y. Jin, I. C. Wu and D. T. Chiu, *J. Am. Chem. Soc.*, 2012, **134**, 7309-7312.
23. Y. Wan, Y. Sun, P. Qi, P. Wang and D. Zhang, *Biosens. Bioelectron.*, 2014, **55**, 289-293.
24. L. Lu, F. H. Rininsland, S. K. Wittenburg, K. E. Achyuthan, D. W. McBranch and D. G. Whitten, *Langmuir*, 2005, **21**, 10154-10159.
25. T. S. Corbitt, J. R. Sommer, S. Chemburu, K. Ogawa, L. K. Ista, G. P. Lopez, D. G. Whitten and K. S. Schanze, *ACS Appl. Mater. Interf.*, 2008, **1**, 48-52.
26. S. Chemburu, T. S. Corbitt, L. K. Ista, E. Ji, J. Fulghum, G. P. Lopez, K. Ogawa, K. S. Schanze and D. G. Whitten, *Langmuir*, 2008, **24**, 11053-11062.
27. C. Zhu, Q. Yang, L. Liu, F. Lv, S. Li, G. Yang and S. Wang, *Adv. Mater.*, 2011, **23**, 4805-4810.
28. C. Xing, Q. Xu, H. Tang, L. Liu and S. Wang, *J. Am. Chem. Soc.*, 2009, **131**, 13117-13124.
29. X. Zhang, J. Yu, C. Wu, Y. Jin, Y. Rong, F. Ye and D. T. Chiu, *ACS Nano*, 2012, **6**, 5429-5439.
30. D. P. Levine, *Clin. Infect. Dis.*, 2006, **42**, S5-S12.
31. M. Teuber and J. Bader, *Arch. Microbiol.*, 1976, **109**, 51-58.
32. M. van Oosten, T. Schäfer, J. A. C. Gazendam, K. Ohlsen, E. Tsompanidou, M. C. de Goffau, H. J. M. Harmsen, L. M. A. Crane, E. Lim, K. P. Francis, L. Cheung, M. Olive, V. Ntziachristos, J. M. van Dijl and G. M. van Dam, *Nat. Commun.*, 2013, **4**.
33. D. Bhattacharya, S. P. Chakraborty, A. Pramanik, A. Baksi, S. Roy, T. K. Maiti, S. K. Ghosh and P. Pramanik, *J. Mater. Chem.*, 2011, **21**, 17273-17282.
34. R. Ladj, A. Bitar, M. Eissa, Y. Mugnier, R. Le Dantec, H. Fessi and A. Elaissari, *J. Mater. Chem. B*, 2013, **1**, 1381-1396.
35. T.-Y. Liu, J.-Y. Ho, J.-C. Wei, W.-C. Cheng, I. H. Chen, J. Shiue, H.-H. Wang, J.-K. Wang, Y.-L. Wang and J.-J. Lin, *J. Mater. Chem. B*, 2014, **2**, 1136-1143.
36. H. N. Abdelhamid and H.-F. Wu, *J. Mater. Chem. B*, 2013, **1**, 3950-3961.
37. M. Vaara and T. Vaara, *Nature*, 1983, **303**, 526-528.
38. G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams and G. A. Smith, *Nature*, 1978, **271**, 223-225.
39. H. J. Chung, T. Reiner, G. Budin, C. Min, M. Liong, D. Issadore, H. Lee and R. Weissleder, *ACS Nano*, 2011, **5**, 8834-8841.
40. J.-G. Lee, C. Sagui and C. Roland, *J. Am. Chem. Soc.*, 2004, **126**, 8384-8385.
41. B. J. Johnson, J. B. Delehanty, B. Lin and F. S. Ligler, *Anal. Chem.*, 2008, **80**, 2113-2117.
42. Y. Wan, D. Zhang and B. Hou, *Biosens. Bioelectron.*, 2010, **25**, 1847-1850.
43. H. Gu, P.-L. Ho, K. W. T. Tsang, L. Wang and B. Xu, *J. Am. Chem. Soc.*, 2003, **125**, 15702-15703.
44. A. J. Kell, G. Stewart, S. Ryan, R. Peytavi, M. Boissinot, A. Huletsky, M. G. Bergeron and B. Simard, *ACS Nano*, 2008, **2**, 1777-1788.
45. S. K. Choi, A. Myc, J. E. Silpe, M. Sumit, P. T. Wong, K. McCarthy, A. M. Desai, T. P. Thomas, A. Kotlyar, M. M. B. Holl, B. G. Orr and J. R. Baker, *ACS Nano*, 2013, **7**, 214-228.
46. M. Mahmoudi and V. Serpooshan, *ACS Nano*, 2012, **6**, 2656-2664.
47. E. M. Hetrick, J. H. Shin, N. A. Stasko, C. B. Johnson, D. A. Wespe, E. Holmuhamedov and M. H. Schoenfish, *ACS Nano*, 2008, **2**, 235-246.
48. A. W. Carpenter, D. L. Slomberg, K. S. Rao and M. H. Schoenfish, *ACS Nano*, 2011, **5**, 7235-7244.
49. M.-C. Wu, A. R. Deokar, J.-H. Liao, P.-Y. Shih and Y.-C. Ling, *ACS Nano*, 2013, **7**, 1281-1290.
50. Y. Li, W. Zhang, J. Niu and Y. Chen, *ACS Nano*, 2012, **6**, 5164-5173.
51. S. Some, S.-M. Ho, P. Dua, E. Hwang, Y. H. Shin, H. Yoo, J.-S. Kang, D.-k. Lee and H. Lee, *ACS Nano*, 2012, **6**, 7151-7161.
52. N. Sémiramoth, C. D. Meo, F. Zouhiri, F. Saïd-Hassane, S. Valetti, R. Gorges, V. Nicolas, J. H. Poupaert, S. Chollet-Martin, D. Desmaële, R. Gref and P. Couvreur, *ACS Nano*, 2012, **6**, 3820-3831.
53. Y. Wan, D. Zhang, Y. Wang, P. Qi, J. Wu and B. Hou, *J. Hazard. Mater.*, 2011, **186**, 306-312.
54. H. Gu, P. L. Ho, E. Tong, L. Wang and B. Xu, *Nano Lett.*, 2003, **3**, 1261-1263.
55. G. B. Qi, L. L. Li, F. Q. Yu and H. Wang, *ACS Appl. Mater. Interf.*, 2013, **5**, 10874-10881.
56. S. Park, H. Chibli, J. Wong and J. L. Nadeau, *Nanotechnology*, 2011, **22**, 185101.

Colour graphic and highlight



Multifunctional semiconducting polymer dots probe, which can detect bacteria with high sensitivity and selective damage pathogen.