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Unique Fluorescent Imaging Probe for Bacterial Surface Localization and Resistant Enzyme Imaging

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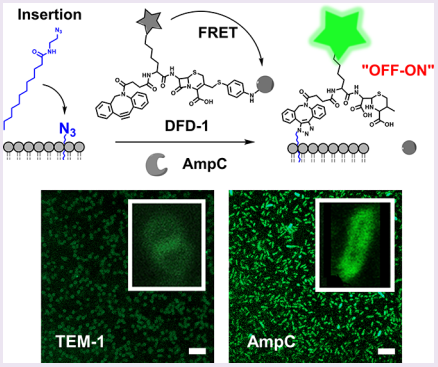
Supporting Information

ABSTRACT: Emergence of antibiotic bacterial resistance has caused serious clinical issues worldwide due to increasingly difficult treatment. Development of a specific approach for selective visualization of resistant bacteria will be highly significant for clinical investigations to promote timely diagnosis and treatment of bacterial infections. In this article, we present an effective method that not only is able to selectively recognize drug resistant AmpC β-lactamases enzyme but, more importantly, is able to interact with bacterial cell wall components, resulting in a desired localization effect on the bacterial surface. A unique and specific enzyme-responsive cephalosporin probe (**DFD-1**) has been developed for the selective recognition of resistance bacteria AmpC β-lactamase, by employing fluorescence resonance energy transfer with an "off−on" bioimaging. To achieve the desired localization, a lipid−azide conjugate (**LA-12**) was utilized to facilitate its penetration into the bacterial surface, followed by copper-free click chemistry. This enables the probe **DFD-1** to be anchored onto the cell surface. In the presence of AmpC enzymes, the cephalosporin β-lactam ring on **DFD-1** will be hydrolyzed, leading to the quencher release, thus generating fluorescence for real-time resistant bacterial screening. More importantly, the bulky dibenzocyclooctyne group in **DFD-1** allowed selective recognition toward the AmpC bacterial enzyme instead of its counterpart (*e.g.*, TEM-1 β-lactamase). Both live cell imaging and cell cytometry assays showed the great selectivity of **DFD-1** to drug resistant bacterial pathogens containing the AmpC enzyme with significant fluorescence enhancement (∼67-fold). This probe presented promising capability to selectively localize and screen for AmpC resistance bacteria, providing great promise for clinical microbiological applications.



Widespread antibiotic resistance among pathogenic bacteria is a critical problem currently faced by both hospitals and community health worldwide, thus placing individuals' health at stake on both an international and national scale.[1−3] One of the primary defense mechanisms that antibiotic resistance bacteria possess is the production of drug resistant enzymes, *e.g.*, β-lactamases (Blas), a family of bacterial enzymes that specifically break down β-lactam antibiotic structure.[4,5] Generally, such resistant Blas enzymes can hinder the antibiotic ability to inhibit penicillin-binding proteins for cell wall biosynthesis, hence rendering these drugs ineffective for antibacterial treatment.[6,7] Therefore, extensive investigation is still required. Additionally, the development of specific approaches for selective recognition of resistance bacteria to gain a better understanding of the biochemical processes conferring bacterial drug resistance will be highly desirable.

In terms of distinct expression patterns and related catalytic mechanisms, Blas enzymes can be categorized into four different Ambler classes: A, B, C, and D, each with its unique enzyme sequence conferring intrinsic antibiotic bacterial resistance.[8−10] Among these enzyme classes, class A and C Blas are considered the most significant in hydrolysis of β-lactam drugs by resistance bacteria transmitting in human communities.[11,12] So far, extensive investigations specific to class A Blas have been widely conducted.[13−17] Although a similar serine hydroxyl group and geometries of active sites are observed in both class A and C Blas enzymes, their detailed secondary structures indicated a significant difference in the pocket size and the amino acid residue arrangement.[18−20] Critically, recent outbreaks of resistance in bacterial species previously not expressing class C Blas have emerged due to conjugative transmission of a bacterial plasmid, which led to a heightened clinical risk of antibiotic resistance in hospitals globally.[21−24] Therefore, a simple and unique approach that is capable of selectively identifying class C Blas enzyme activities will be clinically important in combatting microbial resistance.

Over the years, various effective strategies have been developed to facilitate Blas drug resistant enzyme investiga-

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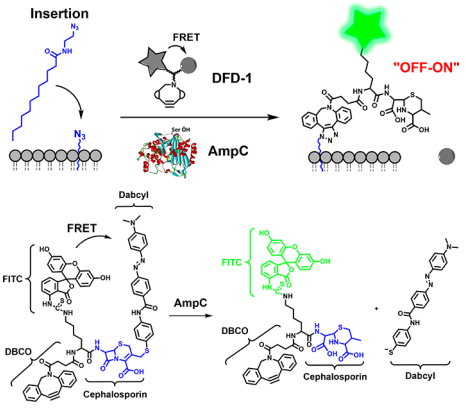
tions.[25−29] Among which, fluorescence optical imaging has been widely employed due to its promising possibilities in offering accurate and reliable results in real-time monitoring of biochemical functions and resolving drug-resistant bacterial pathogens *in vitro* and *in vivo*.[30,31] Despite great dependability, most of the established methods through chemical-based probe molecules may have concerns for real-time monitoring of the dynamics of enzyme functions, or limited precision for the accurate detection of bacterially resistant processes within a targeted and localized region which is mainly attributed to the multidimensional, heterogeneous, and spatial complexity of the cellular environment. Moreover, the inevitable diffusion of the chemical probe during the imaging process may present a potential issue of specificity or limited spatial resolution for real-time imaging analysis in living systems. Although several localization strategies based on covalent labeling or enzyme triggered probe molecule aggregation have been previously proposed to selectively report different resistant enzyme expression in bacterial pathogens;[32−35] these methods may potentially induce perturbation toward bacterial structure and functions in living settings. Therefore, the development of a simple and efficient method that allows effective probe localization on the bacterial surface, especially for the analysis of drug resistant bacteria, remains a technical challenge in the field, and extensive studies are required.

Fatty acid lipid molecules are naturally expressed cell surface components that are well recognized as integral membrane structures with a close relation to cell wall stiffness and susceptibility to various types of cell functions.[36,37] So far, incorporation of fatty acid lipidated groups with therapeutic moieties and contrast agents have been proposed to achieve an enhanced pharmacokinetic profile, improved treatment efficacy, as well as cellular structure-localized imaging to track real-time enzymatic dynamics and subcellular organelle position.[38−47] Inspired by the promising capability of such unique cell surface components, in this study, we present an effective method that can selectively recognize class C drug resistant Blas and, more importantly, interacts with bacterial cell components, allowing the desired localization effect to be obtained (Scheme 1). Effective immobilization of the probe onto the bacterial surface was achieved through insertion of the lipidated fatty acid chain, which is a part of the cell membrane component's favorable structure. Typically, a FRET pair, fluorescein isothiocyanate (FITC) fluorophore as a donor and 4-(4,2-dimethylaminophenylazo) benzoic acid (DABCYL) as a quencher, can be utilized to amplify enzyme activity. Under the presence of class C Blas (*e.g.*, AmpC), the cephalosporin β-lactam ring in **DFD-1** was cleaved, releasing the 3′-position conjugated DABCYL. Such enzyme triggered probe release led to fluorescence enhancement and allowed real-time visualization of resistance bacteria. To attain AmpC enzyme selectivity, a bulky dibenzocyclooctyne (DBCO) group was attached to the cephalosporin β-lactam ring 7′-position to introduce steric hindrance, which may enable selective recognition toward AmpC Blas. More importantly, through fatty acid chain exploitation, insertion of the lipid into the bacterial surface can allow the observation of a desired localization. To achieve such a localization property, we employed a lipid-azide conjugate for its penetration into the bacterial cell surface, followed by copper-free click chemistry, which immobilized the fluorescence signal onto the bacterial surface. Such a unique strategy can promote efficient localization of the fluorescence probe **DFD-1** and can thus greatly reduce the active diffusion of the probe molecules in bacterial structures, therefore providing great promise for performing precise and reliable screening of bacterial resistance in clinical practice.

Scheme 1. Bacterial Surface Localization and Enzymatic Responsive Fluorescence Changes upon the Reaction of Probe DFD-1 and AmpC Blas



RESULTS AND DISCUSSION

Synthetic Strategy. The synthetic route of probe **DFD-1** is depicted in Scheme 2. Cephalosporin β-lactam **1** was first linked with Mtt-Lys **3** at the 7′-position to yield Mtt-Lys-Lac **4** (step a). Next, at the 3′-position, cephalosporin was coupled with Thio-DABCYL **8** (prepared from trityl-protected 4-aminothiophenol with DABCYL) to afford **9** (step b). Subsequently, at the 7′-position of **9**, FITC was conjugated under basic reaction conditions, which was then followed by installation of the DBCO moiety through amide coupling with dibenzocyclooctyne-*N*-hydroxysuccinimidyl ester (DBCO-NHS ester) to give the desired product **DFD-1**. The reaction mixture was purified by high performance liquid chromatography (HPLC) and characterized by mass spectroscopy analysis (MS).

Enzymatic Properties. To study the enzyme activity of the reporter molecule **DFD-1**, the fluorescence emission was recorded upon the addition of AmpC Blas in phosphate buffered saline (PBS) solution (0.1 M, pH = 7.4). As shown in Figure 1A, under the absence of the Blas enzyme, little fluorescence was observed with **DFD-1** alone due to the efficient FRET quenching. Notably, with the treatment of the AmpC enzyme in PBS, significant enhancement in the fluorescence intensity at 516 nm was detected (~67-folds) before and after the enzyme reaction. This indicates the ability of the AmpC Blas enzyme to efficiently cleave the β-lactam ring and release the quencher from the cephalosporin structure, thereby resulting in the observed intensity increment. Moreover, such enzyme cleavage is further supported by the LC-MS spectral analysis of the fragments corresponding to the hydrolyzed cephalosporin ring and quencher Dabcyl moieties at 1062.73 and 377.23, respectively (Supporting Information Figure S1). In addition, a low fluorescence enhancement was found in the presence of a typical AmpC inhibitor, aztreonam (AZT; Figure 1B). Such effective suppression of the AmpC enzymatic activity resulted in a substantial reduction in the fluorescence readout, which clearly demonstrates the specificity of **DFD-1** to the AmpC enzyme instead of the spontaneous degradation of the probe or nonspecific interactions. As a control, a similar enzymatic analysis was carried out with the

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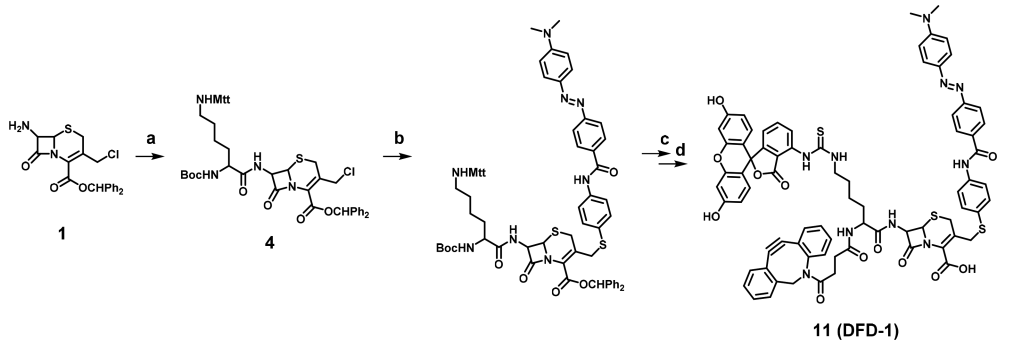
Scheme 2. Synthesis of the Enzyme Responsive Probe DFD-1[a]



[a]Reagents and conditions: (a) Mtt-Lys **3**, EDC·HCl, HOBt, TEA, CH_3CN/dioxane (1:1), 0–23 °C, 12 h; (b) Thio-DABCYL **8**, 2,6-lutidine, NaI, DMF 23 °C, 13 h; (c) (i) 2% v/v TFA, CH_2Cl_2, 23 °C, 1 h; (ii) FITC, TEA, DMF, 23 °C, 10 h; (d) (i) 12.5% v/v TFA, 2.5% v/v TIPS, CH_2Cl_2, 23 °C, 4 h; (ii) EDC·HCl, 4-DMAP, DBCO-NHS, DMF, 23 °C, 10 h.

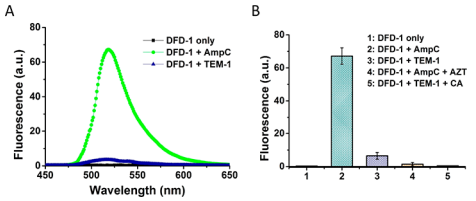


Figure 1. Emission spectra of (A) probe **DFD-1** (10 μM) before and after incubation with AmpC and TEM-1 Blas, respectively (30 nM). (B) Fluorescence enhancement of **DFD-1** incubated with AmpC, TEM-1 enzymes, inhibitor AZT, and CA (100 μM) in 0.1 M PBS (pH = 7.4).

use of TEM-1 enzyme, a typical class A Blas, to investigate the reaction selectivity. As shown in Figure 1A and B, treatment with the TEM-1 enzyme showed weak fluorescence activity after enzyme reaction. Further analysis of enzyme kinetics was also conducted to determine the activity of both AmpC and TEM-1 Blas (Supporting Information Figure S2). The probe **DFD-1** cleavage was identified with the Michaelis constant, K_m = 7.4 μM and 10.0 μM, and the catalytic constant, k_{cat} = 142.9 min^{-1} and 47.6 min^{-1}, for AmpC and TEM-1, respectively. Hence, these results clearly demonstrated that the synthesized **DFD-1** exhibited a greater specificity and selectivity recognition toward AmpC Blas rather than to the TEM-1 counterpart, mostly attributed to the additional bulky DBCO moiety within the **DFD-1** cephalosporin 7′-position that could accommodate well into the larger binding pocket located in the AmpC Blas enzyme structure.[18−20]

Ability of Lipids to Anchor onto Bacterial Surface. To investigate the feasibility of the enzyme responsive probe molecule to be efficiently anchored onto the bacterial surface for facilitated cell structure immobilization, we first employed a fatty acid molecule, an intrinsic lipid component of the bacterial surface as the targeting moiety modified with an azide group, for incubation with the bacterial pathogens. As a proof of concept in this study, two commonly used bacterial strains, the Gram-negative *Pseudomonas aeruginosa* PAO1 (*P. aeruginosa* PAO1) and Gram-positive *Enterococcus faecium* (*E. faecium*) were chosen. Both are well-known as typical pathogens for bacterial resistance studies.[48−51] Upon bacterial incubation with the lipid, a simple fluorescence molecule, DBCO-FITC (**DF**), was subsequently conjugated through a copper-free click reaction. The fluorescence images were recorded to investigate the effective bacterial surface insertion by using confocal microscopy with the excitation at 488 nm (Figure 2A). Furthermore, to examine whether the lipid moiety chain length

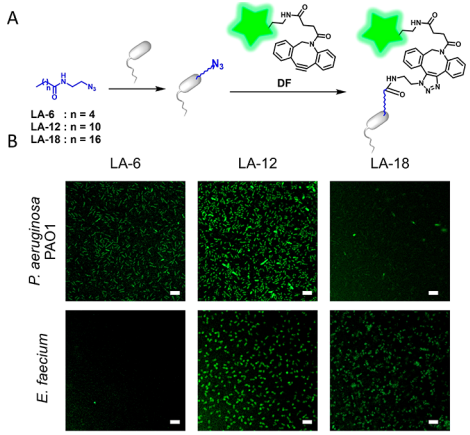


Figure 2. (A) Scheme of bacterial insertion with lipid of **LA-18**, **LA-12**, and **LA-6**, followed by click reaction with **DF**. (B) Confocal imaging of lipid screening in *P. aeruginosa* PAO1 and *E. faecium* bacterial strains upon treatment of lipid moieties (**LA-18**, **LA-12**, and **LA-6**) (2 μM) and **DF** (2 μM) in 0.1 M PBS, pH = 7.4. Scale bar: 5 μm.

may affect bacterial insertion, the azido-coupled fatty acids with different carbons (N-(2-azidoethyl) stearamide, N-(2-azidoethyl) dodecanamide, and N-(2-azidoethyl) hexanamide abbreviated as **LA-18**, **LA-12**, and **LA-6**, respectively; Supporting Information Scheme S2) were also synthesized, and their subsequent bacterial incubations were carried out for imaging analysis.

As shown in Figure 2B, the *P. aeruginosa* PAO1 strain, there was a weak fluorescence observed in fatty acid moiety **LA-18** incubated bacteria when compared to the pathogens treated with **LA-6** and **LA-12** structures. Although both bacteria treated with **LA-6** and **LA-12** moieties led to an increment in fluorescence signal, **LA-12** exhibited a stronger fluorescence intensity than that of **LA-6**. Similarly, for the *E. faecium* bacteria, among three fatty acids moieties, **LA-12** demonstrated the strongest fluorescence for lipid facilitated bacterial imaging (Figure 2B and Supporting Information Figure S3). The results indicated that the increased length of the carbon chain could likely enhance the immobilization of the probe onto the bacterial surface. The shorter hydrophobic chain in **LA-6** would

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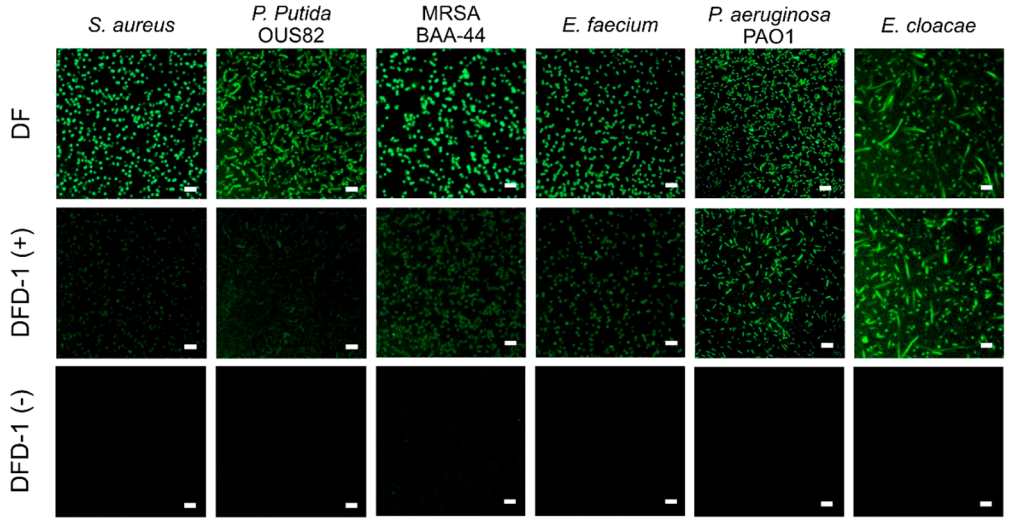

Figure 3. Fluorescence imaging of selective enzyme reaction and bacterial surface localization in living bacteria: antibiotic susceptible strains *S. aureus* and *P. putida* OUS82, TEM-1 Blas producing MRSA BAA-44 and *E. faecium*, as well as AmpC Blas producing *P. aeruginosa* PAO1 and *E. cloacae*, with subsequent treatment of **LA-12** (50 μM) and **DFD-1** (10 μM) in 0.1 M PBS, pH = 7.4. Scale bar: 5 μm.

lead to less staining, which could be easily washed away, giving decreased fluorescence intensity. Although **LA-18** could also show an enhanced tracking ability on the bacterial surface, this azido-coupled lipid moiety indicated limited solubility in aqueous solutions, which may potentially affect cell activity and reduce the effective concentration for bacterial imaging. These fluorescent staining studies in both *P. aeruginosa* PAO1 and *E. faecium* bacteria clearly showed the lipid moiety **LA-12** as the ideal tracer to specifically immobilize **DF** onto the bacterial cell surfaces, which may thus greatly facilitate dynamic visualization of drug-resistant enzymes in living bacteria. Additionally, staining of **LA-12** treated bacterial strains with propidium iodide (PI) showed minimum bacterial perturbation (Supporting Information Figure S4). These studies unequivocally indicated the great possibilities of **LA-12** as a promising localizing agent for bacterial imaging under living conditions.

Imaging Resistant Bacterial with LA-12 and DFD-1. Encouraged by the specific enzymatic hydrolysis of **DFD-1** as well as the promising bacterial immobilization ability of the **LA-12** lipid moiety, we investigated the possibility for localization of the reporter molecular **DFD-1** onto the bacterial surface for real-time imaging of drug resistant enzymes in living bacterial pathogens (Figure 3). In this typical study, two antibiotic resistant bacterial strains, *Enterobacter cloacae* (*E. cloacae*, ATCC 13047) and *P. aeruginosa* PAO1 (ATCC 15692), were selected as our main targets primarily due to their native capability to produce class C AmpC Blas enzyme. Another two drug resistant bacterial pathogens *E. faecium* (ATCC 51559) and methicillin-resistant *Staphylococcus aureus* (MRSA BAA-44, ATCC BAA44) were utilized as control strains owing to their high expression levels of class A TEM-1 Blas. In addition, two antibiotic susceptible strains *Pseudomonas putida* OUS82 (*P. putida* OUS82) and *Staphylococcus aureus* (*S. aureus*, ATCC 29213) without Blas expression were selected as the negative control. Typically, these bacterial strains were separately treated with lipid **LA-12** for 1 h at 37 °C. After washing and subsequent incubation with the **DFD-1** probe for another 30 min, the bacterial samples were subjected to confocal microscopy for fluorescence imaging analysis. As shown in Figure 3, strong fluorescence emissions were observed upon the incubation of the **DFD-1** probe with the AmpC Blas enzyme producing bacterial strains in *P. aeruginosa* PAO1 and *E. cloacae*. On the other hand, similar bacterial treatment with **LA-12** and **DFD-1** in the TEM-1 Blas enzyme expressing MRSA BAA-44 and *E. faecium* strains only resulted in weak fluorescence signals. Importantly, the pretreatment of targeted strains producing the AmpC enzyme (*e.g.*, *P. aeruginosa* PAO1) with a typical class C Blas AZT inhibitor greatly reduced the fluorescence intensity in bacterial imaging, while similar incubation of *P. aeruginosa* PAO1 bacteria with class A TEM-1 enzyme inhibitor CA demonstrated little effect on the fluorescence signal (Supporting Information Figure S5). In the negative control, there was almost no fluorescence detected in antibiotic susceptible *S. aureus* and *P. putida* OUS82 bacteria under treatment with the probe molecule (Figure 3). Importantly, there was minimum effect observed in inhibition of bacterial growth during the bacterial imaging by using the **DFD-1** probe molecule (Supporting Information Table S1). These results clearly enforced the capability of the designed enzymatic substrate **DFD-1** as a safe probe molecule for the selective recognition of AmpC enzyme activities in live bacterial strains. Moreover, compared to the imaging of bacteria strains treated with the **LA-12** lipid moiety and **DF**, the bright fluorescence signals observed in *P. aeruginosa* PAO1 and *E. cloacae* strains further confirmed the localization ability of **LA-12** that facilitated immobilization of the enzyme responsive probe onto the bacterial surface (Figure 3 and Supporting Information Figure S6).

Enzyme Activity and Click Reaction Contribution toward Imaging. In order to investigate the fluorescent staining effects of enzyme hydrolysis and click chemistry triggered bacterial localization contributed to live bacterial imaging, we compared these individual performances in the drug resistance bacteria (e.g., *P. aeruginosa* PAO1) with AmpC Blas expression. In this typical study, the bacterial strain *P. aeruginosa* PAO1 was first incubated with the **LA-12** lipid moiety for 1 h at 37 °C, followed by subsequent addition of **DF**. The bacterial surface immobilization triggered by the copper-free click chemistry reaction was determined by measuring the fluorescence change at different time durations. Alternatively, the enzyme cleavage activity was also carried out in a similar manner, through incubation of **LA-12** lipid-labeled

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P. aeruginosa PAO1 bacteria with the probe **DFD-1**; the fluorescence change was recorded to evaluate surface localized enzyme activities. As shown in Figure 4B, under the same

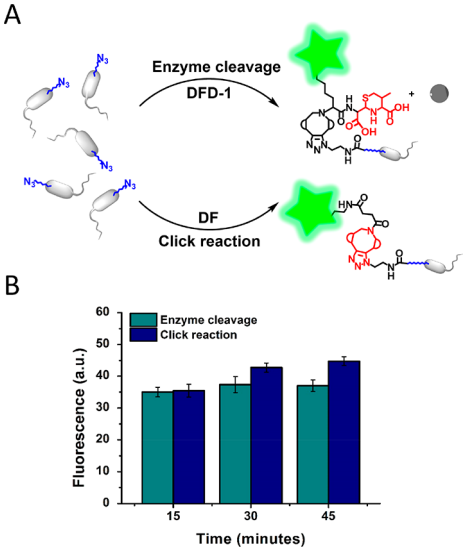

Figure 4. (A) Scheme of the different staining effects from the enzyme hydrolysis and click reaction toward fluorescence imaging in live bacteria. (B) Comparison of fluorescence intensity between the enzyme hydrolysis and click chemistry in live bacteria. **LA-12** lipidated (50 μM) *P. aeruginosa* PAO1 strains were separately treated with **DF** and **DFD-1** (10 μM), under different time intervals in 0.1 M PBS, pH = 7.4.

reaction conditions, **DF** labeling triggered by click reaction exhibited a relatively higher fluorescence intensity than the fluorescence generated from the enzyme reaction. The fluorescence profiles implied that both enzyme hydrolysis and click chemistry triggered surface insertion would contribute to the bacterial imaging, and the click coupling would facilitate the imaging probe staining on the bacterial surface for effective imaging studies. (Figure 4).

Flow Cytometry Analysis. Additionally, we studied the possibility to quantify the specific labeling of AmpC Blas expressing resistant bacteria (*e.g.*, *P. aeruginosa* PAO1) using flow cytometer analysis (FCM). The antibiotic susceptible *S. aureus* and resistant MRSA BAA-44 strains with expression of TEM-1 Blas were used as the negative control (Figure 5). In this study, the different strains were first incubated with the **LA-12** moiety and subsequently treated with **DFD-1** for flow cytometry analysis. Fluorescence signals were collected at 525 nm. As shown in Figure 5A, a stronger intensity of fluorescence was observed in the AmpC expressing *P. aeruginosa* PAO1 strain compared to the negative control *S. aureus* bacteria that do not produce β-lactamase. Meanwhile, a lower fluorescence change was detected for the TEM-1 producing MRSA BAA-44 strain. Moreover, a similar flow cytometry analysis with the treatment of AmpC enzyme inhibitor AZT showed a significant decrease in the fluorescence intensity (Figure 5B). Therefore, these data clearly demonstrate the specificity of **DFD-1** as a reliable reporter molecule for quantification of AmpC activities in antibiotic resistant bacteria.

In conclusion, this work presented a specific and selective approach toward efficient bacterial surface localization and real-time imaging of drug resistant bacteria with AmpC enzyme expression. In this study, the optimized lipid moiety (**LA-12**)

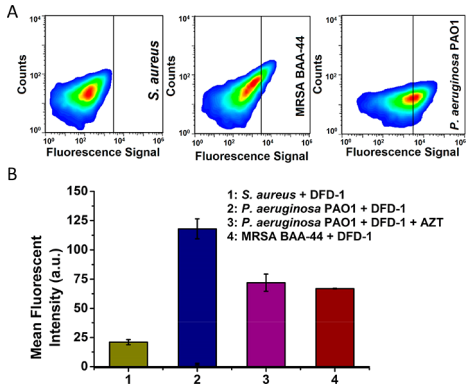

Figure 5. Flow cytometry analysis of resistant bacterial strains incubated with (A) lipid **LA-12** (50 μM) and probe **DFD-1** (10 μM) or (B) lipid **LA-12** (50 μM) and inhibitor AZT (100 μM), followed by probe **DFD-1** (10 μM) staining in 0.1 M PBS, pH = 7.4. Concentration of bacterial was approximately 10^8 cells mL^{-1}.

could efficiently be inserted into the bacterial surface and thus greatly facilitated the localization of the enzyme triggered fluorescent signal onto the bacterial surface. By taking advantage of the bulky DBCO group at the 7′-position of cephalosporin structure, the fluorescent probe **DFD-1** could selectively recognize the AmpC Blas enzyme. The significant fluorescence enhancement toward selective detection of the AmpC Blas enzyme indicated a promising strategy for direct observation of resistant bacterial infections under living conditions. More importantly, such lipid facilitated surface localization of fluorescence labeling could also provide the promising capability for dynamic monitoring of bacterial development and assessing effective antibacterial therapeutics *in vitro* and *in vivo*.

METHODS

See the Supporting Information for a detailed description of the experimental methods.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.8b00172.

Supplemental experimental procedures, figures, data, and spectra for compounds (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Braine, T. (2011) Race against time to develop new antibiotics. *Bull. World Health Organ 89*, 88−89.
(2) Fisher, J. F., Meroueh, S. O., and Mobashery, S. (2005) Bacterial resistance to beta-lactam antibiotics: compelling opportunism, compelling opportunity. *Chem. Rev. 105*, 395−424.
(3) Kahne, D., Leimkuhler, C., Lu, W., and Walsh, C. (2005) Glycopeptide and lipoglycopeptide antibiotics. *Chem. Rev. 105*, 425−448.
(4) Kong, K. F., Schneper, L., and Mathee, K. (2010) Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS 118*, 1−36.
(5) Bush, K., and Jacoby, G. A. (2010) Updated functional classification of beta-lactamases. *Antimicrob. Agents Chemother. 54*, 969−976.
(6) Lim, D., and Strynadka, N. C. J. (2002) Structural basis for the β lactam resistance of PBP2a from methicillin-resistant Staphylococcus aureus. *Nat. Struct. Biol. 9*, 870.
(7) Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A., and Charlier, P. (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis,. *FEMS Microbiol Rev. 32*, 234−258.
(8) Alekshun, M. N., and Levy, S. B. (2007) Molecular mechanisms of antibacterial multidrug resistance. *Cell 128*, 1037−1050.
(9) Bebrone, C., Lassaux, P., Vercheval, L., Sohier, J. S., Jehaes, A., Sauvage, E., and Galleni, M. (2010) Current challenges in antimicrobial chemotherapy: focus on ss-lactamase inhibition. *Drugs 70*, 651−679.
(10) Drawz, S. M., and Bonomo, R. A. (2010) Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev. 23*, 160−201.
(11) Bush, K., and Jacoby, G. (1997) Nomenclature of TEM beta-lactamases. *J. Antimicrob. Chemother. 39*, 1−3.
(12) Coudron, P. E., Moland, E. S., and Thomson, K. S. (2000) Occurrence and detection of AmpC beta-lactamases among Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates at a veterans medical center. *J. Clin Microbiol 38*, 1791−1796.
(13) Mizukami, S., Watanabe, S., Hori, Y., and Kikuchi, K. (2009) Covalent protein labeling based on noncatalytic beta-lactamase and a designed FRET substrate. *J. Am. Chem. Soc. 131*, 5016−5017.
(14) Mizukami, S., Hori, Y., and Kikuchi, K. (2014) Small-molecule-based protein-labeling technology in live cell studies: probe-design concepts and applications. *Acc. Chem. Res. 47*, 247−256.
(15) Shao, Q., and Xing, B. (2012) Enzyme responsive luminescent ruthenium(II) cephalosporin probe for intracellular imaging and photoinactivation of antibiotics resistant bacteria. *Chem. Commun. 48*, 1739−1741.
(16) Xing, B., Khanamiryan, A., and Rao, J. (2005) Cell-permeable near-infrared fluorogenic substrates for imaging beta-lactamase activity. *J. Am. Chem. Soc. 127*, 4158−4159.
(17) Xie, H., Mire, J., Kong, Y., Chang, M., Hassounah, H. A., Thornton, C. N., Sacchettini, J. C., Cirillo, J. D., and Rao, J. (2012) Rapid point-of-care detection of the tuberculosis pathogen using a BlaC-specific fluorogenic probe. *Nat. Chem. 4*, 802−809.
(18) Lobkovsky, E., Moews, P. C., Liu, H., Zhao, H., Frere, J. M., and Knox, J. R. (1993) Evolution of an enzyme activity: crystallographic structure at 2-A resolution of cephalosporinase from the ampC gene of Enterobacter cloacae P99 and comparison with a class A penicillinase. *Proc. Natl. Acad. Sci. U. S. A. 90*, 11257−11261.
(19) King, D., and Strynadka, N. (2011) Crystal structure of New Delhi metallo-beta-lactamase reveals molecular basis for antibiotic resistance. *Protein Sci. 20*, 1484−1491.
(20) Lahiri, S. D., Johnstone, M. R., Ross, P. L., McLaughlin, R. E., Olivier, N. B., and Alm, R. A. (2014) Avibactam and class C beta-lactamases: mechanism of inhibition, conservation of the binding pocket, and implications for resistance. *Antimicrob. Agents Chemother. 58*, 5704−5713.
(21) Philippon, A., Arlet, G., and Jacoby, G. A. (2002) Plasmid-determined AmpC-type beta-lactamases. *Antimicrob. Agents Chemother. 46*, 1−11.
(22) Jacoby, G. A. (2009) AmpC beta-lactamases. *Clin Microbiol Rev. 22*, 161−182.
(23) Doi, Y., and Paterson, D. L. (2007) Detection of plasmid-mediated class C beta-lactamases. *Int. J. Infect. Dis. 11*, 191−197.
(24) Liebana, E., Carattoli, A., Coque, T. M., Hasman, H., Magiorakos, A. P., Mevius, D., et al. (2013) Public health risks of enterobacterial isolates producing extended-spectrum beta-lactamases or AmpC beta-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. *Clin. Infect. Dis. 56*, 1030−1037.
(25) Zhang, J., Shen, Y., May, S. L., Nelson, D. C., and Li, S. (2012) Ratiometric fluorescence detection of pathogenic bacteria resistant to broad-spectrum beta-lactam antibiotics. *Angew. Chem., Int. Ed. 51*, 1865−1868.
(26) Hart, K. M., Reck, M., Bowman, G. R., and Wencewicz, T. A. (2016) Tabtoxinine-beta-lactam is a ″stealth″ beta-lactam antibiotic that evades beta-lactamase-mediated antibiotic resistance. *MedChemComm 7*, 118−127.
(27) Yang, Z., Ho, P. L., Liang, G., Chow, K. H., Wang, Q., Cao, Y., Guo, Z., and Xu, B. (2007) Using beta-lactamase to trigger supramolecular hydrogelation. *J. Am. Chem. Soc. 129*, 266−267.
(28) Staub, I., and Sieber, S. A. (2008) Beta-lactams as selective chemical probes for the in vivo labeling of bacterial enzymes involved in cell wall biosynthesis, antibiotic resistance, and virulence. *J. Am. Chem. Soc. 130*, 13400−13409.
(29) Moya, B., Dotsch, A., Juan, C., Blazquez, J., Zamorano, L., Haussler, S., and Oliver, A. (2009) Beta-lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. *PLoS Pathog. 5*, e1000353.
(30) Roy, R., Hohng, S., and Ha, T. (2008) A practical guide to single-molecule FRET,. *Nat. Methods 5*, 507−516.
(31) Lovell, J. F., Liu, T. W., Chen, J., and Zheng, G. (2010) Activatable photosensitizers for imaging and therapy. *Chem. Rev. 110*, 2839−2857.
(32) Shao, Q., Zheng, Y., Dong, X., Tang, K., Yan, X., and Xing, B. (2013) A covalent reporter of beta-lactamase activity for fluorescent imaging and rapid screening of antibiotic-resistant bacteria,. *Chem. - Eur. J. 19*, 10903−10910.
(33) Aw, J., Widjaja, F., Ding, Y., Mu, J., Liang, Y., and Xing, B. (2017) Enzyme-responsive reporter molecules for selective localization and fluorescence imaging of pathogenic biofilms. *Chem. Commun. 53*, 3330−3333.
(34) Bandyopadhyay, A., McCarthy, K. A., Kelly, M. A., and Gao, J. (2015) Targeting bacteria via iminoboronate chemistry of amine-presenting lipids. *Nat. Commun. 6*, 6561.
(35) Liang, H., DeMeester, K. E., Hou, C. W., Parent, M. A., Caplan, J. L., and Grimes, C. L. (2017) Metabolic labelling of the carbohydrate core in bacterial peptidoglycan and its applications. *Nat. Commun. 8*, 15015.
(36) Lingwood, D., and Simons, K. (2010) Lipid rafts as a membrane-organizing principle. *Science 327*, 46−50.
(37) Phillips, R., Ursell, T., Wiggins, P., and Sens, P. (2009) Emerging roles for lipids in shaping membrane-protein function. *Nature 459*, 379−385.
(38) Sautrey, G., Zimmermann, L., Deleu, M., Delbar, A., Souza Machado, L., Jeannot, K., Van Bambeke, F., Buyck, J. M., Decout, J. L., and Mingeot-Leclercq, M. P. (2014) New amphiphilic neamine derivatives active against resistant Pseudomonas aeruginosa and their interactions with lipopolysaccharides. *Antimicrob. Agents Chemother. 58*, 4420−4430.

DOI: 10.1021/acschembio.8b00172
ACS Chem. Biol. 2018, 13, 1890−1896

(39) Fosso, M. Y., Shrestha, S. K., Green, K. D., and Garneau-Tsodikova, S. (2015) Synthesis and Bioactivities of Kanamycin B-Derived Cationic Amphiphiles. *J. Med. Chem. 58*, 9124−9132.
(40) Lyu, S. Y., Liu, Y. C., Chang, C. Y., Huang, C. J., Chiu, Y. H., Huang, C. M., Hsu, N. S., Lin, K. H., Wu, C. J., Tsai, M. D., and Li, T. L. (2014) Multiple complexes of long aliphatic N-acyltransferases lead to synthesis of 2,6-diacylated/2-acyl-substituted glycopeptide antibiotics, effectively killing vancomycin-resistant enterococcus. *J. Am. Chem. Soc. 136*, 10989−10995.
(41) Yarlagadda, V., Akkapeddi, P., Manjunath, G. B., and Haldar, J. (2014) Membrane active vancomycin analogues: a strategy to combat bacterial resistance. *J. Med. Chem. 57*, 4558−4568.
(42) Benhamou, R. I., Shaul, P., Herzog, I. M., and Fridman, M. (2015) Di-N-Methylation of Anti-Gram-Positive Aminoglycoside-Derived Membrane Disruptors Improves Antimicrobial Potency and Broadens Spectrum to Gram-Negative Bacteria. *Angew. Chem., Int. Ed. 54*, 13617−13621.
(43) Benhamou, R. I., Bibi, M., Steinbuch, K. B., Engel, H., Levin, M., Roichman, Y., Berman, J., and Fridman, M. (2017) Real-Time Imaging of the Azole Class of Antifungal Drugs in Live Candida Cells. *ACS Chem. Biol. 12*, 1769−1777.
(44) Oshikawa, Y., Furuta, K., Tanaka, S., and Ojida, A. (2016) Cell Surface-Anchored Fluorescent Probe Capable of Real-Time Imaging of Single Mast Cell Degranulation Based on Histamine-Induced Coordination Displacement. *Anal. Chem. 88*, 1526−1529.
(45) Cobos-Correa, A., Trojanek, J. B., Diemer, S., Mall, M. A., and Schultz, C. (2009) Membrane-bound FRET probe visualizes MMP12 activity in pulmonary inflammation. *Nat. Chem. Biol. 5*, 628−630.
(46) Fan, Q., Cheng, K., Hu, X., Ma, X., Zhang, R., Yang, M., Lu, X., Xing, L., Huang, W., Gambhir, S. S., and Cheng, Z. (2014) Transferring biomarker into molecular probe: melanin nanoparticle as a naturally active platform for multimodality imaging. *J. Am. Chem. Soc. 136*, 15185−15194.
(47) Mu, J., Liu, F., Rajab, M. S., Shi, M., Li, S., Goh, C., Lu, L., Xu, Q. H., Liu, B., Ng, L. G., and Xing, B. (2014) A small-molecule FRET reporter for the real-time visualization of cell-surface proteolytic enzyme functions,. *Angew. Chem., Int. Ed. 53*, 14357−14362.
(48) Morita, Y., Tomida, J., and Kawamura, Y. (2014) Responses of Pseudomonas aeruginosa to antimicrobials. *Front. Microbiol. 4*, 422.
(49) Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., et al. (2000) Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. *Nature 406*, 959−964.
(50) Smith, J. R., Barber, K. E., Raut, A., Aboutaleb, M., Sakoulas, G., and Rybak, M. J. (2015) beta-Lactam combinations with daptomycin provide synergy against vancomycin-resistant Enterococcus faecalis and Enterococcus faecium. *J. Antimicrob. Chemother. 70*, 1738−1743.
(51) Sakoulas, G., Bayer, A. S., Pogliano, J., Tsuji, B. T., Yang, S. J., Mishra, N. N., Nizet, V., Yeaman, M. R., and Moise, P. A. (2012) Ampicillin enhances daptomycin- and cationic host defense peptide-mediated killing of ampicillin- and vancomycin-resistant Enterococcus faecium. *Antimicrob. Agents Chemother. 56*, 838−844.

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ACS Chem. Biol. 2018, 13, 1890−1896