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# Surface-Bound Antibiotic for the Detection of $\beta$ -Lactamases

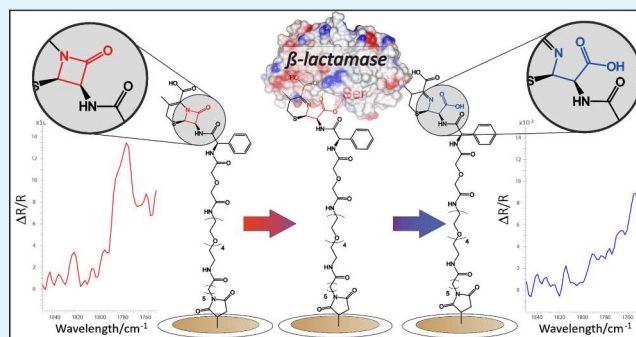
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## Supporting Information

**ABSTRACT:** Antimicrobial resistance (AMR) has been identified as a major threat to public health worldwide. To ensure appropriate use of existing antibiotics, rapid and reliable tests of AMR are necessary. One of the most common and clinically important forms of bacterial resistance is to  $\beta$ -lactam antibiotics (e.g., penicillin). This resistance is often caused by  $\beta$ -lactamases, which hydrolyze  $\beta$ -lactam drugs, rendering them ineffective. Current methods for detecting these enzymes require either time-consuming growth assays or antibiotic mimics such as nitrocefin. Here, we report the development of a surface-bound, clinically relevant  $\beta$ -lactam drug that can be used to detect  $\beta$ -lactamases and that is compatible with a range of high-sensitivity, low-cost, and label-free analytical techniques currently being developed for point-of-care-diagnostics. Furthermore, we demonstrate the use of these functionalized surfaces to selectively detect  $\beta$ -lactamases in complex biological media, such as urine.

**KEYWORDS:** antibiotics, antimicrobial resistance,  $\beta$ -lactamases, surface chemistry, surface-sensitive biosensor



## INTRODUCTION

Antimicrobials are vital for the treatment and prevention of bacterial infections. However, infections that were once simple to remedy are becoming increasingly resistant to treatment.<sup>1</sup> Although the emergence of antimicrobial resistance (AMR) is inevitable through evolution of bacteria via natural selection, increased exposure as a result of the misuse and overuse of antibiotics has accelerated the development of AMR globally.<sup>2,3</sup> Conventional methods used to identify antimicrobial susceptibility can take days to complete and often require isolation and culturing of the pathogenic bacteria.<sup>4</sup> In order to preserve the efficacy of antimicrobial agents, it is imperative that the prescribing practice for antibiotics is improved by allowing clinicians to perform rapid and reliable tests of susceptibility.  $\beta$ -Lactam-based antimicrobials are one of the most commonly used classes of antibiotic.<sup>5</sup> As a defense against these drugs, many bacteria are able to produce enzymes that hydrolyze the drug's pharmacophore (the  $\beta$ -lactam), rendering the drug inactive. Resistance to these classes of antibiotics is common, resulting in the restricted use of previously effective antibiotics, such as the earlier generations of penicillins. By detecting the presence (or absence) of  $\beta$ -lactamases, a susceptibility profile to these drugs can be deduced for a given infection and thus a suitable antibiotic can be prescribed. There are a number of methods currently available for the detection of  $\beta$ -lactamases, but these are limited to solution-phase assays.<sup>6–11</sup> A surface-based assay of  $\beta$ -lactam resistance would be compatible with a range of high-

sensitivity, low-cost, and label-free analytical techniques developed for point-of-care diagnostics.<sup>12–16</sup>

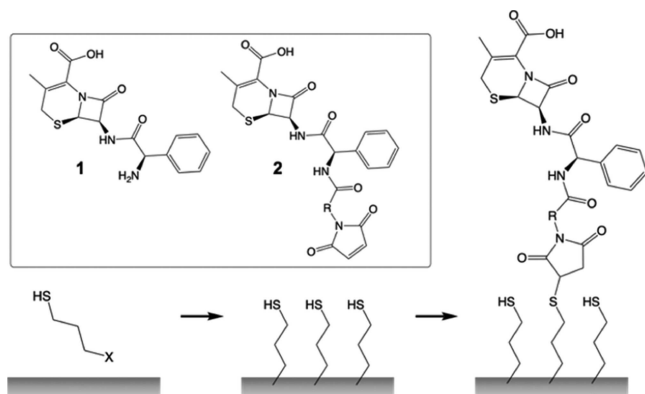
Herein, we report the development of a surface-bound antibiotic able to respond to the presence of  $\beta$ -lactamases, which is compatible with the wide range of emerging surface-sensitive biosensing technologies. Hydrolysis of the surface-bound  $\beta$ -lactams by  $\beta$ -lactamases indicates resistance to the drug. Here, cephalexin (1) was identified as a suitable substrate for immobilization. This first-generation cephalosporin is used widely for the treatment of bacterial infections.<sup>17</sup> Furthermore, the amine present in the drug's structure provides an ideal functional moiety for addition of a chemical tether (2), remote from the pharmacophore. This allows the compound to be anchored to a variety of surfaces, while minimizing the effect on the drug's activity (Scheme 1).<sup>18</sup>

## RESULTS AND DISCUSSION

A previous report has shown the preparation of a  $\beta$ -lactam-modified gold surface using a tether with a cyclic disulfide.<sup>19</sup> However, as many of the  $\beta$ -lactam antimicrobials feature a sulfur group within the core structure of the molecule, the orientation in which these molecules are bound to the surface remains ambiguous.<sup>20</sup> To ensure that the cephalexin molecules used here are immobilized on the surfaces in the optimal

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Scheme 1. Design of the Surface-Bound Antibiotic<sup>a</sup>

<sup>a</sup>SAM formation and attachment of cephalosporin via a maleimide tether. For Au surfaces, X = SH; for SiO<sub>2</sub> surfaces, X = Si(OEt)<sub>3</sub>. Inset: Structure of cephalosporin (1) and cephalosporin-R-maleimide (2), where R = CH<sub>2</sub>OCH<sub>2</sub>C(O)NHCH<sub>2</sub>PEG<sub>4</sub>CH<sub>2</sub>NHC(O)(CH<sub>2</sub>)<sub>5</sub>.

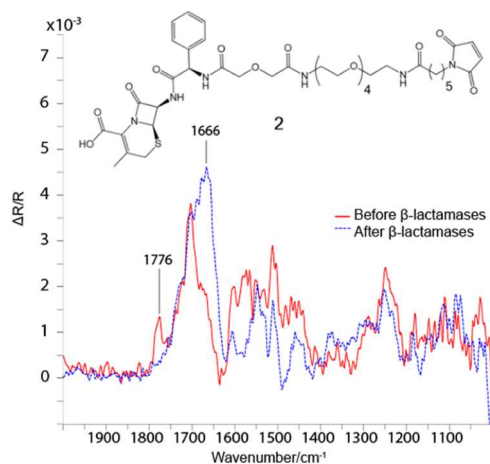
orientation, a two-step process was employed. Initially, the surface was modified with a self-assembled monolayer (SAM) with thiol terminal groups. The antibiotic was subsequently anchored to the thiolated surface via the maleimide tethered to the amine of cephalosporin (2) (Scheme 1).

Ideally, the surface should enable  $\beta$ -lactamases to access the drug, hydrolyze the  $\beta$ -lactam, and then ultimately detach from the surface. A previously reported co-crystal structure of a  $\beta$ -lactamase bound to a  $\beta$ -lactam-based inhibitor was employed in the design of compound 2, to ensure that the tether was of sufficient length to distance the cephalosporin from the surface and allow enzyme access (S1.1).<sup>21</sup> A polyethylene glycol (PEG) chain was chosen as these molecules are well known to inhibit physisorption of proteins onto surfaces, while providing conformational flexibility.<sup>22,23</sup> The thiol SAM was formed on an Au surface using 1,3-propanedithiol (Scheme 1). Control reactions confirmed that thiol addition was selective for the maleimide over the  $\beta$ -lactam (S1.7).

Polarization modulation infrared reflection adsorption spectroscopy (PM-IRRAS) allows direct chemical analysis of SAMs<sup>24</sup> and was employed here to confirm hydrolysis of this surface-bound antibiotic by  $\beta$ -lactamases. PM-IRRAS of a cephalosporin-PEG functionalized Au surface revealed a band at around 1776 cm<sup>-1</sup>, consistent with the carbonyl of the  $\beta$ -lactam, confirming that the pharmacophore of the immobilized antibiotic remained intact (Figure 1). With a surface of cephalosporin successfully prepared, the sample was immersed in a solution of potassium phosphate buffer (KPi) spiked with  $\beta$ -lactamases and incubated for 2 h to allow for the enzymes to hydrolyze the surface-bound cephalosporin. PM-IRRAS showed the loss of the  $\beta$ -lactam carbonyl band, confirming that hydrolysis had occurred (Figure 1).

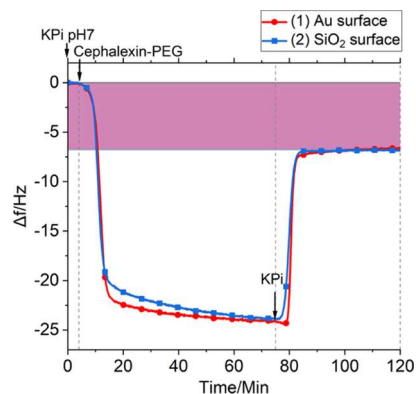
Control reactions demonstrated the stability of the surface-bound drug in the absence of  $\beta$ -lactamases, verifying that hydrolysis was a result of the enzyme-catalyzed reaction (S3.1 IR08). Whereas PM-IRRAS confirmed that  $\beta$ -lactamases were able to hydrolyze the immobilized cephalosporin, IR absorption in the amide I band region (1666 cm<sup>-1</sup>) indicated that protein fouling of the surface was occurring.

Quartz crystal microbalance with dissipation (QCM-D) monitoring was employed to further study the surface-bound drug, enabling quantification of the surface concentration of proteins and other molecules [calculated using the Sauerbrey



**Figure 1.** Structure of cephalosporin-PEG-maleimide (2). PM-IRRAS spectra of the cephalosporin-PEG surface before (red) and after (blue) exposure to  $\beta$ -lactamases.

equation (S3.2)]. For clarity, the frequency data are discussed herein, with dissipation data included in the Supporting Information (S3.3). To explore the versatility of the surface-bound antibiotic, we investigated two different QCM-D sensors coated with Au or SiO<sub>2</sub>. Cephalosporin-PEG (2) was immobilized on the Au-coated sensors via a thiol SAM formed using 1,3-propanedithiol. The surface chemistry was adapted for the SiO<sub>2</sub> sensors using 3-mercaptopropyltriethoxysilane (MPTES) to thiolate the surface. The surface concentration of cephalosporin-PEG (2) was found to be comparable on both the Au- and SiO<sub>2</sub>-coated sensors (Figure 2). Both coatings were



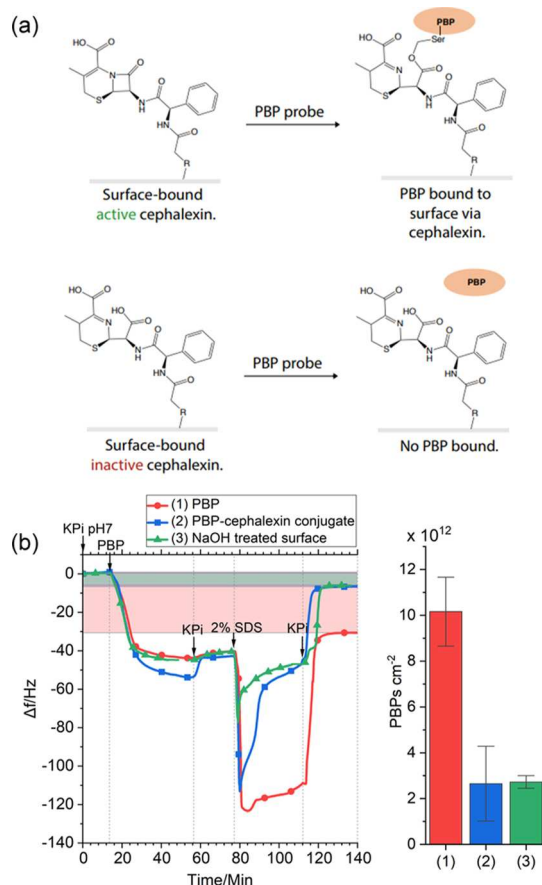
**Figure 2.** QCM-D experiment monitoring cephalosporin-PEG (2) binding to the thiolated surfaces of SiO<sub>2</sub> and Au sensors. The introduction of each solution is indicated by labeled arrows and dashed lines.

calculated to have approximately  $1.77 \times 10^{14}$  molecules/cm<sup>2</sup> (typical for a PEG monolayer)<sup>25,26</sup> of cephalosporin-PEG (2) bound, demonstrating the generality of the surface chemistry.

The two surfaces (Au and SiO<sub>2</sub>) were studied further to examine the interaction of  $\beta$ -lactamases with the surface-bound antibiotics. We found that physisorbed  $\beta$ -lactamases could be effectively removed from both the Au and SiO<sub>2</sub> surfaces functionalized with cephalosporin-PEG (2) by washing with a 2% sodium dodecyl sulfate (SDS) solution (S3.3 QCM02). Complementary experiments using a cephalosporin analogue with a short alkane tether showed increased biofouling, confirming the effectiveness of the PEG linker in minimizing

protein physisorption (S3.3 QCM02). We note that the cephalixin-PEG monolayer was found to be stable following exposure to 2% SDS (S3.1 IR08). Additional QCM-D analyses (discussed below) were performed using both Au and SiO<sub>2</sub> sensors to demonstrate the versatility of the surface-bound antibiotic. For clarity, the Au data are discussed herein, with data for the SiO<sub>2</sub> surfaces included in the Supporting Information (S3.3).

As the drug–enzyme complex is short-lived and there is no significant change in mass of the surface-bound drug after  $\beta$ -lactam hydrolysis, a suitable probe is required to confirm the hydrolysis reaction when using QCM-D. A penicillin-binding protein (PBP), the therapeutic target for  $\beta$ -lactam antibiotics, was chosen as it binds covalently to the active site of the protein. Because of the specificity of this protein, it allows for detection of the orientation and state of the surface-bound  $\beta$ -lactam (Figure 3a). Suitability of this probe was confirmed using a set of experiments in which three QCM-D sensors were functionalized with cephalixin-PEG (2). One sensor was exposed to PBP, the second was challenged with PBP which had been pre-incubated with cephalixin (1) to block the binding site, thus deactivating it, while the third was pre-exposed to 1 M NaOH in order to chemically hydrolyze the  $\beta$ -

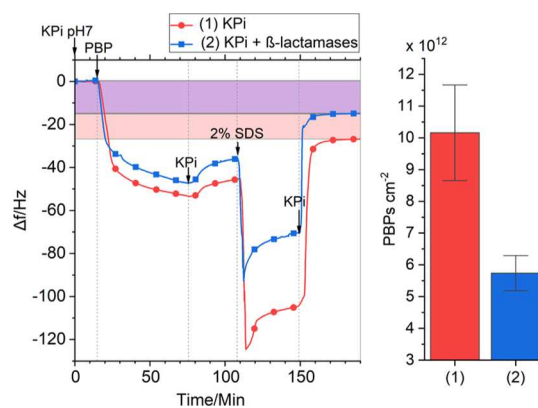


**Figure 3.** (a) Use of PBP as a probe for QCM-D studies. (b) QCM-D experiment of surface-bound cephalixin-PEG (2) on Au: (1) PBP only; (2) PBP preincubated with cephalixin; (3) surface antibiotics pre-treated with NaOH, a gap in the data indicates a skipped buffer step. The introduction of each solution is shown by labeled arrows and dashed lines. The number of PBPs bound after the final wash is shown for each sensor. Data reported as mean value  $\pm$  standard deviation.

lactams. All sensors were then washed using a 2% SDS solution in order to remove noncovalently bound PBPs.

A significant shift in resonant frequency was observed only when both the  $\beta$ -lactam was intact and the PBP was active (Figure 3b). The average value of this frequency shift ( $-27$  Hz) suggests that approximately  $1 \times 10^{13}$  PBPs/cm<sup>2</sup> are covalently bound to intact  $\beta$ -lactams on the surface. This surface density is typical of a protein monolayer,<sup>27</sup> suggesting that around 7% of surface-immobilized cephalixin molecules are bound to PBPs. This experiment confirmed the suitability of PBPs as a probe for the intact  $\beta$ -lactams. We note that when the PBPs were covalently bound to the surface-bound antibiotics, a large shift in frequency was observed following the introduction of SDS (Figure 3b(1)). Because of the covalent nature of the protein–drug bond, the surfactant interacts with the PBPs, but is unable to remove the proteins from the surface, causing a build-up of surfactant and a concomitant increase in mass.

Having confirmed that a PBP can function as a suitable probe for  $\beta$ -lactam hydrolysis, additional measurements were performed to further confirm the activity of  $\beta$ -lactamases against the cephalixin-PEG surface. As reported, a significant shift in frequency was observed following exposure of a cephalixin-PEG surface to PBPs. In contrast, a significantly lower concentration of PBPs ( $5.7 \times 10^{12}$  PBPs/cm<sup>2</sup>) was observed after the cephalixin-PEG surface was first challenged with  $\beta$ -lactamases (Figure 4). It was also noted that the



**Figure 4.** QCM-D experiment of surface-bound cephalixin-PEG (2) on Au: (1) PBPs only and (2)  $\beta$ -lactamases followed by PBPs. The introduction of each solution is shown by labeled arrows and dashed lines. The number of PBPs bound after the final wash is shown for each sensor. Data reported as mean value  $\pm$  standard deviation.

frequency spike associated with the SDS wash was much reduced for the sensor exposed to  $\beta$ -lactamases, indicating that PBPs physisorb rather than covalently bind to the surface. Control measurements using a  $\beta$ -lactamase inhibitor confirmed that the reduction in PBP binding was a result of  $\beta$ -lactam hydrolysis, rather than nonspecific adsorption of the  $\beta$ -lactamases blocking the surface (S3.3 QCM07/08). These results combined with the PM-IRRAS data (Figure 1) confirm that  $\beta$ -lactamases are capable of hydrolyzing the surface-bound antibiotics.

A stage often neglected in the development of novel biosensors is the compatibility of the device, and thus the sensor surface, with clinical samples. However, in order for rapid testing to be a possibility, tests should ideally be performed directly on clinical samples.<sup>28</sup> To this end, we



explored the robustness of the cephalexin-PEG surface for the detection of  $\beta$ -lactamases in physiological samples, specifically urine. Urine is analyzed routinely for diagnosis and susceptibility testing of urinary tract infections but, as a complex biological medium, it contains intact human cells, proteins, and small molecules creating a challenge for in-matrix detection. Gold samples were prepared with cephalexin-PEG (2) for analysis by PM-IRRAS. These samples were then exposed to urine spiked with  $\beta$ -lactamases or, as a control, challenged with urine only. Both surfaces showed excellent selectivity in response to  $\beta$ -lactamases, with reduction in the  $\beta$ -lactam carbonyl band only being observed in the samples exposed to urine spiked with  $\beta$ -lactamases (Figure 5a). The preservation of the  $\beta$ -lactams in the control samples (i.e., without  $\beta$ -lactamases, Figure 5b) demonstrates the stability of the surface-bound drug in urine over an extended time period. We note that hydrolysis of the  $\beta$ -lactam was incomplete after 24 h. In contrast, experiments performed using  $\beta$ -lactamases in buffer (Figure 1) showed loss of the  $\beta$ -lactam carbonyl band after 2 h. It is likely that the high protein concentration of urine resulted in increased surface biofouling, evidenced by a large IR band with maximum absorbance at  $1664\text{ cm}^{-1}$ , which could inhibit access to the surface-bound drug, thus inhibiting complete hydrolysis.

Complementary experiments were performed using QCM-D. The cephalexin-PEG monolayer, assembled on Au-coated QCM-D sensors, was exposed either to urine spiked with  $\beta$ -lactamases or to urine only. All sensors were washed in flow during the QCM-D measurement to ensure that each sample was treated to the same washing procedures, allowing a consistent baseline to be established (S3.3 QCM09/10). Significantly more binding of PBPs was observed on surfaces that had not previously been exposed to  $\beta$ -lactamases (Figure 5c). Comparing the urine studies (Figure 5c) to previous QCM-D experiments performed in buffer (Figure 4), it is clear that the number of PBPs binding to the cephalexin-PEG exposed to urine was reduced. This reduction in binding is likely due to the large number of nontarget proteins in urine; however, the surface still provides a robust and detectable response despite these challenging conditions.

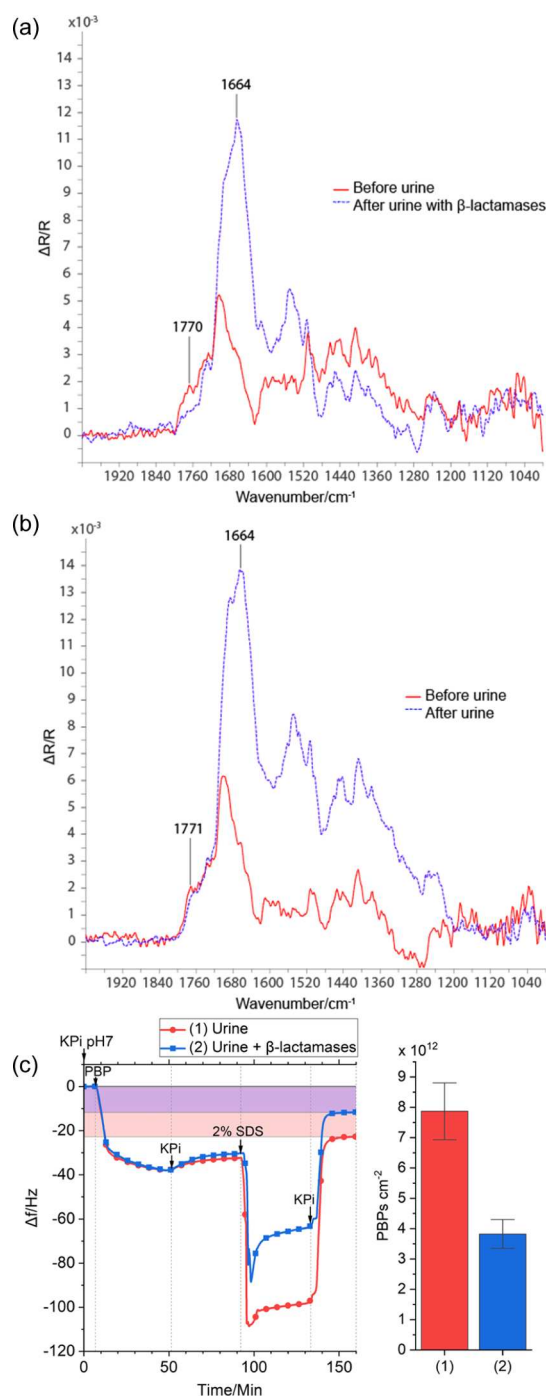
## CONCLUSIONS

In conclusion, this work details the development of a surface-bound antibiotic, able to detect the presence of  $\beta$ -lactamases in urine. Studies using PM-IRRAS and QCM-D demonstrated the successful hydrolysis of the surface-bound drugs by  $\beta$ -lactamases and confirmed the stability of the drug surface in the absence of these enzymes, even in urine. Results obtained from the antibiotic-functionalized Au and  $\text{SiO}_2$  surfaces were comparable, demonstrating the versatility of this method. This versatility could be exploited to allow integration of our novel cephalexin-PEG (2) with a wide range of current and emerging surface affinity biosensor technologies that ultimately could provide evidence-based diagnostics for the rational and targeted prescription of antibiotics.

## MATERIALS AND METHODS

**Compound Synthesis.** Details of the synthetic procedures, compound characterization, and spectra are provided in the Supporting Information (S1).

**Materials.** Planar gold surfaces were prepared for studies using PM-IRRAS. Briefly, samples were fabricated by electron beam evaporation of 25 nm Ti/100 nm Au onto cleaned Si wafers



**Figure 5.** PM-IRRAS and QCM-D studies in urine. (a) PM-IRRAS test: before and after exposure to urine spiked with  $\beta$ -lactamases. (b) PM-IRRAS control: before and after exposure to urine only. (c) QCM-D experiment of surface-bound cephalexin-PEG (2) on Au: (1) urine only and (2) urine spiked with  $\beta$ -lactamases. The introduction of each solution is shown by labeled arrows and dashed lines. The number of PBPs bound after the final wash is shown for each sensor. Data reported as mean value  $\pm$  standard deviation.

purchased from IDB Technologies. Gold (QSX 301)- and silicon dioxide (QSX 303)-coated QCM-D sensors were purchased from Biolin Scientific. Unless otherwise stated, solvents and reagents purchased commercially were used without further purification. 1,3-Propanedithiol was purchased from Sigma-Aldrich and MPTES was purchased from TCI Chemicals. DMSO was purchased from Fisher Scientific.

**Instrumentation.** PM-IRRAS spectra were acquired using a Bruker Vertex 70 spectrometer coupled with a PMA50 polarization modulation unit (Hinds Instruments, Oregon, USA). QCM-D spectra were recorded on a QSense E4, QFM 401. The surface area exposed to solution was equal to 0.95 cm<sup>2</sup> as defined by a Viton O-ring. The temperature in the QCM-D chamber was controlled by a Peltier device and was set to 37 °C (standard deviation 5 × 10<sup>-3</sup> °C) for all measurements.

**Surface Functionalization.** Gold wafers for PM-IRRAS were cleaned by immersion in piranha solution (H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> 70:30) for 10 min, followed by sonication in water and ethanol for 10 min each. QCM-D sensors were cleaned by UV-ozone treatment (10 min), followed by sonication in a 2% Hellmanex III solution (Hellma Analytics, Müllheim, Germany) (10 min) and then sonication in ultrapure water (2 × 10 min), followed by UV-ozone treatment (30 min) and immersion in EtOH (30 min). Cleaned samples were functionalized according to the type of surface employed. Cleaned gold samples were functionalized with 1,3-propanedithiol by 24 h immersion in a 10 μM MeOH solution. However, cleaned silicon dioxide samples were functionalized with MPTES by 24 h immersion in 4% v/v MPTES/IPA solution. Following the formation of the thiol SAM, samples were gently rinsed with ultrapure water and dried with N<sub>2</sub> gas. The samples were then immersed in the required cephalaximaleimide analogue solution (2 μM in 25% DMSO/H<sub>2</sub>O) for 24 h to allow formation of the thiol-maleimide conjugate. Finally, the samples were gently rinsed with 50% DMSO/H<sub>2</sub>O followed by ultrapure water, then dried with N<sub>2</sub> gas.

**β-Lactamase Stock Solution.** A blend of recombinant β-lactamase proteins, expressed in *Escherichia coli*, was purchased from Sigma-Aldrich (L7920) and used without further purification. The lyophilized powder was dissolved in 50 mM KPi (pH 7) and aliquoted into 1 mL aliquots, each containing 40–70 IU β-lactamase I and 6–10 IU β-lactamase II. The enzyme stock solutions were stored below 0 °C. For experiments testing the hydrolysis by β-lactamase, stock enzyme solution was diluted ×6 in the required test solution. Concentrations were maintained at this dilution throughout all experiments.

**Expression and Purification of PBP.** A truncated version of the ftsI gene formed of residues W44-S588, encoding only the soluble domain, was amplified from *E. coli* BW25113. The resulting construct was inserted into the vector pBADnLIC2005,<sup>29</sup> introducing an N-terminal deca-histidine tag when expressed. The resulting vector was transformed into *E. coli* MC1061 for expression. Cultures for protein expression were grown in 1 L of Luria-Bertani broth at 37 °C on an orbital shaker. Expression was induced by addition of 0.01% L-arabinose during mid-log phase of growth. Cultures were further incubated for 4 h and cells were harvested by centrifugation. Cell pellets were resuspended in 50 mM KPi pH 7.8, 200 mM NaCl, 10 mM imidazole, 20% glycerol with 1 mM phenylmethylsulfonyl fluoride followed by sonication. The lysate was clarified by centrifugation before loading onto a HisTrap HF column (GE Healthcare). To remove any pre-bound ligands, refolding purification was performed by initially washing with the protein unfolding buffer [2 M guanidine HCl, 50 mM KPi (pH 7.8), 200 mM NaCl, 20% glycerol, and 20 mM imidazole] and then performing a gradient wash to the protein refolding buffer [50 mM KPi (pH 7.8), 200 mM NaCl, 20% glycerol, and 20 mM imidazole] before the elution of the protein using the elution buffer [50 mM KPi (pH 7.8), 200 mM NaCl, 20% glycerol, and 500 mM imidazole]. The eluted protein was buffer-exchanged to the buffer 50 mM KPi (pH 7.8), 200 mM NaCl using a HiTrap Desalting (GE Healthcare) column. Binding activity of the purified *E. coli* PBP3 was confirmed using a thermal shift assay; details can be found in the Supporting Information (S2).

**PBP Stock Solution.** Protein concentration was determined using the Beer-Lambert law from the absorbance at 280 nm with an extinction coefficient of 58 790 M<sup>-1</sup> cm<sup>-1</sup>. For surface experiments using PBP, solutions were prepared to 1 μM protein in 50 mM KPi (pH 7).

**Urine Samples.** Urine samples were collected anonymously from 13 healthy adults that had not shown symptoms of infection or taking

antibiotics within 1 month prior to the sample collection. Participants were recruited from within the University of York. Samples were filter-sterilized using 0.22 μm syringe filters to remove any cells. The urine samples were combined to form an “average” human urine, which was aliquoted and stored in a -80 °C freezer. The pH of the resulting urine was measured to be pH 6.7. Control experiments did not detect any β-lactamases within the urine stock (S1.8).

**PM-IRRAS Surface Studies.** Following formation of the functionalized surfaces, the gold wafers were immersed in the test solution as described in the experimental procedures (Supporting Information). Once the surface reactions were complete, the gold wafers were cleaned according to the procedure detailed in the Supporting Information and then loaded into the PM-IRRAS spectrometer. The incident angle was set at 80° with a 4 cm<sup>-1</sup> spectral resolution, while the PEM controller operated at 2000 cm<sup>-1</sup>. Average measurement time was 15 min, collecting 1000 scans. Spectra and experimental details of each sample analyzed are provided in the Supporting Information (S3.1).

**QCM-D Surface Studies.** Following formation of the functionalized surfaces, each sensor was then installed into the flow modules of the Q-Sense E4 system. Each chamber was then filled with Milli-Q water at a flow rate of 100 μL/min controlled by a four-channel peristaltic pump. To achieve a stable baseline, the running buffer was left to flow through the modules at 20 μL/min for 60–80 min until the drift in frequency was <±1 Hz over 10 min. For all experiments, the temperature of the modules was kept at 37 °C (standard deviation 5 × 10<sup>-3</sup> °C) and the flow rate was kept constant at 20 μL/min. Buffer was allowed to flow over the sensor surface between sample injections until a stable level was achieved, corresponding to a drift in frequency <±1 Hz over 10 min. QCM-D spectra showing both the frequency and dissipation shifts for each experiment, with noteworthy shifts in frequency being indicated by shaded horizontal bars, are provided in the Supporting Information (S3.3). Surface concentrations of proteins were calculated using the Sauerbrey equation (S3.2). We accept that this method of analysis assumes a thin, rigid, and uniform layer, and is therefore only an estimated value.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.9b05793.

Compound synthesis and characterization, protein binding assay, PM-IRRAS, and additional QCM-D data (PDF)

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L.M.M. and C.D.S. contributed equally. All authors have given approval to the final version of the article.

### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

AMR, antimicrobial resistance  
KPi, potassium phosphate buffer  
MPTES, 3-mercaptopropyltriethoxysilane  
PBP, penicillin binding protein  
PEG, polyethylene glycol  
PM-IRRAS, polarization modulation infrared reflection adsorption spectroscopy  
QCM-D, quartz crystal microbalance with dissipation  
SAM, self-assembled monolayer

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