



# Covalent capture and electrochemical quantification of pathogenic *E. coli*†

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**Pathogenic *E. coli* pose a significant threat to public health, as strains of this species cause both foodborne illnesses and urinary tract infections. Using a rapid bioconjugation reaction, we selectively capture *E. coli* at a disposable gold electrode from complex solutions and accurately quantify the pathogenic microbes using electrochemical impedance spectroscopy.**

Pathogenic strains of *Escherichia coli* are the most common cause of urinary tract infections (UTIs)<sup>1,2</sup> and are a major contributor to foodborne diseases,<sup>3–6</sup> both of which cause millions of illnesses each year. Unfortunately, current methods to identify bacterial pathogens are limited to techniques performed in centralized laboratory facilities that require trained personnel and specialized equipment. The most prevalent methods to detect bacteria in complex samples such as bodily fluids or foods are colony-based detection and the polymerase chain reaction (PCR).<sup>7–11</sup> Despite the sensitivity of these methods, they cannot be deployed for point-of-contamination detection of foodborne pathogens, nor can they be used for the rapid point-of-care diagnosis of UTIs. Colony-based detection often requires several days of growth to determine accurate colony counts, and, though PCR can be used to identify bacteria, it cannot discern between living and dead cells. In order to combat the spread of these dangerous pathogens, the development of a rapid-readout, field-deployable test is paramount.

Electrochemical detection offers a rapid and inexpensive alternative to traditional methods for detection and quantitation of pathogenic bacteria. For *E. coli* sensing, antibody- and

aptamer-based electrochemical sensors have been reported that enable highly sensitive monitoring of these microbes. Such technologies have enabled detection limits of 10 colony-forming units (CFU) mL<sup>-1</sup> or lower.<sup>12–18</sup> However, the incorporation of biorecognition elements increases the cost and complexity of these platforms and decreases their stability, often necessitating highly controlled storage and transportation conditions. Additionally, neither antibodies nor aptamers covalently capture bacteria, which can lead to variability in measurements due to washes or interactions with components of the analyte solution.

Here, we report an electrochemical sensor to detect *E. coli* from complex food samples and bodily fluids. By combining covalent capture and electrochemical impedance spectroscopy (EIS) for sensing,<sup>19</sup> quantitative detection of the bacteria was achieved with a limit of detection (LOD) of 12 CFU mL<sup>-1</sup>. To accomplish this, a fast and selective bioorthogonal conjugation reaction between the *E. coli* cells and the electrochemical surface was needed. As demonstrated by Bandyopadhyay *et al.*,<sup>20</sup> the synthetic amino acid 2-acetylphenylboronic acid can be incorporated into the *E. coli* cell wall *via* peptidoglycan remodelling, which can then be reacted with a fluorescently-labelled carbazide. The bioconjugation reaction proceeds rapidly, with rates faster than 10<sup>3</sup> M<sup>-1</sup> s<sup>-1</sup>, to form a stable diazaborine linkage. Following the reported synthetic protocol,<sup>21</sup> the synthesis of the amino acid was completed, albeit with significant racemization of the final product, as observed and quantified by chiral HPLC (ESI,† Fig. S5). The enantiomeric ratio is important to note as the D-enantiomer has been shown to incorporate into the *E. coli* peptidoglycan at higher levels than the L-enantiomer. However, specificity over other bacterial species was retained for both configurations of the synthetic amino acid.<sup>20</sup> To capture the cell at the gold electrode surface, a PEGylated thiol with a terminal carbazide group was synthesized in four steps (Fig. 1a). The reactivity of this terminal carbazide with 2-acetylphenylboronic acid incorporated in the *E. coli* peptidoglycans was confirmed by flow cytometry (ESI,† Fig. S7).

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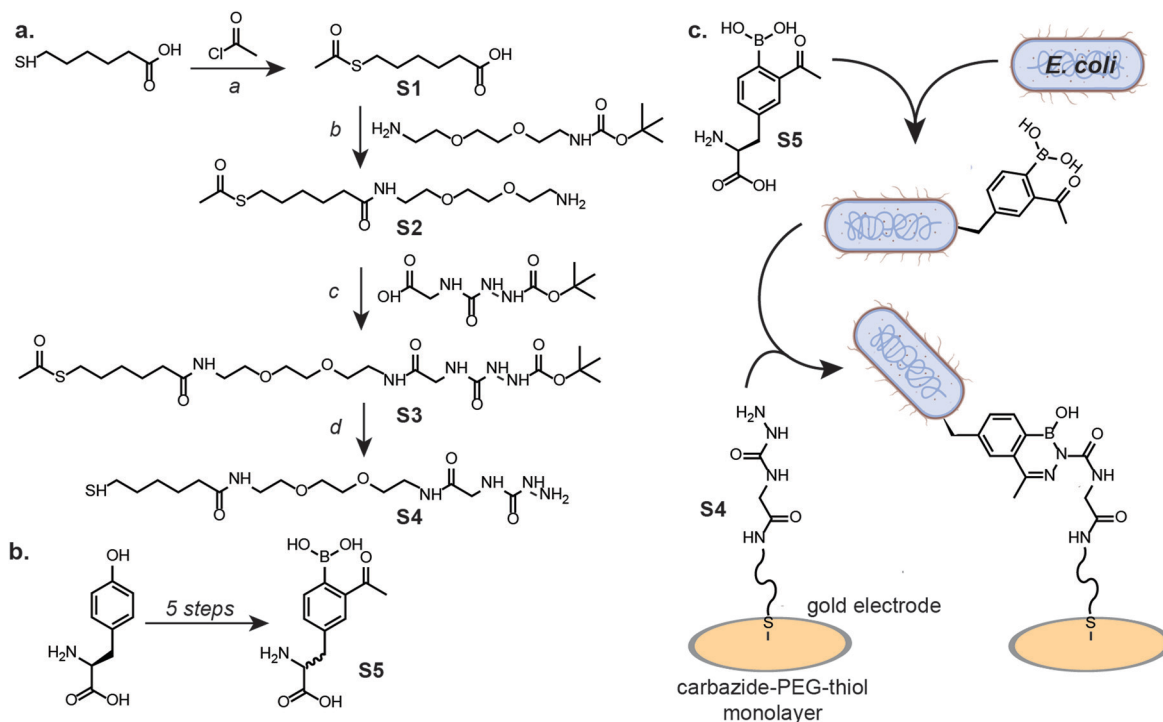
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**Fig. 1** Synthetic route for *E. coli* capture at a modified electrode. (a) The carbazide-PEG-thiol small molecule (S4) was synthesized in 4 steps (a–d). Acetyl chloride was reacted with 6-mercaptohexanoic acid, generating S1 (a). To S1 was added tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate, followed by deprotection, generating S2 (b). S2 was reacted with (2-(tert-butoxycarbonyl)hydrazine-1-carbonyl)glycine to generate S3 (c), followed by reaction with  $K_2CO_3$  and deprotection to generate S4 (d). (b) 2-Acetylphenylboronic acid (S5) was synthesized in five steps following a previously-published protocol<sup>21</sup> to yield a mixture of the enantiomers. (c) The reaction between 2-acetylphenylboronic acid-labelled *E. coli* and the carbazide-PEG-thiol monolayer self-assembled on the gold surface yields a covalent diazaborine linkage to capture cells on the electrode.

For electrochemical detection, *E. coli* cells were labelled through the initial incorporation of 2-acetylphenylboronic acid into the peptidoglycan cell wall. As the synthetic amino acid must be enzymatically incorporated into the peptidoglycan, only viable cells of specific species are able to incorporate it.<sup>20</sup> Further specificity for the labelling of *E. coli* over other bacterial species (*S. aureus*) is due to the rate of incorporation of the 2-acetylphenylboronic acid, as was previously demonstrated.<sup>20</sup> To capture the 2-acetylphenylboronic acid-labelled *E. coli* cells on the electrode surface, the carbazide-PEG-thiol was self-assembled on the gold electrode, forming a monolayer through gold-thiol bonds. When exposed to the reactive monolayer, the labelled *E. coli* were covalently captured by the rapid bioconjugation reaction, resulting in stable diazaborine linkages (Fig. 1c).

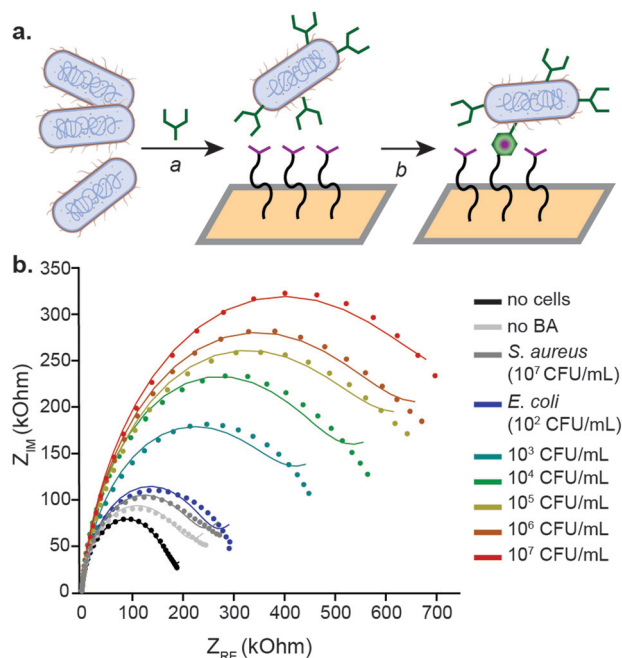
Combining the covalent capture of cells with an EIS-based readout, which is a facile and highly sensitive electrochemical sensing technique, enabled the quantification of *E. coli* in multiple complex solutions.<sup>19</sup> As the platform is intended for point-of-contamination and point-of-care measurements, the capture and detection strategy was developed using inexpensive, disposable gold electrodes, which necessitate only 10  $\mu$ L of sample volume for sensing (ESI,† Fig. S6).<sup>22</sup> The workflow for labelling, capture, and quantification requires less than two hours. The cells are initially incubated with the synthetic amino acid for one hour at 37 °C, followed by washing and a five-minute heat killing step.

The labelled cells are then incubated on the electrode for 30 minutes, followed by electrochemical detection, which requires less than five minutes (Fig. 2a).

For sensitive electrochemical detection, faradaic EIS in the presence of ferricyanide and ferrocyanide was performed. Example Nyquist plots of the detection of known *E. coli* concentrations in buffer are shown (Fig. 2b). The data were fit with a Warburg impedance model, which is overlaid with the raw plots. From the resulting model fits, the charge transfer resistance ( $R_{CT}$ ) was extrapolated and correlated to the number of cells in samples of known concentration. *E. coli* were measured and quantified over a range of  $10^2$ – $10^7$  CFU  $mL^{-1}$ . The  $R_{CT}$  increased by an average of 46.5% in the presence of 100 cells, with an order of magnitude larger increase for  $10^7$  cells (315% change). From these data, the LOD was calculated to be 12 CFU  $mL^{-1}$  in buffer. Based on the measured range of bacterial concentrations, cells can be detected and quantified over a range of  $10^2$ – $10^7$  CFU  $mL^{-1}$ , which can be seen in the normalized  $R_{CT}$  values for *E. coli* quantification in buffer, shown in (Fig. 3a). This range is well within what is necessary for both foodborne pathogen detection and UTI diagnosis.<sup>23</sup>

The specificity of the platform was then evaluated for *E. coli* quantification. As can be seen in Fig. 3a, the sensor does not respond to the presence of *S. aureus*, independent of the concentration of cells. Even at the highest concentration of *S. aureus* evaluated,  $10^7$  CFU  $mL^{-1}$ , the change in the  $R_{CT}$  for

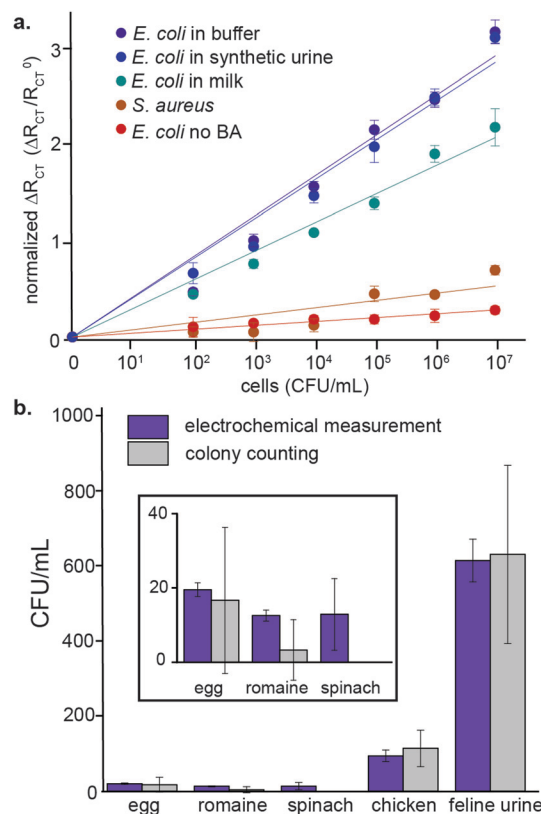




**Fig. 2** Capture and electrochemical detection of *E. coli*. (a) *E. coli* are incubated with the synthetic amino acid for one hour at 37 °C (a). Cells that incorporate the 2-acetylphenylboronic acid (green) are captured at the electrode surface (b), where they can be quantified electrochemically using electrochemical impedance spectroscopy (EIS). (b) EIS is used to quantify bacteria captured at the electrode. Nyquist plots of bacterial detection with the platform at concentrations ranging from  $10^2$  to  $10^7$  CFU mL<sup>-1</sup>, along with the curve fits used to determine the charge transfer radius ( $R_{CT}$ ).

these cells was only 69%, as compared to 315% for *E. coli*. Similarly, if the *E. coli* are not exposed to the 2-acetylphenylboronic acid prior to incubation with the electrode surface, non-specific adhesion is not observed. Only a 28% change in the normalized  $R_{CT}$  occurred, even upon treatment with  $10^7$  unlabelled cells mL<sup>-1</sup>. The lack of electrochemical response from *S. aureus* as well as from unlabelled *E. coli* confirms the specificity of the platform for cells that rapidly incorporate 2-acetylphenylboronic acid into their peptidoglycan cell wall. One limitation of the platform, though, is that it cannot discern between *E. coli* strains. The electrochemical results were further confirmed by flow cytometry. *E. coli* and *S. aureus* were exposed to varying concentrations of the synthetic amino acid, followed by secondary labelling with a fluorescent semicarbazide. Incubating with 2-acetylphenylboronic acid concentrations between 20–100  $\mu$ M showed labelling of *E. coli* with minimal background labelling of *S. aureus* (ESI,† Fig. S7). Consistent with prior literature,<sup>20</sup> the phenylboronic acid labelling is specific for viable *E. coli*, which are capable of rapidly integrating the synthetic amino acid through peptidoglycan remodelling.

Following the initial sensor characterization with pathogens detected from buffer, the ability of the sensor to detect *E. coli* in complex solutions was further investigated. *E. coli* were detected and quantified in artificial urine (obtained from Innovating Science™) with similar sensitivity to buffer; the LOD was also 12 CFU mL<sup>-1</sup> in this matrix. Although the percent change in the  $R_{CT}$  was slightly lower for detection of these



**Fig. 3** Electrochemical quantification of cells in complex matrices. (a) From the electrochemical impedance spectroscopy data, the charge transfer resistance ( $R_{CT}$ ) for each condition is extrapolated. For *E. coli* in buffer, artificial urine, and milk, cells are detected over a range of  $10^2$ – $10^7$  CFU mL<sup>-1</sup>. Minimal response is observed for *S. aureus* and for *E. coli* that are not pre-treated with the 2-acetylphenylboronic acid (*E. coli* no BA). (b) Endogenous *E. coli* are detected from egg, raw chicken, spinach, and romaine lettuce rinsings, as well as from feline urine. These values are compared to cell counts as measured by colony counting on agar. Error bars represent standard error for  $n = 3$  replicates.

bacteria in milk (216% change in the normalized  $R_{CT}$  for  $10^7$  cells), a similar LOD and range of detection is maintained (Fig. 3a). This slightly smaller change is likely due to proteins from the milk adhering to the electrode surface or sugars in solution interfering with the coupling, as a slight decrease in signal is similarly observed by flow cytometry.

Finally, detection of endogenous *E. coli* was performed with samples commonly contaminated with this pathogen. Rinsings from the surface of eggs, raw chicken, spinach, and romaine lettuce<sup>24–26</sup> were measured electrochemically and compared to colony counts following growth on agar plates. Additionally, a sample of UTI-contaminated feline urine was obtained. As can be seen in Fig. 3b, CFU counts of *E. coli* measured using the electrochemical platform were consistent with values determined by colony counting, but with significantly lower error. The bacteria present in the samples were confirmed to be of the *Escherichia* genus by 16S sequencing. Thus, the electrochemical impedance sensor is capable of competing with established technologies to detect contaminants from complex samples.



*E. coli* pose a serious threat to public health, both as a foodborne pathogen and a leading cause of UTIs. Accepted methods to detect these microbes generally require centralized laboratory facilities with specialized equipment, trained personnel, and hours or days to complete. A sensitive electrochemical sensor has been developed to covalently capture, detect, and quantify *E. coli* from complex sample matrices with an LOD of 12 CFU mL<sup>-1</sup> and a linear range of detection up to 10<sup>7</sup> CFU mL<sup>-1</sup>. The technology enables quantification of these pathogens within two hours on disposable electrodes, even from complex matrices including milk and artificial urine. Detection of endogenous *E. coli* was successful in commonly-contaminated samples, including eggs, raw chicken, spinach, and romaine lettuce, in addition to an infected urine sample. The quantification of *E. coli* from these samples tracked well with the current gold-standard of colony counting on agar plates. This platform represents significant progress in the development of field-deployable sensors to detect these dangerous pathogenic microbes.

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## Conflicts of interest

There are no conflicts to declare.

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