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Glycosylation of Quinone-Fused Polythiophene for Reagentless and Label-Free Detection of *E. coli*

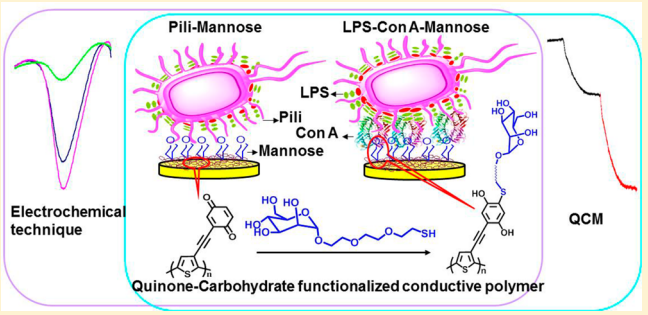
Fen Ma,[†] Abdul Rehman,[†] Haiying Liu,[‡] Jingtuo Zhang,[‡] Shilei Zhu,[‡] and Xiangqun Zeng*[,†]

[†]Department of Chemistry, Oakland University, Rochester, Michigan 48309, United States

[‡]Department of Chemistry, Michigan Technological University, Houghton, Michigan 49931, United States

Supporting Information

ABSTRACT: In this report, a new polythiophene interface is fabricated containing fused quinone moieties which are then glycosylated to form a carbohydrate platform for bacterial detection. Very importantly, this interface can be used for label-free and reagentless detection, both by electrochemical and Quartz Crystal Microbalance (QCM) transducers and by using the direct pili-mannose binding as well as Concanavalin A (Con A) mediated lipopolysaccharides (LPS)-mannose binding. The conductive polymer's unique collective properties are very sensitive to very minor perturbations, which result in significant changes of electrical conductivity and providing amplified sensitivity and improved limits of detection (i.e., 25 cell/mL for electrochemical sensor and 50 cells/mL for QCM sensor), a widened logarithmic range of detection (i.e., 3−7 for pili-mannose binding and 2−8 for Con A mediated binding), high specificity and selectivity, and an extraordinary reliability by a mechanism of internal validation. With these analytical performances, the described biosensor is envisaged for being capable of differentiating Gram-negative bacterial strain and species, for many important applications.



Early diagnosis of bacterial infections increases the possibility of successful medical treatment.[1] Furthermore, homeland security threats escalate the need for point-of-care (PoC) detection and identification of potentially hazardous bacterial pathogens.[2] The major requirements for PoC testing systems are real time sensing and miniaturization into a portable, autonomous, and inexpensive platform so that the immediate results can be obtained without a laboratory setting.[3] Currently, the detection and identification of causative pathogens utilizes classic microbiological techniques that rely on the culture and biochemical identification of the pathogen. Depending upon the source of the culture, the pathogen growth rate, specific nutritional needs, and the inoculum size, the presence of a pathogen may not be confirmed for up to 5 days, with another 24−48 h needed to biochemically identify the bacterial species.[4] These delays lead to empiric use of broad spectrum and often multiple antibiotics,[5] which in turn lead to increased rates of antibiotic resistance.[6] To date, rapid identification of pathogens from sources such as blood has had limited success. The Septifast system[7] uses a series of real-time polymerase chain reactions (PCR) to determine the presence of pathogens directly from blood samples, but it requires a large instrument, complex sample processing, and precious time to perform. FDA approved Matrix-Assisted Laser Desorption Ionization (MALDI) based systems have significantly shorter identification time once a culture has turned positive, but they also have shown little use in identification of pathogens directly from clinical samples. Moreover, the identification of pathogens can often be quite difficult if the organism is fastidious and difficult to culture.[8] In these cases, detection is often limited to serology, which implies but does not confirm actual active infection, or antigen detection, thereby having low sensitivity and specificity. In general, existing molecular techniques have not fulfilled their promise for rapid diagnosis of infections and are still in the realm of experimental or nonstandardized testing.

In order to develop a PoC system for rapid pathogen detection, it is critical to eliminate the time-consuming culture step and minimize sample preparation requirements. Recently, it was shown that detection of bacteria can be achieved with biosensors that monitor the presence of protein and carbohydrate biomarkers within the organism or on the bacterial cell surface.[4b] However, identification of bacteria based on a single physical property may not provide the same degree of discrimination as molecular based nucleic acid analysis techniques due to phenotypic variation among bacteria.[9] To improve the discrimination power, multiple physicochemical properties of bacterial cells need to be measured simultaneously. For instance, the bacterial discrimination is shown to be enhanced by use of carbohydrate[10] and lectin[11] biosensor interfaces that target fimbriae proteins and O-antigens of lipopolysaccharides (LPS), respectively. In order

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to measure these binding events, various transduction mechanisms, including optical,[12] electrochemical,[13] thermal,[14] and acoustic,[4b] have been applied using both labeled and nonlabeled techniques.[15] Nonlabeled techniques offer significant advantages, including elimination of time- and cost-demanding labeling steps and avoidance of interference due to binding of labeling agents. In this regard, we have already demonstrated reagentless and label-free carbohydrate, boronic acid, and lectin based biosensing interfaces that measure a multitude of discrete carbohydrate−protein interactions on bacterial cells using either quartz crystal microbalance (QCM) or electrochemical readout. However, the electrochemical processes, despite being extremely sensitive, often require the use of a redox probe such as $K_3Fe(CN)_6/K_4Fe(CN)_6$, which is not desirable for PoC systems, as this is an additional reagent and needs a timely replacement. $Fe(CN)_6^{4-/3-}$ in the electrolyte can be metamorphic as well.[16] Therefore, efforts are made for designing a solid state redox probe by immobilizing the redox probe on the electrode surface.[16] QCM sensors, on the other hand, are very sensitive and label-free, but that sensitivity is universal, therefore requiring a validation that the obtained sensor response has truly come from the binding event. Thus, if we can combine these two approaches in a fashion that allows cross-validation of each other and can measure multiple bacterial biomarkers simultaneously, the resulting sensing profile can provide a virtual fingerprint that may enable discrimination equal to or better than nucleic acid based methods and thus enable identification of organism species and differentiation of organism strains. However, the most critical limitation in this regard is the development of a sensing interface that allows the facile integration of these approaches while still having the same or better sensing attributes.

In this work, we have described a new approach exploring the benefits of functionalized conducting polymers as a solid state redox probe and the well-established quinone based coupling chemistry for incorporating carbohydrate functionality, thereby fabricating an innovative carbohydrate biointerface. Conductive polymers containing heterocyclic aromatics as monomeric units can be ideal precursors in this regard, providing uniform and strongly adherent films.[17] Moreover, these films can be electrochemically deposited onto a small area with a high degree of geometrical conformity and controllable thickness,[18] justifying their application in microsensors. We used thiophene in this work because of its good thermal and chemical stability and relative ease of functionalization[19] with quinone (Q)/hydroquinone (H_2Q) functionality. Polythiophene with π-conjugated quinone moieties also displays low formal potential around 0.42 V vs an Ag/AgCl reference electrode. By taking advantage of the addition and substitution reactions of quinones with nucleophiles, particularly thiol and amino groups, we were then able to incorporate carbohydrate functionality to the polythiophene units. In contrast to monosaccharide-quinone for electrochemical probing of protein binding reported earlier,[20] which can be time-consuming (about 12−36 h) due to slow self-assembled monolayer (SAM) immobilization, the instability of the S−Au bond at reductive potentials, and the high mobility of the SAM monolayer due to the weakness of the S−Au bond. In this work, the thiophene containing fused quinone moieties (abbreviated TQ), synthesized according to the literature,[21] were immobilized on the gold electrode by electrochemical polymerization to form polythiophene, which enables the coupling of the thiol-modified mannose, α-D-mannopyranoside, 2-[2-(2-mercaptoethoxy)ethoxy]ethyl (SM), to gold electrode based on 1,4-reductive addition of the Michael type. The SM/TQ-glycosurface thus generated[22] allowed label-free detection of two major bacterial cell surface biomarkers, the fimbriae proteins on bacterial pili and the LPS on Gram-negative bacteria using orthogonal QCM and electrochemical readouts (EQCM). A systematic study of this new biointerface using these two different transduction mechanisms provided complementary information that can be used for cross-validation of the data in addition to having enhanced sensitivity, selectivity, and linear range. Together with a panel of conductive polymer based biointerfaces targeting LPS and pili proteins, the described biosensors will be able to measure the subtle differences among Gram-negative microorganisms based on their surface lectins and LPS biomarkers for differentiating Gram-negative bacterial strains and species.

MATERIALS AND METHODS

Chemicals and Materials. 4-(2-Hydroxythyl)-1-peperazineethaneslfonic acid (HEPES) was purchased from VWR International. Concanavalin A (Con A) and Erythrina cristagelli (ECL) were purchased from Sigma. *Staphylococcus aureus* serotype 1 (*S. aureus*) (ATCC 12598), *E. coli* O86: K61 (B7) (ATCC 12701), and *E. coli* W1485 (ATCC 12435) were obtained from ATCC. All other reagents and material were analytical grade and solvents were purified by standard procedures. Biograde and deionized water was used throughout the experiments. The details of the synthesis of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene (**3**) (abbreviated TQ) and α-D-mannopyranoside, 2-[2-(2-mercaptoethoxy)ethoxy]-ethyl (**7**) (abbreviated SM) are described in the Supporting Information. The cultures of *E. coli* strains were grown in sterilized Luria−Bertani broth (LB broth)[23] prepared by adding 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl into 1 L of biograde water adjusted to pH 7.4 at 37 °C for 18 h in a shaking incubator. The viable cell number was determined by conventional agar plate counting. The crude cultured bacteria sample was directly diluted with 10 mM HEPES buffer to the desired concentrations and frozen for further use without any washing steps.[4b] The unit, cells/mL, is used in our work to quantify the number of bacteria in the HEPES buffer rather than colony-forming unit per milliliter.

Biosensor Interface Fabrication and Characterization. The electrochemical polymerization of TQ in CH_3CN with 0.1 M $LiClO_4$ as supporting electrolyte was performed by repeatedly scanning in the potential region from 0 to 1.2 V (vs Ag/AgCl wire) at a scan rate of 20 mV/s for 20 cycles. The yellow-brown film modified gold electrode was washed with CH_3CN and water,[21] and then it was incubated in a solution of 4 mg/mL thiol mannose (SM) in 10 mM HEPES buffer containing a catalytic amount of triethanolamine.[24] The mannosylation of quinone-fused polythiophene was obtained by repeatedly scanning the potential region from −0.2 to 0.8 V at a scan rate of 20 mV/s.[22a] The EQCM measurement was performed with a single QCM electrode rather than the dual QCM electrodes in which a reference nonmodified electrode is simultaneously used in each experiment. The geometric area of the gold QCM electrode is 0.22 cm^2. We determined the gold QCM surface roughness factor to be 2.92 by integrating the anodic peak of the gold oxidation in 0.5 M H_2SO_4 and comparing it with the standard value (390 $\mu C/cm^2$) of the Au oxidation processes in 0.5 M aqueous H_2SO_4 (limit to extent of

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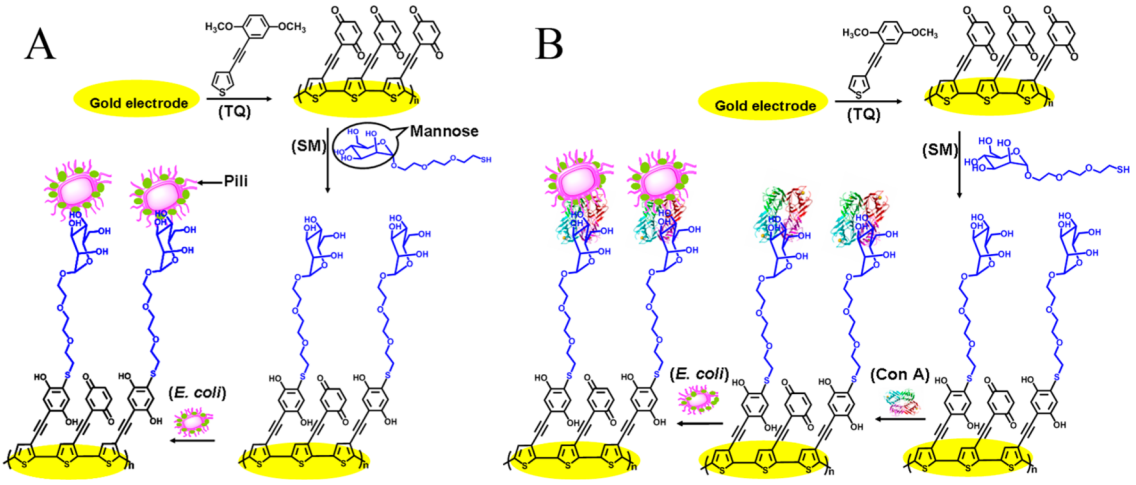
Scheme 1. Schematic Representation of Different Modes of *E. coli* Detection Used in This Work: (A) a Direct *E. coli* Detection Using Pili-Mannose Binding and (B) a Con A-Mediated *E. coli* Detection Using LPS-Mannose Binding



formation of the quasi-two-dimensional oxide state on Au electrodes).[25] The detailed experiments and characterization of the fabrication of the mannosylated quinone fused polythiophene on a gold electrode were described in Supporting Information Figures S1−S4. RQCM (Research Quartz Crystal Microbalance, MAXTEK Inc.) and a *GAMRY* electrochemical workstation were used to characterize the biointerface and its binding events with proteins and bacteria, respectively.

RESULTS AND DISCUSSION

This work demonstrates the fabrication and validation of the mannosylated polythiophene biointerface for detection of Gram-negative bacteria *E. coli* by combined EQCM methods as summarized in Scheme 1. The system thus generated has multiple orthogonalities: (1) the mannosylated interface can directly detect fimbriae proteins or can detect LPS via lectin mediator, (2) the innovative interface with built-in solid state redox probe allows label-free and reagentless transduction of both electrochemical and QCM mechanisms, and (3) the signals generated by these mechanisms are by themselves orthogonal; the electrochemical measurement is a signal OFF approach (i.e., an increase of analyte concentration results in a decrease of the signal) while QCM is a signal ON approach (i.e., an increase of analyte concentration results in an increase of the signal) for this interface. All these functions of the interface positively interact to provide enhanced sensitivity, broader dynamic range of detection, and greater reliability via cross-validation. *E. coli* W1485, a wild type of *E. coli* K-12,[26] was used as the model Gram-negative bacterial analyte in the work, in order to demonstrate the described improvements in the detection system. *E. coli* W1485 is a "semirough" bacterial strain in which the intact LPS core is capped by a single O-antigen subunit consisting of glucose and *N*-acetylglucosamine, which could be recognized by specific Con A.[27]

As detailed in the Supporting Information and Figure S1−S4, we first characterized the proposed glycosurface chemistry for the biointerface fabrication including the electrochemical polymerization of TQ at a Au electrode and the coupling of mannose thiol (SM) with quinone derivatized polythiophene via 1, 4-reductive Michael-type addition to form an SM/TQ modified gold electrode as sensing interface. As shown in Figure S1b, the anodic peak of TQ (+0.48 V) lies between +0.15 V (the characteristic peak of quinone, Figure S2) and 0.75 V (the characteristic peak of polythiophene,[28] which indicates that the redox process of TQ is the result of the conjugated thiophene and quinone moieties in TQ, not thiophene or quinone alone. The estimated surface coverage of TQ (Γ_{TQ}) of 1.54×10^{-10} mol/cm^2 was calculated by integrating the cathodic peak area shown in the curve (Figure S1b) and considering the two-electron process for the Q/H_2Q couple and the gold surface roughness factor 2.92. In comparison with the literature values for a full monolayer coverage of hydroquinone thiols and hydroquinone alkanethiols (5.0×10^{-10} and 5.6×10^{-10} mol/cm^2, respectively),[29] this suggests that our surface is a monolayer. Figure S3 displays CVs as a function of scan rate for a TQ modified gold electrode in 10 mM HEPES (pH 7.4). From Figure S3, it can be calculated that the anodic and cathodic peak currents for the H_2Q/Q redox couple scaled linearly with scan rate from 20 to 100 mV/s, which is consistent with a surface-bound redox moiety. Interestingly, as shown in Figure S4b, $E^{o\prime}$ negatively shifts from +0.37 to +0.35 V along with the ΔE decreasing from 0.18 to 0.12 V after introducing mannose thiol to TQ, which is possibly attributed to the increased electron transfer rate (ET). The detection of *E. coli* via binding of SM/TQ with the type 1 pili protein directly (carbohydrate sensor, Scheme 1A) or upon binding of LPS on the bacterial surface via the Con A bridge at the SM/TQ modified gold electrode (carbohydrate-lectin sensor Scheme 1B) was characterized by electrochemical and QCM readouts. The use of a thiophene biointerface enables the flexibility of control of the polythiophene polymerization process and the rapid construction of an array of carbohydrate functionalized biointerfaces for subsequent assays, with two distinguishable biomarkers (pili proteins and LPS) at the bacterial surface.

EQCM Detection of *E. coli* via Pili-Mannose Binding. Once we have fully characterized the biointerface, the analytical sensing parameters need to be evaluated in order to draw quantitative performance outcomes. Our previous work has described in detail that there are two important elements in the Gram-negative bacterial cell wall for specific bacterial adhesion.[4b] One is carbohydrate binding lectins in many bacteria, usually in the form of fimbriae (or pili). The other one is glycoconjugates called LPS in all Gram-negative bacterial cell walls, which are present in the outer monolayer of the outer membrane along with phospholipids (inner leaflet) and

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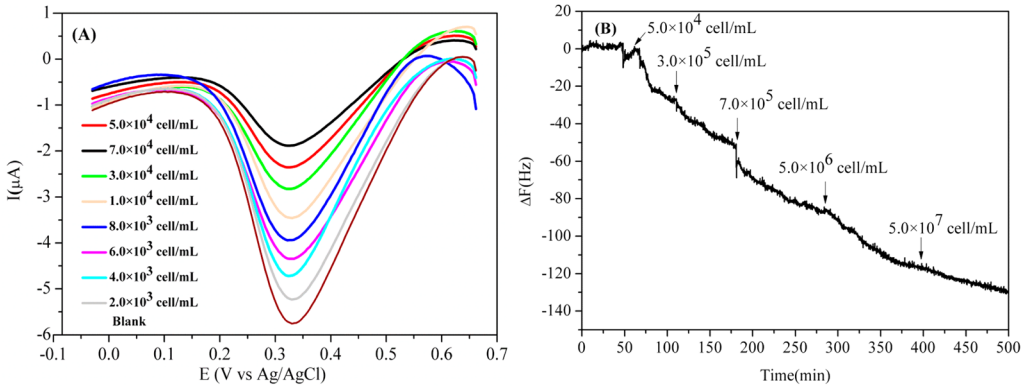
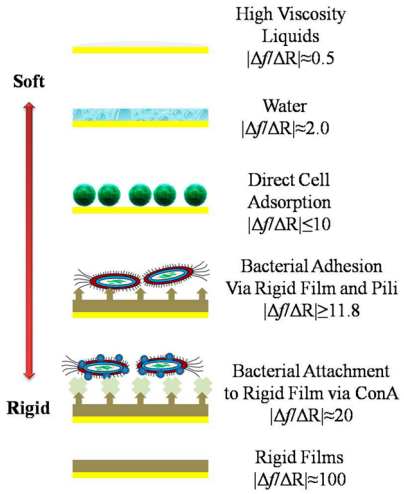


Figure 1. (A) Square wave voltammetry (SWV) responses of an SM/TQ modified gold electrode after incubation with different concentrations of *E. coli* cells/mL, and (B) QCM frequency change vs time curve when SM/TQ was exposed to different concentrations of *E. coli* cells in HEPES buffer (pH 7.4) with 1 mM Mn^{2+} and 1 mM Ca^{2+}.

proteins, which are lectin binding sites. The designed glycosylated polythiophene biointerface can explore both types of these binding activities. In the first case, the binding between *E. coli* and mannose receptor on the SM/TQ surface can occur, which provides simultaneous detection signals for both electrochemical and QCM readouts, as shown in Figure 1. This direct binding of cells leads to the formation of an adsorption layer, which in turn should lead to a change at the electrode interface. The adsorption layer hinders the electron transfer process of quinone/thiophene moieties in the conducting polymer modified electrode, which quantitatively depends upon the binding sites occupied as well as the binding strength of each individual interaction. This is consistent with the partially blocked electrode reported by Amatore[30] and generates changes of electrochemical signals. It has been shown experimentally that square wave voltammetry (SWV) is more sensitive than cyclic voltammetry (data not shown); therefore, SWV was used to characterize the biointerface for determination of *E. coli* (Figure 1A) via electrochemical readout. When an increasing number of *E. coli* cells were introduced into the sensor chamber, a consistent decrease in the peak current was observed, which indicates a signal OFF readout mechanism. By using the electrochemical approach, we were able to detect bacteria *E. coli* in the concentration range of 2.0×10^3 cells/mL to 7.0×10^4 cells/mL with a detection limit calculated according to S/N = 3 to be 8.0×10^2 cells/mL.

At the same time, QCM measurements were performed to evaluate the same binding process. The cells are soft structures; and their attachment to the interface can generate viscoelastic changes at the interface that can confuse the mass sensing results. In most cell based studies, frequency-dissipation analysis or frequency-resistance analysis is typically used to exclude viscoelastic changes. In this work we have opted for the frequency-resistance analysis, to justify our approach. Scheme 2 gives a comparison of such analyses for typical soft and rigid interfaces and their corresponding $|\Delta f_0/\Delta R_1|$ values from the literature. When a highly viscous fluid (e.g., silicone oils, ionic liquids) is present at the QCM interface, the damping resistance of the acoustic waves is too high, thereby decreasing the decay length. Thus, the resulting $|\Delta f_0/\Delta R_1|$ values are less than 1 (e.g., 0.2 for silicone oil).[31] When the interface is changed to typical fluids such as water, the $|\Delta f_0/\Delta R_1|$ value is typically around 2.0, which indicates that the resistance changes are very high in comparison to mass changes. Cho et al. have shown that this value can be increased to even 4.1 by increasing

Scheme 2. Comparison of Typical Soft and Rigid Interfaces with the Ones Described in This Work and Their Corresponding Frequency-Resistance Ratios



the roughness of the surface.[32] This clearly shows that the rigidity of the interface is related to the slip behavior of the contacting fluid or film. When we increase the roughness of the surface, we decrease the slip, thereby increasing the value of $|\Delta f_0/\Delta R_1|$, even for the real fluids. Thus, the fluids can also be made to show a bit of rigidity by roughness manipulations. For the direct cell adsorption on the QCM surface, with the cells being very soft structures, the slip effect is reasonably large, and thus the $|\Delta f_0/\Delta R_1|$ values are still less than 10.[33] Even in that case, it has been shown by us that the external load (cancer cells), if connected to the interface, can be sensed by the QCM while they are lying outside the decay length of the acoustic wave. However, in this work, the cells are not directly attached to the QCM. Rather, we first generated a polymer film which is typically rigid. Especially, when these films are produced by the electrochemical polymerization, the homogeneity of the film makes it even more rigid than those made by casting or spin coating. Polymer surfaces are much rougher than the QCM surface, thereby decreasing the slip and increase the rigidity of the interface. When the bacterial cells are attached to this film via the pili on their surface, the resulting interface is not completely rigid. However, because of the presence of string-like attachments, the $|\Delta f_0/\Delta R_1|$ values are $\geq$11.8 (Figure S5),

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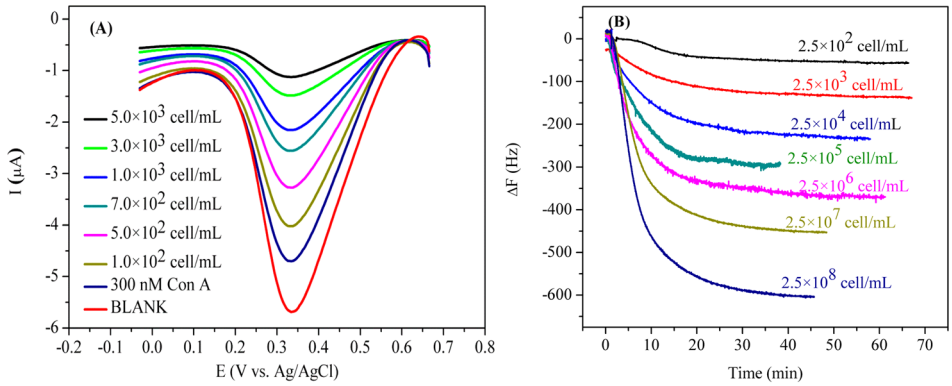

Figure 2. (A) Square wave voltammetry (SWV) responses of an SM/TQ gold electrode after incubation with 300 nM Con A and different concentrations of *E. coli*., and (B) QCM frequency change vs time curve when Con A/SM/TQ electrodes were exposed to different concentrations of *E. coli* in 1 mL of stirred HEPES with 1 mM Mn^{2+}, 1 mM Ca^{2+}.

which is larger than the threshold value reported by Xie et al.[34] At this value, the damping resistance is so small that the viscoelastic effects despite their presence can be ignored. Thus, the cells can be detected by the QCM while lying outside the extinction depth of the acoustic wave. When we move onto bacterial binding to the same polymer film via Con A mediation, the bacterial attachment is in glued-like fashion, making it even more rigid, thereby increasing the $|\Delta f_0/\Delta R_1|$ values to ~20 (Figure S6). Table S1 shows the changes (%) in damping resistances when SM/TQ or Con A/SM/TQ modified gold electrodes were exposed to different concentrations of *E. coli* cells. From the Table S1, it can also be seen that the values of $\Delta R/R_0$ are smaller than 15%; this suggests that the bacterial attachment can be considered as rigid, rather than a viscoelastic behavior. These results also show that the Con A/SM/TQ film is more rigid than the SM/TQ film and the binding between LPS of *E. coli* and Con A is stronger than that of fimbriae of *E. coli* and mannose attached to the gold surface. However, this theoretical threshold $|\Delta f_0/\Delta R_1|$ value (i.e., 11.6 for 10 MHz QCM) cannot be considered as the digital switch; rather the above analysis gives an indication that there is a gradual change in this value while moving from a soft to more and more rigid interfaces. At the threshold level, there is no sudden shift in behavior but only the viscosity effects are quite minimal. We have also shown if the attachment of the cells to the interface is intact, the cells can be detected while lying outside the decay length of the acoustic wave generated by QCM.[35] Thus, in this case, although the length of the pili is comparable to the extinction depth of the acoustic wave, yet the anchoring of the cells at a rigid polymer film by the pili will not significantly affect their detection by the QCM.

Despite all these reasons to believe that the responses shown in this work are predominantly due to mass changes at the interface, the viscosity effects might be present and thus cannot be considered as true Sauerbrey's responses. We have circumvented the possible error in our calculation by avoiding the application of Sauerbrey's equation in calculating the affinity constant and by using the signal vs the concentration of cells for our calibration. For the quantitative analysis, different concentrations of *E. coli* were introduced into the measuring chamber and the frequency shift of the QCM was recorded as shown in Figure 1B. With increasing number of *E. coli* cells, the shift in frequency increases as well, which indicates a signal ON readout mechanism. By using this approach, we were able to detect bacteria *E. coli* in a different concentration range than the electrochemical approach, i.e., 5.0×10^4 cells/mL to 5.0×10^7 cells/mL with a detection limit calculated according to S/N = 3 as 1.7×10^4 cells/mL. This linear range is broader than that in our previous work (i.e., 2.9×10^7 to 2.7×10^8 cells/mL) using mannose SAM as the glycosurface,[4b] thereby indicating that the SM/TQ as the biointerface has higher mannose binding sites than those in mannose SAM.

Theoretically speaking, if QCM is regarded as a mass sensor, the binding of the bacterial cells should generate a much larger response considering the length and weight of one *E. coli* are about 2 μm and 1×10^{-12} g, respectively. The theoretical mass of a close packed monolayer of *E. coli* at a 0.22 cm^2 gold electrode is about 125 μg. However, the observed response is a few orders smaller than this value. The plausible explanation for such a smaller response is that the surface area of the gold electrode is only a rough estimate of the polythiophene biointerface area. Furthermore, the QCM mass sensor senses surface binding phenomena and requires rigid and tight binding. As discussed earlier, the fimbriae-mediated adhesion is relatively weak and flexible. With high mobility of the bacteria, it creates a large freedom of movement of the bacterial cells on the QCM surface. This weak and flexible binding of the fimbriae to the mannose may also result in a displacement of one species with another.[36] Consequently, the surface is only a temporary host to the *E. coli* and the net change of mass is very small. This is why the measured mass is often smaller than expected when QCM was used to detect big targets such as bacteria. Therefore, we also evaluated the second type of recognition events which are based on tight and rigid binding via Con A mediated sandwich assay.

EQCM Detection of *E. coli* via Con A Mediated LPS-Mannose Binding. As discussed above, a binding on the sensor surface which is not rigid is not as sensitive for bacterial detection, especially if the mass sensitive sensing mechanism is utilized. Thus, the key principle in these measurements is to ensure adequate bacterial binding by using recognition molecules with high affinity and multiple binding sites. Distinct LPS structures present on Gram-negative bacteria are particularly advantageous in this regard that can be recognized by lectins as shown in our previous studies. For this purpose, we used lectin Con A as an *E. coli* adhesion promoter to strongly attach *E. coli* to the mannose so a rigid binding layer would be formed on the electrode surface, thereby generating the Con A/SM/TQ interface depicted in Scheme 1B. Figure S7 and Figure S8 depict the similar characterization for fabricating

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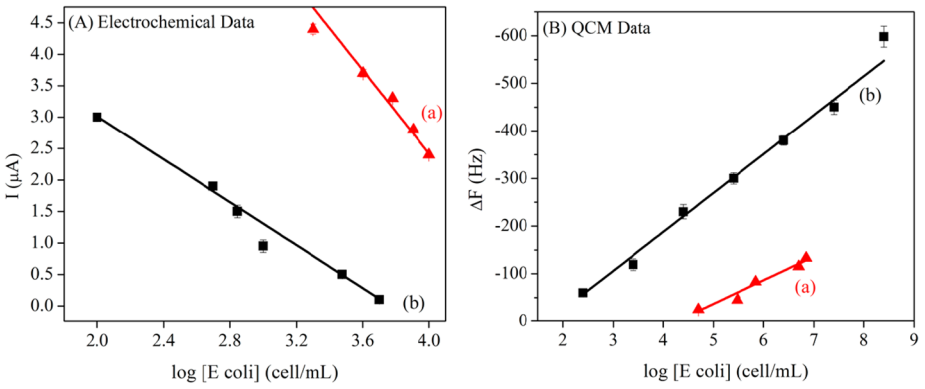

Figure 3. Calibration curves of sensor signals drawn against logarithm of *E. coli* concentrations, where (A) represent SWV electrochemical signals while (B) represent QCM frequency signals. Moreover, (a) depicts the the pili-mannose binding via the SM/TQ interface and (b) depicts Con A mediated binding via the Con A/SM/TQ interface.

the Con A/SM/TQ (the carbohydrate-lectin sensor) interface whereas Figure S9 and S10 in the Supporting Information describe in detail the characterization of Con A binding with the SM/TQ interface to obtain the optimum conditions. When this surface was exposed to different concentrations of *E. coli* cells and the SWV measurements were performed again, the obtained results are shown in Figure 2A. For the Con A/SM/TQ mode too, the peak currents decreased upon increasing of the concentration of *E. coli* in the concentration range of 1.0×10^2 to 5.0×10^3 cells/mL and the calculated detection limit was 25 cells/mL. This result on one hand shows a 32-fold enhancement in the detection limit with a totally different linear detection range, but also supports the fact that a tight binding of the target is beneficial for the electrochemical measurements as well.

In order to perform the QCM measurements using this binding mechanism, the SM/TQ modified QCM sensor was first exposed to the 300 nM Con A solution for 1 h to reach binding equilibrium as a Con A/SM/TQ modified QCM sensor. This step ensures consistency between each experiment, and then *E. coli* samples ranging from 2.5×10^2 to 2.5×10^8 cells/mL were injected into the Con A/SM/TQ modified QCM sensor chambers, which also contained 1 mL of 10 mM HEPES buffer with 1 mM Mn^{2+}, 1 mM Ca^{2+}. Much faster and larger responses were observed for this interface as compared to the SM/TQ only interface as shown in Figure 2B. The detection limit was calculated to be 50 cells/mL, with three decades wider detection range than the early SM/TQ modified QCM sensor. For a quantitative comparison, calibration curves were drawn for all these measurements using the plot of sensor signals against logarithm of bacterial cell concentrations. Figure 3 shows the well fitted linear curves for both the electrochemical (Figure 3A) and QCM data (Figure 3B), whereas (a) and (b) notations represent the SM/TQ and Con A/SM/TQ interfaces, respectively.

From Figure 3, three important conclusions can be drawn and discussed:

(1) From the comparison of (a) and (b) curves both in the electrochemical as well as in QCM data, it is quite clear that the signals are much stronger for the Con A/SM/TQ interface, and thus, the detection limit for this interface is 32-fold lower for electrochemical measurements and more than 300-fold lower for QCM measurements, as detailed in Table 1. This significant increase in sensitivity of the interface is due to rigid binding of the bacteria via LPS on its surface and by using Con A

Table 1. Comparison of the Analytical Performance of Two Orthogonal Sensing Interfaces, One Based on Direct Binding of Bacteria and the Other Based on Con A Mediation

		Mannose/*E. coli* (Scheme 1)	Mannose/Con A/*E. coli* (Scheme 2)
Linear range	SWV	2.0×10^3 to 1.0×10^4 cells/mL	1.0×10^2 to 5.0×10^3 cells/mL
	QCM	5×10^4 to 5×10^7 cells/mL	2.5×10^2 to 2.5×10^8 cells/mL
Sensitivity	SWV	−3.33 (μA/log(cells/mL))	−1.71 (μA/log(cells/mL))
	QCM	−49.52 (Hz/log(cells/mL))	−81.79 (Hz/log(cells/mL))
Detection limit	SWV	8.0×10^2 cells/mL	25 cells/mL
	QCM	1.7×10^4 cells/mL	50 cells/mL

mediation as described earlier. However, the question arises here, in the presence of this tight binding mechanism, why it is important to build and analyze the flexible binding interface i.e., SM/TQ interface. The answer to this question lies in the futuristic development of this interface for multiple type bacterial detection using sensor arrays. One major issue that must be considered in this multiple type bacterial detection is the antigenic or phase variation.[37,38] As a result of this phase variation, type 1 *E. coli* bacteria might shift from a fimbriated phase to a nonfimbriated phase and back spontaneously,[39] which might affect the fimbriated *E. coli* attachment. Various environmental factors, such as centrifuge, can also change the phase of the type 1 *E. coli* bacteria from a fimbriated phase to a nonfimbriated phase. Therefore, in order to accommodate these variations in the sensor, and to detect multiple types of bacteria at the same time, the bacterial pili must also be targeted for binding, which can be done by the direct binding of the pili to the mannosylated interface. The data from these measurements and from the LPS binding processes can then be fed into multidimensional data analysis tools in order to draw conclusions about the nature and type of bacteria.

(2) From the comparison of the electrochemical data (A) and QCM data (B) for both SM/TQ and Con A/SM/TQ interfaces, it is again quite clear that the range of analysis is quite different: rather orthogonal for the two methods. For the SM/TQ interface, the logarithmic range for the SWV data starts from ∼3 and goes until 4, whereas for the same interface the logarithmic range is ∼4.5−7 for the QCM data. This depicts that the overall range is broadened from 3 to 7 by

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utilizing these orthogonal methods, which otherwise was not that simple. The electrochemical method is a signal OFF approach, thus providing at least a signal at logarithmic range of 4, and cannot go further up in concentration unless a new interface is designed. Contrarily, the QCM is a signal ON approach and cannot measure less than 4.5 of logarithmic concentration. In the similar manner, the logarithmic ranges in the case of the ConA/SM/TQ interface (b curves) are 2−3.7 and 2.4−8 for electrochemical and QCM detection, respectively. Thus, it is evidently shown that the proposed strategy can be used to increase the detection dynamic range of the two orthogonal sensing platforms, thereby enhancing the overall sensing performance. Moreover, at many points, the two sensing mechanisms supplement each other as well in terms of detection range. For example the logarithmic range of 2.4−3.7 can be covered by both electrochemical and QCM sensor based on Con A/SM/TQ interface. These points can serve as an internal validation system, thereby eliminating the need for expensive external validations. The reliability of each and every measurement is enhanced too, in this manner. Furthermore, as shown in Supporting Information Figure S7, if $Fe(CN)_6^{3-}$ is used as the electrochemical probe for the SM/TQ modified electrode, the I_{pa} of $Fe(CN)_6^{3-}$ continuously decreased during the process of the sensor fabrication, particularly, when the *E. coli* bound to the SM/TQ modified gold electrode pretreated by the Con A. The result indicates that an SM/TQ modified gold electrode can be used to detected *E. coli* using $Fe(CN)_6^{3-}$ as well. However, the lower concentration (500 cells/mL) of *E. coli* and greatly decreased I_{pa} (from 12 μA Ma to 2.1 μA) indicated that the detection range of *E. coli* possibly is very narrow if using $Fe(CN)_6^{3-}$ as a probe. Therefore, it can be safely concluded that this new interface with built-in electrochemical probe has much better prospects in bacterial detection.

(3) Although, in comparison to the Con A/SM/TQ sensor, the SM/TQ sensor exhibited much lower sensing performances, they are still better than those for previously reported interfaces and protocols being used. Table 2 shows the performance evaluation of this sensor and the comparative analysis of the other techniques reported. The detection limit of this sensor is many fold lower than the value of 1000 cfu/mL of *E. coli* O157:H7 obtained from a peptide based impedimetric biosensor,[40] and is even lower than 136 cells/mL of *E. coli* O157:H7 obtained from a DNA based chemiluminescence biosensor.[41] The high sensitivity obtained in our work may be ascribed to employing Con A as the mediator for the binding between LPS of *E. coli* and mannose and TQ as the signal-producing compound. Tables 1 and 2 list the limits of detection (LOD) obtained using antibody or DNA recognition elements as reported in the literature and the reliable quantitative detection via the two methods we used (carbohydrate or carbohydrate/lectin recognition elements). From Table 2, the detection limit and linear response range of this SM/TQ modified QCM sensor using Con A as mediator are impressive in comparison with the detection limit of 8×10^2 cfu/mL obtained from the bacteriophage-impedimetric/loop-mediated isothermal amplification dual-response biosensors,[3] the detection limit of 1000 cfu/mL obtained from the electrochemical sensor using enzymatic amplification combined with electrochemical−chemical−chemical redox cycling,[42] and the detection limit of the QCM sensor combined with the self-assembled thiol modified mannose monolayers we have previously developed.[4b] The present system is also comparable with other sensors or sensing methods developed.[4c,40,43] Although the detection limit of the SM/TQ modified QCM sensor is higher than that of the above electrochemical sensor, the carbohydrate QCM sensor has large linear range. Therefore, the SM/TQ modified interface can be used extensively for detection of biomacromolecule combined QCM and electrochemical techniques. The high sensitivity and broad response range obtained in our work may be ascribed to the new SM/TQ biointerface designed, which has many advantages, such as the following: (1) Compared with the monomolecular layer monosaccharide reported previously, the multilayer of the conducting polymer with a three-dimensional structure could bring in more molecular recognition elements to improve the sensitivity of the sensor. (2) The cross-linked conducting polymer creates unique multivalent recognition sites that complement the analytes in chemical functionality and in size and shape and prevent the nonspecific adsorption of protein to the substrate and ensure that only specific interactions between soluble proteins or bacteria and immobilized ligands occur. (3) Con A, a multivalent specific binding lectin, is used to increase the binding of *E. coli* on the mannose-coated sensor, and a sensitive response is achieved.

Table 2. Comparison of Techniques for the Detection of *E. coli*

refs	*E. coli* strains	Assay principle	LOD (cells/mL)	Linear ranges (cells/mL)
42	O157:H7	electrochemical ELISAs combined with redox cycling	10^3	10^3−10^8
3	B	loop-mediated isothermal amplification with EIS	800 (EIS)	10^3−10^9 (EIS)
			100 (LAMP)	10^2−10^7 (LAMP)
43	CIP 76.24	EIS and SPR	10^7 (SPR)	
			10 (EIS)	
40	O157:H7	Antimicrobial peptides array	10^4	10^4−10^7
40	O157:H7	chemiluminescence flow-through DNA microarray	136 (stop circle 35)	10^2−10^4 (stop circle 35)
			4000 (stop circle 30)	10^5−10^7 (stop circle 30)
4c	K12	T4-modified microarrays using EIS	100	10^2−10^8
Our work	W1485	SM/TQ as receptor using SWV technique	800	2.0×10^3 to 1.0×10^4
Our work	W1485	Con A/SM/TQ as receptor using SWV technique	25	1.0×10^2 to 5.0×10^3
Our work	W1485	SM/TQ modified as receptor using QCM	1.7×10^4	5.0×10^4 to 5.0×10^7
Our work	W1485	Con A/SM/TQ as receptor using QCM	50	2.5×10^2 to 2.5×10^8
Our work	W1485	Con A/SM/TQ as receptor using $Fe(CN)_6^{3-}$ as probe	500	

Sensor Specificity. Several control experiments were performed to characterize sensor specificity. The TQ modified gold electrode (no mannose attached to the TQ modified gold electrode) was first tested. As shown in Figure S11 and Figure S12, QCM gave little frequency change with or without Con A present when *E. coli* was added. In contrast, when the SM/TQ modified gold electrode was first exposed to 300 nM Con A solution for about 1 h and formed a Con A/SM/TQ modified gold electrode, *E. coli* W1485 was added with a final concentration of 2.5×10^5 cells/mL and generated a large signal response (about 240 Hz) (Figure 4 curve a), which was

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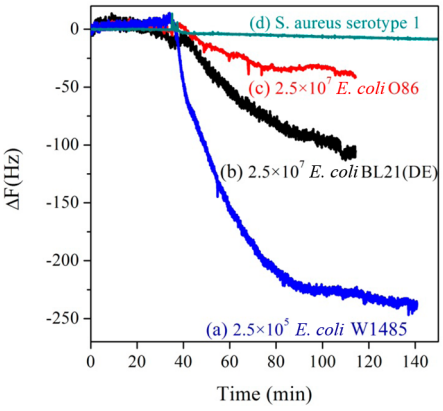

Figure 4. Comparison of sensor specificity. Different electrodes were exposed to (a) *E. coli* W1485, a Con A pretreated mannose electrode with Con A in the binding solution. (b) *E. coli* BL21(DE), (c) *E. coli* O86, and (d) *S. aureus* were added to the Con A pretreated mannose electrode with Con A in the binding solution. All the test chambers contain 1.0 mL of stirred HEPES buffer (pH 7.4) with 1 mM Mn^{2+} and 1 mM Ca^{2+}.

about 2.3 times larger than the adhesion of 2.5×10^7 cells/mL of *E. coli* BL(21)DE onto the SM/TQ modified gold electrode (about 104 Hz, curve b). The possible reason is that *E. coli* BL(21)DE is a descendant of the *E. coli* B strain, which is similar to the K strain.[44] *E. coli* O86 was used additionally to examine the specificity of the above system. With the same experimental condition as for the Figure 4 curve a, a very small signal (39 Hz) was observed for the addition of 2.5×10^7 cells/mL of *E. coli* O86 (curve c) because the O-antigen of *E. coli* O86 contains no Con A specific binding glucose, *N*-acetylglucosamine, or mannose.[45] *S. aureus* , a Gram-positive bacterium, was further used as a negative control. When *S. aureus* was added to the Con A pretreated SM/TQ modified gold electrode, only a negligible frequency change was detected which resulted from no LPS in the bacterial wall of *S. aureus* (curve d). Our control experiments in Figure 4 indicated that the extracellular matrix did not interfere with the *E. coli* detection. They also verified that Con A in the binding solution aggregated onto the *E. coli* W1485 wall and thus enhanced the sensitivity and specificity for mannose binding with *E. coli* W1485 by promoting the formation of rigid attachment to the mannose modified QCM surface.

CONCLUSIONS

In this work, we showed an integrated approach to address the concerns of carbohydrate based biosensors for bacterial detection with the following new strategies: (1) using polythiophene fused quinone moieties for carbohydrate biointerface fabrication for bacterial detection via pili; (2) enhancing bacterial recognition by combining it with an additional binding event, i.e. lectin-LPS binding, thereby enhancing the sensitivity and limit of detection; (3) using two orthogonal label-free transducers for widening the dynamic range of detection and to introduce the internal validation. The sensor fabricated in this way showed the lower detection limits of 25 cells/mL (for SWV) and 50 cells/mL (for QCM), as well as better selectivity and stability as compared to the presently available technologies. By taking advantage of such a polyvalent binding situation in carbohydrate based polymers and the built-in electrochemical transduction of conductive polymers, we were able to substantially minimize possible cross-reactivity that significantly enhanced the specificity and sensitivity of detection. The glycosurface chemistry, with the flexibility of the electrochemical synthesis of polythiophene containing fused quinone moieties alongside the inherent quick, clean, high fidelity characters of coupling chemistry presented here, can be used to rapidly generate an array of sugar biointerfaces for subsequent assay with proteins. Thus, the reported interface can be advantageous for multiple directions of sensor development. Combining such a biointerface with an appropriate electrochemical and QCM transducer yields sensor devices being highly suitable for the fast, low cost, and straightforward online detection of bacterial analytes and harmful pathogens related to agriculture, the food industry, disease control, and biodefense.

ASSOCIATED CONTENT

Supporting Information

Additional information including extensive Figures S1−S12 as noted in text. The details of the synthesis of 3-((2,5-dimethoxyphenyl)ethynyl)thiophene (**3**) (abbreviated TQ). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: zeng@oakland.edu.

Notes

The authors declare no competing financial interest.

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