



## Tethered DNA scaffolds on optical sensor platforms for detection of *hipO* gene from *Campylobacter jejuni*

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### ABSTRACT

DNA biosensors have gained increased attention over traditional diagnostic methods due to their fast and responsive operation and cost-effective design. The specificity of DNA biosensors relies on single-stranded oligonucleotide probes immobilized to a transduction platform. Here, we report the development of biosensors to detect the hippuricase gene (*hipO*) from *Campylobacter jejuni* using direct covalent coupling of thiol- and biotin-labeled single-stranded DNA (ssDNA) on both surface plasmon resonance (SPR) and diffraction optics technology (DOT, dotLab) transduction platforms. This is the first known report of the dotLab to detect targeted DNA. Application of 6-mercapto-1-hexanol as a spacer thiol for SPR gold surface created a self-assembled monolayer that removed unbound ssDNA and minimized non-specific detection. The detection limit of SPR sensors was shown to be 2.5 nM DNA while dotLab sensors demonstrated a slightly decreased detection limit of 5.0 nM (0.005 μM). It was possible to reuse the SPR sensor due to the negligible changes in sensor sensitivity ( $\sim 9.7 \times 10^{-7} \Delta RU$ ) and minimal damage to immobilized probes following use, whereas dotLab sensors could not be reused. Results indicated feasibility of optical biosensors for rapid and sensitive detection of the *hipO* gene of *Campylobacter jejuni* using specific ssDNA as a probe.

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### 1. Introduction

*Campylobacter jejuni* is a significant cause of human bacterial gastroenteritis [1–3] and can be acquired from contact with farm animals or ingestion of contaminated poultry products, raw milk or water. This pathogen has been implicated in 80 outbreaks in the US alone [4], and the Centers for Disease Control and Prevention estimate that campylobacteriosis affects over 2.4 million people annually in the US. In 2008, 5825 cases of campylobacteriosis were reported through FoodNet, with 12.68 cases per 100,000 people [5]. Campylobacteriosis has also been associated with the development of illnesses such as Guillian-Barre syndrome, reactive arthritis, Reiter syndrome, diarrheal infections, intestinal malignancy and dehydration [6,7].

Traditional laboratory procedures are used for *Campylobacter* detection, which include plate culturing or extracting the bacteria from samples followed by biochemical tests and genotype based diagnostic assays [8,9]. Although these methods are sensitive, they can take up to 96 h for the analysis to be completed. Therefore, development of sensors capable of offering a rapid and inexpensive alternative, coupled with real-time monitoring and specific detection of low numbers of *C. jejuni* in food and clinical samples, is needed to ensure public safety.

Numerous biosensor platforms have been developed for the detection of food-borne pathogens [10]. Nucleic acid-based sensing platforms have been widely described, including piezoelectric sensors that measure frequency changes resulting from the hybridization of immobilized probes with a corresponding target [11,12], chemi-luminescence based systems where fluorescently labeled probes are immobilized onto transducer surfaces [13], aptamer-based sensing using DNazymes to design functional chimera structure-like probes [14]. However, these methods are rigorous, time-consuming and possess low sensitivity in comparison to other detection platforms [15,16].

Recently, there have been reports of detection of pathogenic food-borne bacteria by targeting genes that encode for virulence factors involved in pathogenesis [17,18]. Detection using

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polymerase chain reaction (PCR) is very selective but may lack the required sensitivity for the rapid screening of samples [19]. Electrochemical DNA microarrays are advantageous over PCR-based assays for their high sensitivity, portability and also better performance without labeling the probes. They can also be used for multiple target preparation. These methods are quite sensitive but produce high background signals that are difficult to separate, and the sensing platform is destroyed after every measurement. This is problematic for the reusability, reliability and robustness of the surface structures that are developed over the electrodes [20]. An alternative to these methods is DNA-based biosensors, which can be selective, specific and robust. For example, self-assembled monolayers of ssDNA probes specific to targeted ssDNA can be immobilized on sensor surfaces, allowing indirect detection of a bacterium of interest [21].

Hybridization-based sensing platforms have been developed using DNA probes labeled with fluorophores [22]. Although these methods are specific and sensitive, they suffer from photobleaching, have limited differentiation between hybridized and non-hybridized sequences in the mixture [20]. Signals that arise from non-specific adsorption and diffusion across the colloid create background that interferes with the analysis. DNA sequences can also be labeled with nanoparticles to enhance signal production, but DNA may create dense layers around nanoparticles due to thiol linkage, which limits the effectiveness of detection [23]. In addition, the low surface roughness of the nanoparticles limits surface coverage and therefore covalent bond formation on gold, as thiol–gold linkages or avidin–biotin chemistries, is used to develop anti-fouling biosensor surfaces [11,24].

The basis for the difference between *C. jejuni* and any other bacteria is the expression of the *N*-benzoylglucineamidohydrolase (*hippuricase*) gene. The hallmark of the hippuricase activity is the conversion of hippuric acid into benzoic acid and glycine. This gene is the key feature in discriminating *C. jejuni* from other similar *Campylobacter* species, such as *C. coli*. Diffraction optics technology (DOT) sensors are widely used for label-free detection and real-time monitoring of bio-molecular interactions. This technique has been widely employed for the detection of proteins and small molecules [25], and live cells [26,27], and a variety of biotin labeled ligands including proteins, antibodies and DNA can be immobilized on the DOT sensor chip.

Our work describes the development of hybridization-based diffraction optics technology (DOT) and surface plasmon resonance (SPREETA) sensing platforms. False positive responses were eliminated in the DOT and only the diffraction phenomena across the grating was recorded, which are advantageous over other label-free optical biosensor technologies. A range of surface chemistries and the extensive possibilities in label-free and amplified modes, coupled with low cost disposable sensor chips, makes DOT an attractive optical sensing device. The application of this coupling strategy to two devices was conducted with the aim of exploiting its applicability in different instrumentations using the same sensor recognition strategies.

## 2. Experimental

### 2.1. Reagents

Potassium phosphate monobasic, phosphate buffered saline (pH 7.4) containing 0.138 M NaCl and 0.0027 M KCl (PBS) and 6-mercapto-1-hexanol were obtained from Sigma–Aldrich (St. Louis, MO). Tween-20, EDTA, sodium phosphate dibasic and sodium chloride were used as received from Fisher–Scientific (Pittsburgh, PA). Reductactryl™ (Cleland's reagent) was obtained from Calbiochem (San Diego, CA).

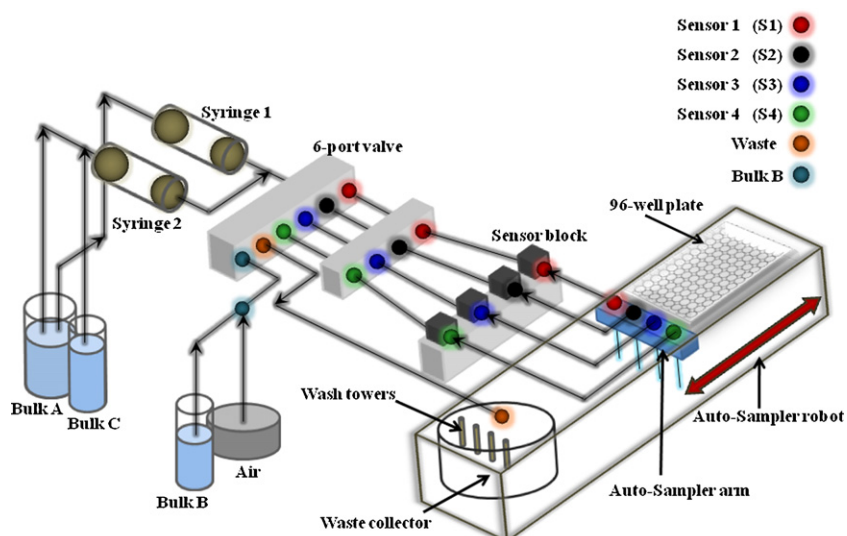
### 2.2. Sensor platforms

DOT sensor chips (Axela Inc., Toronto, Canada) were used for diffraction experiments (Fig. 1). In the DOT system, a coherent light strikes the regular pattern of capture molecules on the sensor surface creating interference patterns that produce a well-defined image. When target molecules interact with the receptor surface, the height of the pattern increases, which in turn increases the diffraction intensity. This change in intensity is monitored by a photo diode. The DOT system is based on a near real-time and total internal reflection scheme that allows for 95% of the signal to be detected. A 670 nm laser diode, class 3R with a maximum output of 4.0 mW and a beam divergence of 5.0 mRad was used as the light source, to produce a diffractive pattern. One end of the sensor was connected to a fluidic panel for circulating the buffer solutions, while the other end was connected to a sensor manifold which aspirates the analyte from the well. The sensor chip was made of optically clear polystyrene with an interspersed prism situated below the flow channel. The linear flow channel, which can hold a volume of 10.0  $\mu$ L, consisted of eight circular assay spots, each 2.0 mm in diameter, with 15.0 femto moles of avidin deposited on each spot [28]. Each sensor had a low cost (US\$ 15 per chip) and up to eight assay features along each linear flow channel. Every spot had a diameter of 2 mm and a depth of 0.25 mm. Neutravidin was immobilized on the diffraction pattern for biotin labeled bioprobes to be attached as receptors for the sensor development. The DOT system introduces the analyte sample through the channel using a high precision fluidic controller, and different reagents can be passed over the surface by introducing an air gap between each reagent, which leads to a transient spike in the signal. The dotLab software, version 1.1.1 (Axela Inc.), monitors the change in diffraction intensity near the sensing surface, calculates the statistical noise in the signal and displays a self-referenced signal relative to biomolecular interactions in all the eight spots. The signal is generally displayed in diffraction intensity unit (DI).

The SPREETA (Texas Instruments, Dallas, TX) is a robust, two-channel SPR system comprised of a three-channel SPR sensor, flow cell and 12-bit three-channel DSP electronic control box (ECB). The sensing region is coated with a semi-transparent gold film (~50 nm) with a Cr-adhesion layer (1–2 nm). A two-channel polypropylene flow cell coupled with a silicone gasket and a peristaltic pump (Ismatec, Cole-Parmer Instrument Co., Chicago, IL) with a flow rate of 25.0  $\mu$ L/min at 25 °C was used to establish a flow system. Multi-channel SPREETA software, version 10.83 (Texas Instruments), monitored the changes in RI near the sensing surface, calculated the statistical noise in the signal and displayed the results. The signal is generally displayed in response unit (RU) (1 response unit =  $10^{-6}$  refractive index unit) [29].

### 2.3. DNA probes

Primers and probes were designed from the hippuricase gene (*hipO*) of *C. jejuni* (accession number Z36940.1). The forward (5'-GAC TTC GTG CAG ATA TGG ATG CTT-3') and reverse (5'-CGA TAT TGA TAG GCT TCT TCG GTA GTA-3') primers amplified a 344 bp segment. The short (SP, 5'-GGT GGT GCT AAG GCA ATG ATA G-3') and long (LP, 5'-GGT GGT GCT AAG GCA ATG ATA GAA GAT GGA TTG TTT GAA AAA TTT GAT AG-3') probes attached to the 344 bp segment. Primers and probes – both thiolated and biotinylated – were purchased from Integrated DNA Technologies (IDT, Coralville, IA). Reverse and complementary ssDNA (purchased from IDT) corresponding to the amplified segment was diluted in TE Buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). The disulfide bonds of thiolated DNA probes were cleaved by resuspending in TE buffer along with Reductactryl™ 1:50, allowing formation of self-assembled monolayers (SAM) with higher surface density and better reproducibility



**Fig. 1.** Detailed schematic of the flow path and tubing connections in a dotLab system. The system is plumbed using a 0.032-in. Teflon tubing. Arrow marks indicate the direction of flow of the buffer and analyte. The analytes are poured in to the respective wells in an order in which they have to be passed over the surface.

[30]. This mixture was then agitated at room temperature (RT) for 15 min followed by syringe filtration (0.2  $\mu\text{m}$ ).

Experiments were also conducted by attaching the long probe (LP) and a second 22-mer ssDNA (5'-CTA TCA TTG CCT TAG CAC CAC C-3'). Equimolar mixture of the probe and target were mixed together and heated to 95 °C followed by slow cooling of the mixture [31]. A DNA probe with a 22-mer double strand and 28-mer single strand was formed (Fig. 2). Disulfide bonds in the long probe were cleaved with Reductacryl™.

#### 2.4. Immobilization of biotinylated DNA probes on dotLab

The analyte was passed at a standard flow rate of 500.0  $\mu\text{L}/\text{min}$ , which was maintained throughout the experiment in the mixer mode. The flow of reagents was controlled by the method that was programmed before the start of the analysis and 500  $\mu\text{L}$  of a mixture of 1 $\times$  PBS and 0.0025% Tween (PBST) was primed over the neutravidin modified polystyrene surface. The non-specific area across the flow channel was blocked with BSA (5.0 mg/mL) for 3 min. Subsequently, biotinylated DNA probes (1.0  $\mu\text{M}$ ) were injected for 45 min followed by washing with buffer. Changes in diffraction intensity were monitored using dotLab™ software. After a sequence of washing steps, the target was injected sequentially in graded

concentrations through a precise fluidic system and pre-defined incubation time.

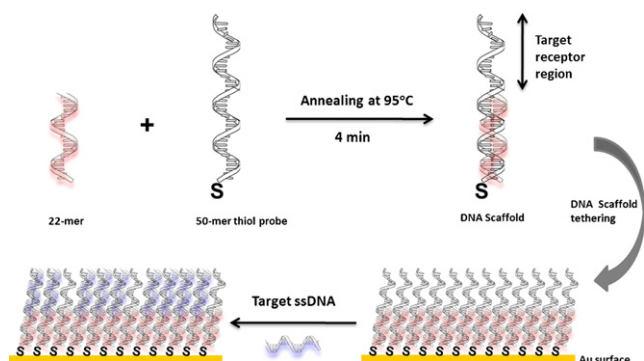
#### 2.5. Immobilization of thiolated DNA probe on SPREETA

Gold surface of SPREETA™ sensors were carefully cleaned with piranha solution followed by argon plasma cleaning. Thiolated probes (1.0  $\mu\text{M}$ ) suspended in the immobilization buffer were passed over the gold surface for 3 h. Short immobilization time ensured that no degradation of DNA occurred during assembly on the surface [32]. Apparently, a low pH of 4.5 is required to form this self-assembled monolayer [33]. After DNA immobilization, the surface was thoroughly washed and 1 mM 6-mercapto-1-hexanol (MCH) was injected. Long DNA probes (1.0  $\mu\text{M}$ ) were resuspended in immobilization buffer and injected over freshly cleaned surface for 3 h. It is not necessary to incorporate 6-mercapto-1-hexanol in the DNA-scaffold monolayer because DNA scaffolds form a tightly packed monolayer that prevents non-specific binding [34]. The surface was washed after immobilization in order to remove loosely bound probes.

#### 2.6. Detection of target DNA

##### 2.6.1. dotLab

To determine the limit of detection, target DNA was serially diluted from 0.005  $\mu\text{M}$  to 1.28  $\mu\text{M}$  and introduced sequentially, starting with the lowest concentration. Complementary target DNA had the sequence 5'-CTA TCA TTG CCT TAG CAC CAC C-3', and the non-complementary DNA was 5'-CCG TCG TAG ACA TAC TAC ATC G-3'. The difference in signal between the PBST baseline and respective intensity was determined for each concentration. The auto sampler, controlled by the two syringe pumps, aspirated the analyte from the tray. Before the molecules bound to the surface, a weak intensity image was produced upon illumination. However, when the probe molecules were immobilized onto the surface, an increase in height of the diffraction pattern was produced, which in turn corresponded to the increase in intensity. The binding phenomenon was monitored using laser light in a total internal reflection mode to acquire 95% of the signal.



**Fig. 2.** Illustration of the formation of DNA scaffolds using a 50-mer long probe and 22-mer short probe by annealing the complimentary regions in both the strands. Annealing at 95 °C binds the short and the long strand and forms a DNA scaffold. This scaffold is then used to form a self-assembled monolayer tethered on the gold surface of the SPR based biosensor in order to detect the complimentary single stranded DNA.

### 2.6.2. SPREETA

Hybridization experiments were conducted in hybridization buffer (150.0 mM NaCl, 20.0 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.1 mM EDTA, 0.005% Tween 20, pH 7.4). Target DNA was serially injected and the surface was washed after every injection to remove loosely bound DNA. Similarly, a non-complimentary nucleotide strand was used to verify specificity and there was no detectable signal from the control channel. Net response was calculated by deducting the control signal from sensing signal.

### 2.7. Electrochemical impedance spectroscopy (EIS)

AC electrochemical impedance spectroscopy was carried out using an FRA2 – μAUTOLAB – TYPE III electrochemical impedance analyzer (Metrohm USA Inc., Westbury, NY). A one-compartment 2.0 mL glass cell with a working volume, Ag/AgCl reference electrode and a platinum wire auxiliary electrode were used for the measurements. The sensing surface was characterized over a wide range of frequency (10 Hz to 100 MHz;  $E^0 = 180$  mV versus Ag/AgCl) in the presence of 5 mM K<sub>3</sub>Fe(CN)<sub>6</sub> + K<sub>4</sub>Fe(CN)<sub>6</sub> in 0.1 M KCl. A linear region for the bare gold electrode implies that the reaction across the electrode is diffusion controlled at low frequencies [35].

## 3. Results and discussion

### 3.1. Probe immobilization

#### 3.1.1. Biotinylated ssDNA probes (dotLab)

The response curve for probe binding at different concentrations is shown in Fig. 3A. A net response (Fig. 3B) was determined by deducting the control signal from the sensing signal. Observed intensity increased with concentration until signal saturation occurred at 10 μM concentration. The optimum probe concentration was selected from the linear working range and was found to be 1 μM. This confirmed the high specificity of the system using biotinylated ssDNA probes. Using the same concentration (1 μM) for 20 cycles (7 days) of hybridization/regeneration without desorption of probe molecules, the average value of refractive index response was  $4.7 \times 10^{-2} \pm 1.16 \times 10^{-2}$  DI units ( $R^2 = 0.981$ ) (data not shown). This is comparable with other diffraction-based assays or surface plasmon resonance platforms [36,37].

#### 3.1.2. Thiolated ssDNA probes (SPREETA)

Using previously established methods [29,38], the surface coverage of DNA probes was predicted to be  $3.64 \times 10^{12} \pm 0.15$  molecules/cm<sup>2</sup>, with a surface coverage density of  $0.44 \pm 0.12$  ng/mm<sup>2</sup> [33].

$$d_a = \left(\frac{l_d}{2}\right) \times \left(\frac{n_{eff} - n_b}{n_a - n_b}\right) \quad (1)$$

As a result, the following parameters were used to calculate the surface coverage from Eq. (1): thickness of the adlayer ( $d_a$ ), SPR decay length ( $l_d = 307$  nm), effective refractive index of the adlayer from the SPR signal ( $n_{eff}$ ), refractive index of water ( $n_b = 1.333$ ) and the refractive index of proteins or nucleic acids ( $n_a = 1.57$ ). Change in RU after washing the surface was found to be  $1380 \pm 15$ . It is speculated that 22 bp ssDNA probes form a densely packed semi-crystalline monolayer, due to Van der Waals forces between the ssDNA [39,40].

#### 3.1.3. Electrochemical impedance spectroscopy

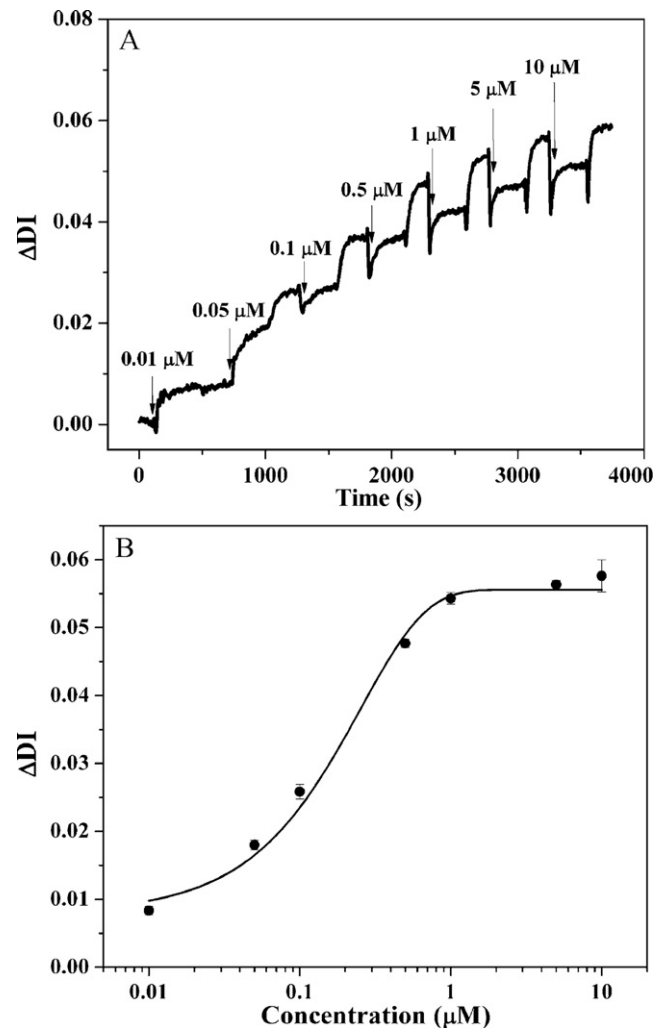
EIS was performed for each step of DNA immobilization and hybridization to better understand the immobilization of thiolated probes on the surface and to characterize the electrical properties

of the interfacial interaction between DNA probes before and after incubation with target DNA (Fig. 4A). Since the electrical resistance and capacitance are sensitive indicators for monitoring the surface properties, this technique has been used to analyze binding phenomena. The charge transfer resistance ( $R_{ct}$ ), after immobilization of DNA on gold electrode was 8.0 kΩ. Incubation of MCH resulted in a decrease in  $R_{ct}$  (4 kΩ), indicating that the inclusion of MCH spacer thiol allows redox molecules to freely reach the electrode, thereby reducing tunneling distance where no redox process occurs. Fig. 4B shows the EIS of the DNA hybridization using complimentary target. Clearly,  $R_{ct}$  increases with target concentration. This is attributed to the increase in repulsive electrostatic interactions along the backbone of the SH-ssDNA which later doubles with double-stranded DNA [41,42]. Additionally, the limit of detection using EIS was verified to be 5 nM (Fig. 4C) from a curve which is a sigmoidal fit with a regression coefficient of 0.94.

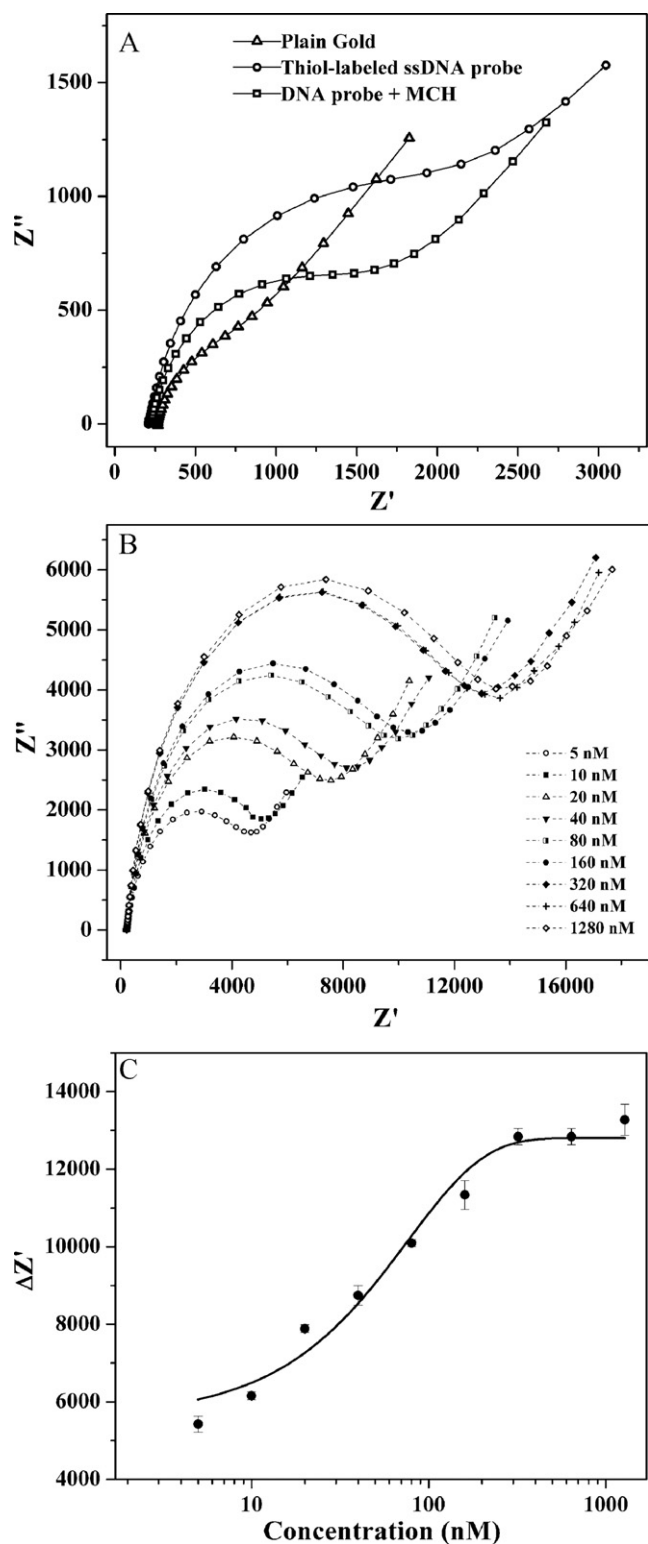
### 3.2. DNA detection

#### 3.2.1. dotLab

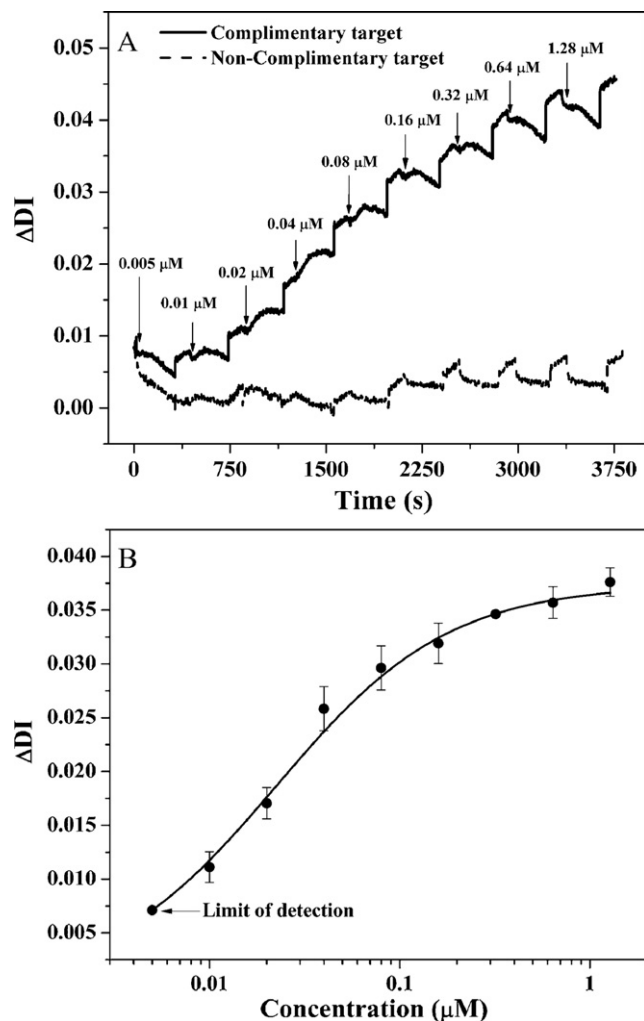
Here, we show the application of dotLab as a biosensor by using ssDNA specific to the *hippuricase* gene of *C. jejuni*. An interaction



**Fig. 3.** (A) Sensor response to probe immobilization plotted for change in diffraction intensity ( $\Delta$ DI) versus time. There was a continuous increase in net change, which was calculated by subtracting the subsequent PBST baseline. (B) Dose-response curve for probe binding. The net response ( $\Delta$ DI) was plotted against concentration by subtracting the response signal from the base line.

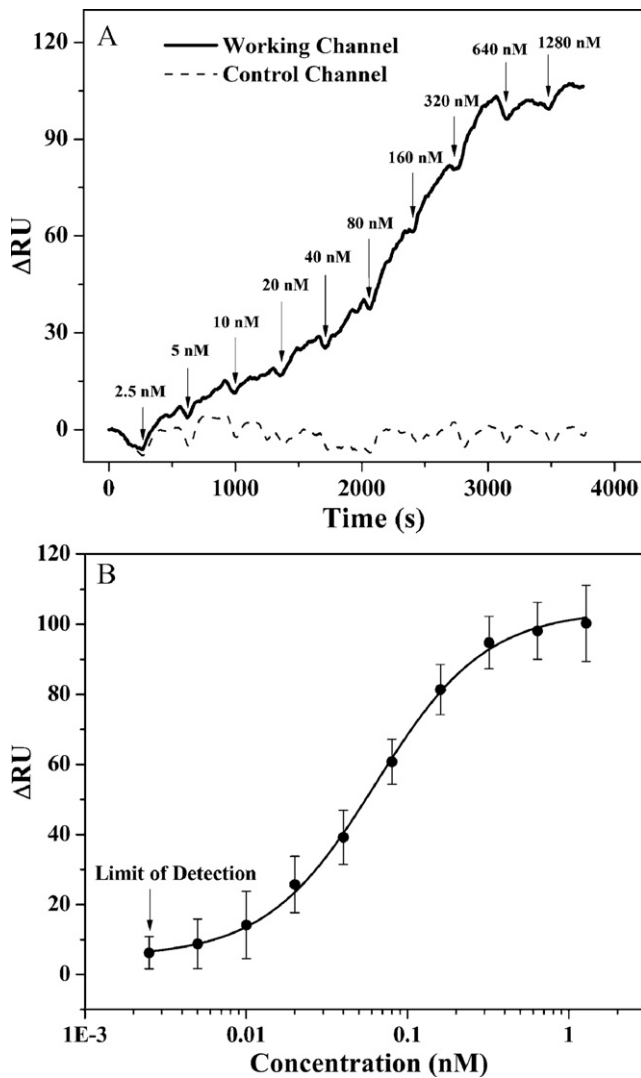


**Fig. 4.** Nyquist plots for the electrode in a  $5.0 \times 10^{-3} \text{ mol L}^{-1}$   $[\text{Fe}(\text{CN})_6]^{3-/4-} + 0.1 \text{ mol L}^{-1}$  KCl aqueous solution. Frequency intervals: 10 mHz to 100 kHz and measurements carried out at 0.32 V vs. Ag/AgCl. The faradaic response is monitored for every step included in the SPREETA platform, including immobilization of thiolated probes (A), detection of target ssDNA (B). (C) Dose–response curve for impedance spectrum obtained during target binding.



**Fig. 5.** (A) Representative response curves for the detection of the *hippuricase* gene. The target was serially diluted and introduced over the sensing surface, starting with the lowest concentration. The net response for each concentration was calculated by subtracting the control channel (non-specific signal) response from sensing channel (specific + non-specific signal). (B) Dose–response curve for *hippuricase* gene target binding. The net response curves were plotted by subtracting the response signal from the base line.

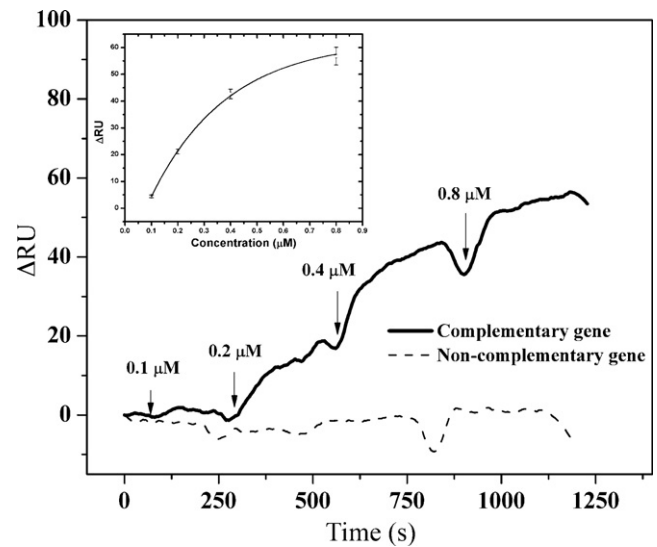
between immobilized probe and target causes a shift in intensity and a gradual increase in signal (Fig. 5A). DNA hybridization occurs through concentrations 0.005  $\mu\text{M}$  and 1.28  $\mu\text{M}$ . When injecting the *hippuricase* target gene at different concentrations, a shift resulting from DNA hybridization was detected. The net response for each concentration was calculated by subtracting the non-detectable response ( $\Delta\text{DI} \sim 2.94 \times 10^{-3}$ ) from specific signal. The limit of detection, determined as the lowest concentration of target DNA that produced a distinguishable net response (signal/noise > 3) was 5.0 nM (Fig. 5B) and demonstrated the high specificity of the dotLab. Spurious signals due to non-specific binding were eliminated, as the diffraction efficiency depends on the difference in height between the patterned and non-patterned areas [43]. Since diffraction phenomena on the dotLab are inherently self-referencing, nonspecific binding to both the patterned and unpatterned regions will not affect the signal. Such characteristic offers an important advantage over other optical biosensor systems in which any surface binding event will cause non-specific binding. Besides, the dotLab biosensor is a cost-effective platform that has the format flexibility to provide a quick and simple solution to both assay development and routine analysis [44,45].



**Fig. 6.** (A) Representative response curves for the detection of the *hippuricase* gene. Graded concentrations of ssDNA were introduced to both, sensing channel and control channel (both blocked with spacer thiol). The net response for each concentration was calculated by subtracting the control channel (non-specific signal) response from sensing channel (specific + non-specific signal). (B) Dose-response curve for *hippuricase* gene target binding. The net response curves were plotted by subtracting the response signal from the base line.

### 3.2.2. SPREETA

In order to evaluate the performance of the device, a calibration curve has been obtained with different concentrations of target ssDNA injected serially from 2.5 nM to 1280 nM (Fig. 6A). The limit of detection was estimated to be 2.5 nM, determined as the lowest concentration of target DNA that produced a distinguishable net response (Fig. 6B). The introduction of target resulted in an initial drop in RU, which is attributed to the bulk refractive index change as the amount of drop is similar in both channels. Following the initial drop, the RU signal in the sensing channel increased with DNA binding, while the reference channel remained constant. For instance, when the target solution was injected, the change in RU at a concentration of 320.0 nM was found to be  $101.45 \pm 10$  ( $n = 10$ ), with a non-detectable signal of  $\Delta DI < 2$  from the non-complimentary target ssDNA. The same surface could be regenerated using 2.5 mM HCl and reused more than 20 times over one week with only a small change in the coefficient of variation (11%). Regeneration efficiency was found to be between 89% and 95% across the concentration range from 2.5 nM to 1280 nM. In the case of longer



**Fig. 7.** A representative response curve for long DNA probes immobilized on gold surface. The concentration of probes used was 1 μM. Inset shows calibration curve for the binding event.

probes, the target DNA was serially passed over the surface from the lowest to highest concentrations (0.1 μM to 0.8 μM) with a complimentary nucleotide on the reference channel. Fig. 7 shows the representative response curve for DNA target binding to the DNA scaffold monolayer. It can be seen that as the concentration of target DNA increased, there was an increase in refractive index in the working channel, and no change in the control channel. Inset shows the dose-response curve for the concentrations that were injected.

Clinical laboratories use methodology based on direct plating or sample enrichment to isolate *Campylobacter* spp. The sensitivity of microbiology methods to detect *Campylobacter* in clinical and food samples is in the order of 10 CFU (colony forming unit microbiology) per gram of fecal material to 1 CFU per 25 g of food. The sensors described here measure nanomolar concentrations of *Campylobacter jejuni* DNA, and therefore a simple comparison cannot be done. It is important to remember that biological samples (feces, food, etc.) have different compositions and there is no universal method for DNA extraction. In addition, the harvest of target DNA from biological samples varies greatly among DNA extraction methods, with the ones yielding more purified DNA being the most expensive (labor and time).

The standard methodology for the testing of foodborne pathogens by the Food and Drug Administration and the Food Safety and Inspection Services of the U.S. Department of Agriculture required a detection limit of 1 CFU per 25 g of food. Currently, this detection limit in retail broiler meat, for instance, is only achieved by enrichment of the samples for at least 48 h [46]. A new performance standard has been implemented to test for the presence of *Campylobacter* spp. in processed broiler and turkey carcasses (<http://www.fsis.usda.gov>). This standard is part of the verification system that the Food Safety and Inspection Services of the United States Department of Agriculture (USDA) have established when the Hazard Analysis and Critical Control Point system was introduced in 1997. Under this new regulation, the USDA will test processing plants for *Campylobacter* spp., and therefore the allied industry, which provides detection kits for rapid identification of bacterial foodborne pathogens, is searching for new methods based on rapid detection of bacterial pathogens, such as *Campylobacter*, using DNA.

Biological samples, such as food matrices, that contain a wide array of microbial communities, yield DNA templates that make

PCR reactions more challenging. The theoretical concepts of PCR are limited in samples that are contaminated with DNA from many different by similar bacterial species. The uniqueness of the DNA segment targeted by PCR is paramount for the specificity (detection) but also for the sensitivity (to avoid false negatives). Another important issue is maintaining the integrity of the target DNA during the DNA extraction step, which is very important to avoid false negative samples. Unfortunately, DNA extraction is a current limitation for all DNA methods, whether they are based on amplification of isothermal hybridization. The yield of DNA from current extraction methods varies widely, and the extraction kits that contain an elution column are the ones that result in the highest yield of good quality DNA. However, the DNA from any commercial kit will not be in the form of intact genomic DNA but as segments of different sizes, and the extraction methods with the best yield will increase the cost of any DNA method which is quite significant for large throughput applications.

In these studies, as observed from the range of concentration and analytical curves, the sensitivity of the SPREETA and dotLab in the detection of the *hipO* gene of *C. jejuni* is higher than that of the BIAcore or amperometric detection platforms or any antibody based detection [47]. The calculated lower limit of detection was 2.5 nM for SPR and 5.0 nM (0.005  $\mu$ M) for dotLab. Further experiments are underway to improve sensitivity and reusability of these optical sensing platforms using ssDNA from asymmetric PCR and biological samples.

#### 4. Conclusion

We have reported a comparative study of immobilization techniques based on direct coupling of thiolated probes and covalent binding of biotinylated DNA to a patterned avidinated surface. Detection of DNA segments from the *hippuricase* gene of *C. jejuni* using diffraction optics technology (dotLab) and surface plasmon resonance was achieved. Development of self-assembled monolayers was used as the primary sensing mechanism, using by covalent immobilization of thiolated and biotinylated probes. Probes were not labeled and were immobilized using simple physical adsorption on to the surface of polystyrene or a gold sensor thereby avoiding complex and expensive surface chemistries for fast, responsive DNA sensors.

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