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Rapid Detection of Pathogenic Bacteria and Screening of Phage-Derived Peptides Using Microcantilevers

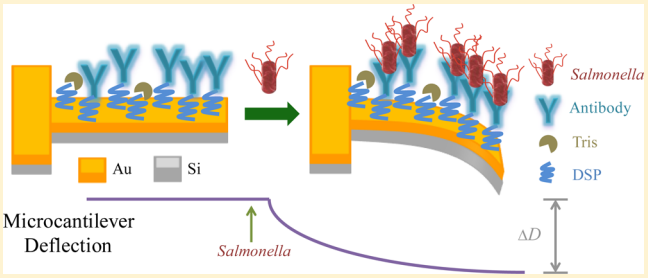
Jinghui Wang,[†] M. Josephine Morton,[‡] Christopher T. Elliott,[‡] Nitsara Karoonuthaisiri,[‡,§] Laura Segatori,[†] and Sibani Lisa Biswal*[,†]

[†]Department of Chemical and Biomolecular Engineering, Rice University, Houston, Texas 77005, United States

[‡]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, United Kingdom

[§]Microarray Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phahonyothin Road, Pathumthani 12120, Thailand

ABSTRACT: We report the use of an array of microcantilevers to measure the specific binding of *Salmonella* to peptides derived from phage display libraries. Selectivity of these phage-derived peptides for *Salmonella* spp. and other pathogens (*Listeria monocytogenes* and *Escherichia coli*) are compared with a commercially available anti-*Salmonella* antibody and the antimicrobial peptide alamethicin. A Langmuir isotherm model was applied to determine the binding affinity constants of the peptides to the pathogens. One particular peptide, MSal 020417, demonstrated a higher binding affinity to *Salmonella* spp. than the commercially available antibody and is able to distinguish among eight *Salmonella* serovars on a microcantilever. A multiplexed screening system to quickly determine the binding affinities of various peptides to a particular pathogen highly improves the efficiency of the peptide screening process. Combined with phage-derived peptides, this microcantilever-based technique provides a novel biosensor to rapidly and accurately detect pathogens and holds potential to be further developed as a screening method to identify pathogen-specific recognition elements.



The rapid and sensitive detection of pathogenic bacteria remains critical for the prevention of foodborne illnesses. Among these pathogens, *Salmonella* is one of the most common causes of food contamination.[1,2] Because of the number of different serovars and the low infectious dose,[3] preventing *Salmonella* contamination requires quick and accurate pathogen testing. Conventional methods, including culture and colony counting methods,[4] immunology-based methods,[5] and polymerase chain reaction (PCR),[6] have been widely used for reliable, sensitive, and inexpensive detection;[1] however, these techniques are either tedious or time-consuming or they require pretreatment or labeling of samples.

Label-free biosensing technologies that promise improved performance and shorter analysis time have been recently developed.[2] These biosensors are composed of a recognition element, such as an antibody specific for the pathogen, and a transducer that translates the binding of the pathogen to the recognition element into an easily detectable output signal.[7] Highly specific recognition elements ensure selectivity, and sensitivity is achieved through transducer-mediated signal amplification. Antibodies are the most commonly utilized recognition elements because of their high binding affinity and specificity; however, antibodies lack stability under harsh environmental conditions and can be expensive to generate.[8,9] DNA and aptamers have been used for pathogen detection;[10,11] however, the processing can be labor-intensive. A high-throughput screening technology is typically needed to first isolate recognition elements with high affinity and low cross-reactivities.[12] Phage display has been widely used to generate combinatorial libraries of antibodies[13] or peptides,[8] which are subsequently screened by ELISA[14] or flow cytometry[15] to isolate recognition elements with desired binding properties, but simplified screening processes remain a priority.[16,17]

Piezoelectric biosensors have been widely explored for pathogen detection. Particularly, quartz crystal microbalance (QCM)-based biosensors have been reported:[18] despite considerable effort being focused on enhancing the limit of the detection through the use of displacement assays,[19] QCM biosensors still lack the sensitivity needed for detection of ultralow pathogen concentrations that can cause foodborne diseases. Surface plasmon resonance has also been successfully used, with high sensitivity, for the detection of microbial species, but it requires an extra pretreatment step to first separate the bacteria using antibody-coated magnetic nanoparticles.[20,10] Electrochemical biosensors promise fast read-out but suffer from limited sensitivity and selectivity.[2,21] Nanomechanical cantilevers provide an alternative transducer for the development of high-sensitivity biosensors for detection of

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bacteria. The attachment of target molecules onto one of the cantilever surfaces causes a surface stress mismatch between the upper and lower surfaces of the cantilever, leading to bending of the cantilever.[22,23] This method offers high sensitivity and holds promise for multiplexed analysis based on simultaneous testing of microcantilever arrays.

In this work, we report the development of a multiplexed microcantilever biosensor that utilizes phage-derived peptides[24] for *Salmonella* detection. An array of cantilevers, individually functionalized with different peptides, enables the detection of multiple pathogens in parallel.[25] The selectivity of the phage-derived peptide was determined by quantifying its cross-reactivity to other relevant bacteria species, such as *Listeria monocytogenes* and *Escherichia coli*, and compared with a commercial anti-*Salmonella* monoclonal antibody. The antimicrobial peptide alamethicin was used as a control because of its ability to bind to bacteria nonselectivly.[26] The selectivity of the peptide toward various *Salmonella* serovars is also reported. A Langmuir adsorption-based model[27] was used to determine the binding affinity constants and characterize the peptide–pathogen interaction. This multiplexed detection enables detection with high selectivity. Moreover, simultaneous testing of multiple peptides has the potential to be further developed into a screening technology for pathogen-specific recognition elements.

2. MATERIALS AND METHODS

2.1. Materials. Anti-*Salmonella* monoclonal antibody was purchased from Abcam (UK Catalogue no. ab8273). The sequences of the peptides MSal 020401, MSal 020404, MSal 020415, and MSal 020417 are listed in Table 1. These peptides

Table 1. *Salmonella*-Specific Phage-Derived Peptides

name	amino acid sequence	theoretical pI[a]	theoretical MW[b]
MSal 020401	S E A Y K H R Q M H M S G G G S C	7.97	1866.07
MSal 020404	V P W V T T Y E P W G M G G G S C	4.00	1827.06
MSal 020415	G P A D N T S K H V I R G G G S C	8.23	1655.81
MSal 020417	N R P D S A Q F W L H H G G G S C	6.91	1869.00

[a]Isoelectric point. [b]Molecular weight.

were derived from phage clones screened against a cocktail of eight commonly found *Salmonella* serovars (Enteritidis, Typhimurium, Dublin, Infantis, Senftenberg, Hadar, Mbandaka, Virchow).[24] They were chemically synthesized by GL Biochem (China) at >85% purity with a glycine–glycine–glycine–serine–cysteine space at the C-terminus, as suggested by the manufacturer (PhD-12 phage display library; New England Biolabs, UK). DSP (dithiobis[succinimidyl propionate]) was purchased from Pierce, and PEG-SH (dithiol-alkane-aromatic PEG3-OH) was from SensoPath Technologies. Alamethicin was purchased from VWR.

Bacteria used in this study include a cocktail of eight commonly found *Salmonella* serovars (Enteritidis, Typhimurium, Dublin, Infantis, Senftenberg, Hadar, Mbandaka, Virchow), *L. monocytogenes*, and *E. coli* K12. Each culture was inoculated from a single colony in 10 mL of nutrient broth (CM-0001, Oxoid Ltd., UK) overnight at 37 °C. The overnight cultures were washed with phosphate-buffered saline (PBS), pH 7.4, by centrifugation (14 400 rpm, 15 min, 4 °C) and resuspended in PBS to a concentration of 2×10^9 cfu/mL, as determined using previously collected optical density measurements (the number of cells per milliliter at an OD of 1 measured at 600 nm for each bacterium). The bacterial suspension was subjected to a 10-kGy dose of γ radiation (Gamma 650 cobalt irradiator) to kill the bacteria while preserving the outer structure of the whole cell.

2.2. Preparation of Microcantilever Surfaces. Microcantilever chips were purchased from Concentris GmbH. Each chip contains eight rectangular silicon cantilevers coated with 3 nm of titanium, followed by a 20 nm gold layer, resulting in a bimetallic structure. The cantilevers are 500 μm in length, 100 μm in width, and 1 μm thick.[28] Before functionalization, the microcantilever chip was washed with a mixture of hydrogen peroxide and ammonia hydroxide at 75 °C and cleaned using a UV–ozone cleaner (Novascan) under 5 psi oxygen. Dithiobis [succinimidyl propionate] (DSP) was used as cross-linker to immobilize the recognition element to the gold surface of microcantilevers.[29] The chip was first immersed in DSP (1 mg/mL in DMSO) for 30 min and then in the recognition element solution (2 μg/mL for antibody, 5 μg/mL for peptide, or 5 μg/mL for alamethicin) for 2 h to form a "sandwich" structure: "Au ↔ DSP ↔ recognition element", on the surface. Finally, the chip was incubated in a 1 M Tris solution overnight to block uncoated sites. The cartoon schematic in Figure 1 depicts the

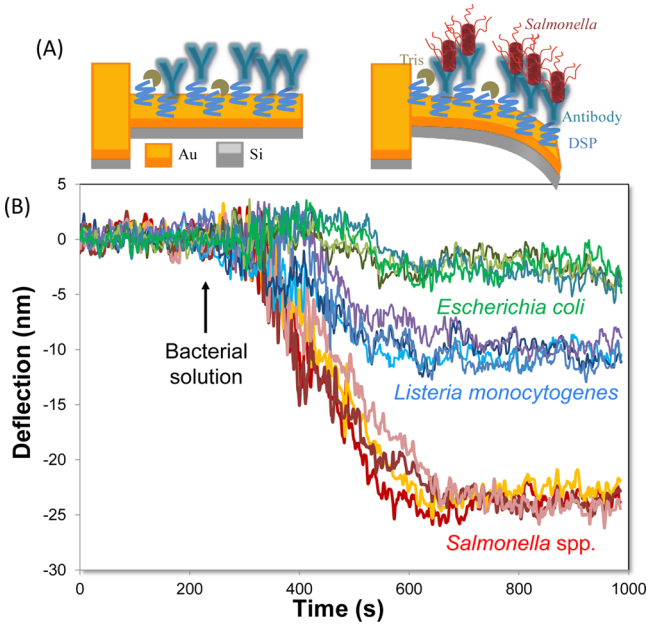

Figure 1. Selective detection of *Salmonella* using microcantilevers. (A) Schematic representation of the microcantilever-based sensor. (B) Anti-*Salmonella* antibody (2 μg/mL) was immobilized on the cantilever surface and three bacterial solutions (1×10^7 cfu/mL; *Salmonella* spp., red lines; *L. monocytogenes*, blue lines; *E. coli*, green lines). Each experiment was conducted in quadruplicate. The arrow represents the time point at which the bacterial solution was added.

chemical functionalization. A reference cantilever was functionalized with PEG-SH (1 mM in ethanol), which has no affinity to the bacterial solutions. For multiplexed detection, each cantilever was individually functionalized with a different peptide using glass microcapillaries. After use, the microcantilever chips were treated with 0.1 M glycine–HCl solution at

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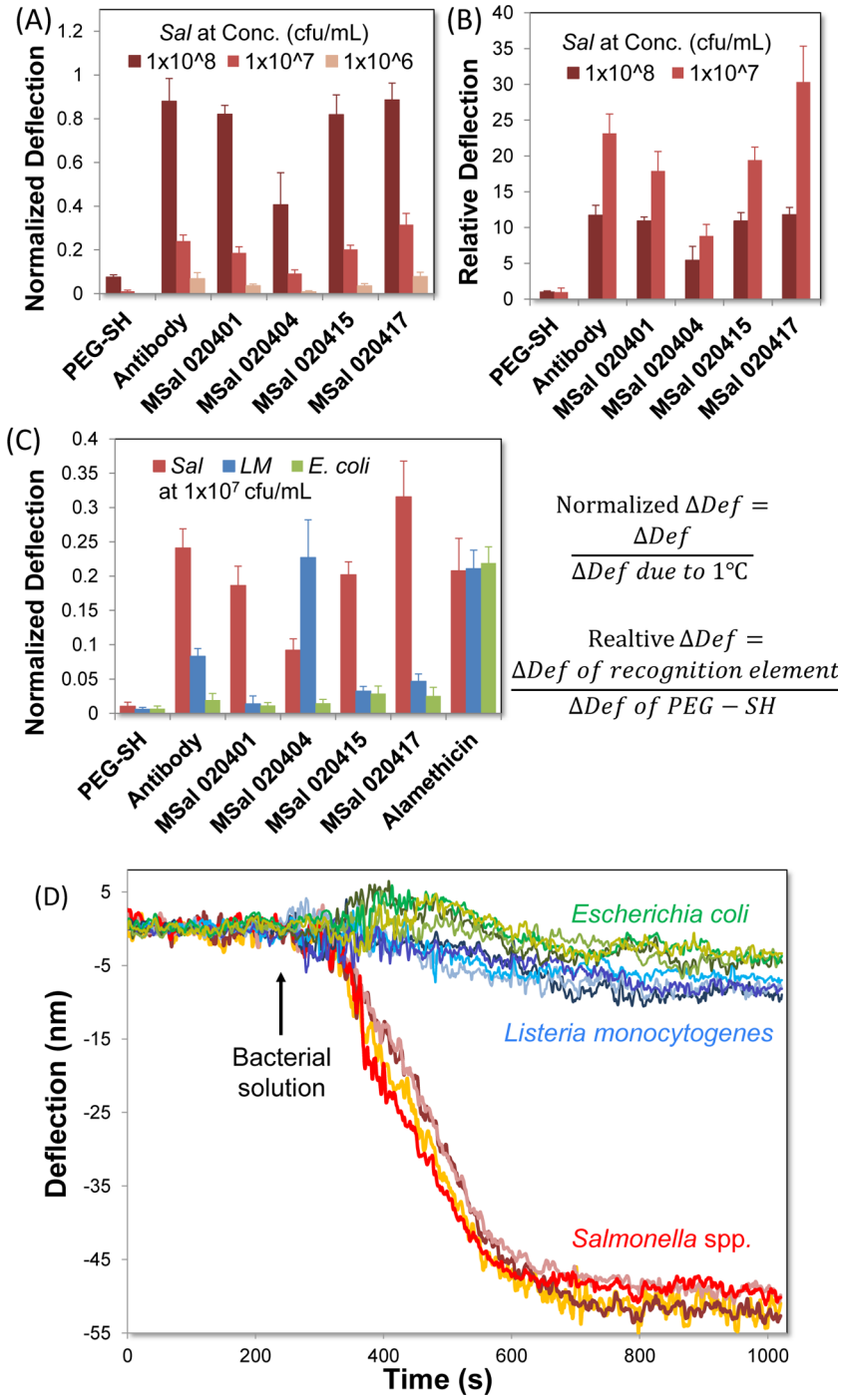

Figure 2. Selective detection of bacterial species using peptide-coated microcantilevers. (A) Normalized deflection of microcantilevers coated with recognition elements and upon binding of *Salmonella* spp. at a concentration of 1×10^6, 1×10^7, or 1×10^8 cfu/mL. (B) Relative deflection of microcantilevers under conditions described in part A. Relative deflection is defined as deflection of the recognition element-coated cantilever normalized to the deflection of the PEG−SH-coated cantilever. (C) Relative affinities of various recognition elements toward different bacteria: *Salmonella* spp. (*Sal*), *L. monocytogenes* (LM), and *E. coli* at 1×10^7 cfu/mL. The equations used to calculate the normalized and relative deflection are also reported. (D) Selective detection of *Salmonella* using peptide MSal 020417. Three bacterial solutions (1×10^7 cfu/mL; *Salmonella* spp., red lines; *L. monocytogenes*, blue lines; *E. coli*, green lines) were tested. Each experiment was conducted in quadruplicate. The arrow represents the time point at which the bacterial solution was added.

pH 2.2 for at least 1 h to release the cells from the immobilized peptides on the cantilever surface, and then regenerated by equilibrating with PBS buffer.[30,31]

2.3. Microcantilever Assay. The microcantilever responds to the change in surface free energy on the sensor surface by monitoring its deflection change. The physico- or chemisorption of molecules to the cantilever surface induces a mismatch in the surface stress, causing the cantilever to bend. The relationship between the cantilever deflection and the change in the surface stress is described by Stoney's equation.[32] Real-time deflection of the microcantilevers was monitored using a Cantisens system from Concentris GmbH, which utilizes a

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scanning laser diode aligned to the tip of the microcantilevers. The position of the reflected laser beam was captured using a position-sensitive detector with a sampling frequency of 1 Hz.[28] The various bacterial solutions (*Salmonella* spp., *L. monocytogenes*, or *E. coli*) were flowed past the functionalized microcantilevers via a syringe pump at 0.42 μL/s at 25 °C. Small variations in the material properties of the cantilevers, such as stiffness or thickness of the gold layer, result in different signals. Thus, the deflections of microcantilevers were normalized by each cantilever's thermomechanical sensitivity, which uses the change in deflection due to a 1 °C change in temperature.[22] Each experiment was repeated at least three times on either the same or different chips, with a minimum of five cantilevers on one chip.

2.4. Mathematical Modeling of Bacteria Binding on Microcantilevers. The binding of the bacteria to the recognition element on the cantilever surface was modeled by assuming a Langmuir isotherm model.[27] The kinetic of the binding is shown as:

$$\text{Binder} + \text{Bacteria} \overset{K_A}{\rightleftarrows} \text{Binder} - \text{Bacteria} \quad (1)$$

In eq 1, "Binder" represents the recognition element: anti-*Salmonella* antibody or a phage-derived peptide. K_A is the binding affinity constant. The affinity constant plays an important role in the adsorption process and can be used to study interaction between two molecules. The deduction steps of the model have been previously reported.[33,34] There are three more assumptions in this model: the deflection is proportional to the coverage of the recognition element on the microcantilever surface, the recognition elements bind only one molecule of the targets (monovalency), and the adsorption of bacteria to the recognition element reaches equilibrium after a certain time. Afterward, the following equation is obtained:

$$k_A[\text{Bacteria}](1 - \theta) = k_{-A}\theta \quad (2)$$

In the equation above, θ is the fraction of the surface with attached *Salmonella*, and $(1 - \theta)$ represents the sites available for further *Salmonella* binding. Define K_A as the binding affinity constant, and we can obtain the expression for θ. Finally the equation with the relation between the *Salmonella* concentration and the deflection signal from the microcantilevers is obtained, as shown in eq 3.

$$\frac{[\text{Bacteria}]}{\Delta\text{Def}} = \frac{[\text{Bacteria}]}{\Delta\text{Def}_{max}} + \frac{1}{\Delta\text{Def}_{max}K_A} \quad (3)$$

In eq 3, [Bacteria] is the molar concentration of bacteria, and ΔDef is the change in the normalized deflection caused by a given concentration of *Salmonella*, and ΔDef_{max} is the maximal change in the normalized deflection when all the recognition elements are occupied. Since [Bacteria]/ΔDef is linear to [Bacteria], K_A is obtained from the slope and intercept of the plot of [Bacteria]/ΔDef with respect to [Bacteria].

3. RESULTS AND DISCUSSION

Phage-derived peptides have increasingly been employed as recognition elements to detect specific analytes.[35] Several rounds of biopanning are typically employed to screen a phage display library and isolate high-affinity binders. Previously, a PhD-12 phage display library containing 10^{13} phage forming units (pfu)/mL expressing 12 amino acid peptides was biopanned against *Salmonella* spp., and several phage clones were selected that present high affinity for *Salmonella* spp. The corresponding peptides from some of the phage clones were chemically synthesized and proven to be specific receptors to *Salmonella* spp. for magnetic separation.[24]

In this study, four peptides of the previously identified phage-derived peptides specific to *Salmonella* spp. (MSal 020401, MSal 020404, MSal 020415, and MSal 020417) were investigated as bacterial recognition elements on microcantilevers. Previous ELISA results using phage-displayed peptides showed that MSal 020401, MSal 020415, and MSal 020417 exhibited higher binding affinity to the *Salmonella* serovars than to other nontarget bacteria tested (*L. monocytogenes* and *E. coli* K12). Although MSal 020404 did not display high affinity binding toward *Salmonella* spp. in phage ELISA analyses, it was found to have the lowest cross-reactivity to the nontarget bacteria in studies conducted using chemically synthesized peptides.

3.1. Microcantilevers for Pathogen Detection. Detection of *Salmonella* was first investigated using a microcantilever functionalized with the anti-*Salmonella* antibody to determine selectivity of the antibody. Three different bacterial solutions at concentrations of 1×10^7 cfu/mL were flowed past the functionalized cantilever, causing a deflection, as shown by Figure 1. A decrease in cantilever deflection of 24 nm is observed upon the binding of *Salmonella* spp. to the anti-*Salmonella* functionalized cantilever, compared with 10 nm for *L. monocytogenes*, and 3 nm for *E. coli*. The extent of binding, which is directly proportional to the magnitude of the deflection change, reflects the relative affinity of each bacterium for the anti-*Salmonella* antibody. The results obtained using microcantilevers are in agreement with the relative affinity measured by ELISA:[24] *Salmonella* spp. > *L. monocytogenes* > *E. coli*.

3.2. Comparison of Antibody and Peptides: A Potential Screening Method of Pathogen Specific Recognition Elements. Four phage-derived peptides (MSal 020401, MSal 020404, MSal 020415, and MSal 020417) were individually immobilized onto microcantilevers. The binding behavior of these peptides was compared with the anti-*Salmonella* antibody, alamethicin, and a PEG-coated microcantilever surface. The anti-*Salmonella* antibody remains the standard for comparing the selectivity of the peptides. Alamethicin serves as a control because it interacts nonspecifically with bacterial cell membranes, mainly through electrostatic attraction.[36] The PEG-coated microcantilever serves as the negative control because it prevents any nonspecific adsorption to the surface. Figure 2A shows the normalized deflections ($\Delta\text{Def}/\Delta\text{Def}_{1°C}$) of the various recognition elements to *Salmonella* spp. at concentrations of 1×10^6, 1×10^7, and 1×10^8 cfu/mL. The extent of deflection increases with increasing concentration of *Salmonella* spp. Deflections at concentrations below 1×10^6 cfu/mL were too small to be accurately detected, suggesting that 1×10^6 cfu/mL *Salmonella* is the limit of detection of this biosensor under the conditions tested in this study. Figure 2B shows the relative deflection compared with the PEG-coated reference cantilever, $\Delta\text{Def}/\Delta\text{Def}_{PEG\text{-}SH}$. The sensitivity of detection at different concentrations of *Salmonella* can be determined by normalizing the normalized signals to that of the reference cantilever. We found that the sensitivity decreased when the concentration of *Salmonella* was increased from 1×10^7 cfu/mL to 1×10^8 cfu/ (Figure 2B), most likely due to multilayer adsorption at concentrations above the absorption capacity of the microcantilever.

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Because 1×10^7 cfu/mL of *Salmonella* proved to be the optimal concentration for detection, the binding behavior of various recognition elements to the three different bacterial species was tested under these conditions (Figure 2C). As expected, minimal deflection was detected on the PEG-SH-coated cantilever, indicating poor binding of any of the bacterial solutions. Alamethicin-coated cantilevers displayed similar binding affinity for *Salmonella* spp., *L. monocytogenes*, and *E. coli*. The anti-*Salmonella* antibody presented high binding affinity for *Salmonella* spp., but low affinity for *L. monocytogenes* and *E. coli*, confirming previously reported results.[24] Three of the four peptides—MSal 020401, MSal 020415, and MSal 020417—exhibited similar relative affinity for the different bacterial species tested. The cantilever deflection for MSal 020417 for the various pathogenic bacteria at 1×10^7 cfu/mL is shown in Figure 2D. The MSal 020417-coated cantilever resulted in the largest deflection upon *Salmonella* spp. binding, indicating that MSal 020417 presents the highest affinity to *Salmonella* spp. compared with the other phage-derived peptides tested and compared with the anti-*Salmonella* antibody, previously shown in Figure 1B. These results confirm previously reported studies using peptide-displayed magnetic beads.[24] As a comparison with the deflections for anti-*Salmonella* antibody in Figure 1B, the direct cantilever deflections for MSal 020417 caused by three bacterial solutions are given in Figure 2D, indicating its better sensitivity and selectivity. The MSal 020401-coated cantilever, on the other hand, displayed the smallest deflection upon binding of *L. monocytogenes* and *E. coli*, suggesting that MSal 020401 presents the highest selectivity toward *Salmonella* spp. and the lowest cross-reactivity with the other bacterial species tested. The only inconsistency between the results obtained with microcantilevers and ELISA is the relative affinity of MSal 020404 toward *L. monocytogenes*. This difference is likely due to differences in affinities of peptides when tested individually or displayed on a phage capsid.

3.3. Detection of Various *Salmonella* Serovars. The experiments described above were conducted using solutions of *Salmonella* spp., which is a mixture of various *Salmonella* serovars. To further evaluate the selectivity of the cantilever-displayed peptide technology for biosensing and biopanning applications, eight different *Salmonella* serovars were tested individually (Figure 3). Microcantilevers were coated with the peptide with highest affinity toward *Salmonella* spp. (MSal 020417) to quantify the relative affinity of MSal 020417 to various *Salmonella* serovars tested at optimal concentration (1×10^7 cfu/mL). The eight serovars induced different signals (normalized deflections), indicating different binding affinities of MSal 020417 to *Salmonella* serovars. The relative normalized deflections detected using microcantilevers are in agreement with the relative binding affinities observed in the previously reported phage-peptide ELISA analyses.[24] Moreover, the deflection observed using each one of the eight *Salmonella* serovars tested was much larger than that observed using *L. monocytogenes* and *E. coli*, demonstrating that the MSal 020417 peptide can be used for detection not only of *Salmonella* spp., but also of individual *Salmonella* serovars. Interestingly, we found that the average normalized deflection induced by the *Salmonella* serovars equals the normalized deflection observed using *Salmonella* spp., underscoring the potential of microcantilever-displayed peptide biosensors for reliable detection of bacteria.

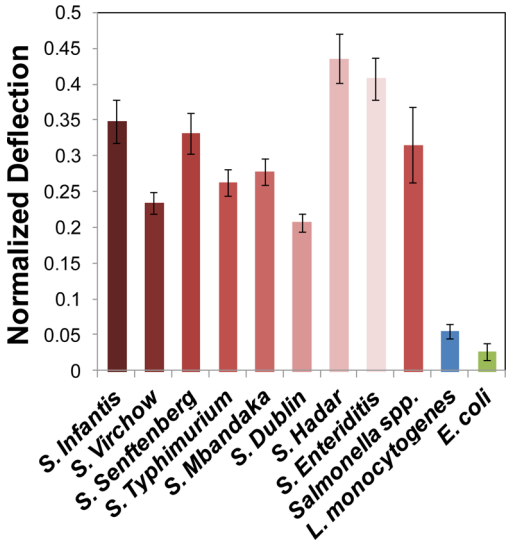

Figure 3. Detection of *Salmonella* serovars using microcantilevers. The peptide MSal 020417 is used as recognition element, and the concentration of bacterial solution is 1×10^7 cfu/mL. *Salmonella* serovars, shades of red; *Salmonella* spp., red; *L. monocytogenes*, blue; *E. coli*, green.

3.4. Mathematical Modeling and Calculation of the Binding Affinity Constants. The binding affinity of the peptide for the different bacterial species can be determined by modeling the process using a Langmuir isotherm model, which has previously been used to describe antigen−antibody interactions on biosensor surfaces.[37,38] The binding affinity constant, K_A, refers to the relative affinity of the peptide to a specific bacterium, as described in eq 3. Figure 4 shows selected plots of [Bacteria]/ΔDef as a function of [Bacteria] for the four peptides tested in this study. In each plot, at least six data points were used to optimize the data fitting. The binding affinity constant, K_A, was calculated from the slope and intercept of each plot (Table 2): the values of K_A represent the relative affinity of a recognition element for each bacterium, with the largest value corresponding to the highest affinity. Thus, the relative affinity values obtained using the Langmuir isotherm model recapitulate the experimental results described above for cantilevers coated with the anti-*Salmonella* antibody and MSal 020417 (*Sal* > *LM* > *E. coli*), MSal 020401 and MSal 020415 (*Sal* > *LM* ≈ *E. coli*), MSal 020404 (*LM* > *Sal* > *E. coli*), and almethecin and PEG (*Sal* ≈ *LM* ≈ *E. coli*). The model was therefore considered reliable and applied to evaluate the affinity constants of various recognition elements. MSal 020417 was found to have the highest affinity constant for *Salmonella* spp., confirming the superior binding properties of this peptide, even compared with the commercially available antibody. These results, taken together, suggest that the mathematical model developed in this study can be used to reliably determine the relative affinities of various recognition elements for different bacterial species and to determine the kinetics properties of phage-derived peptides screened using microcantilevers.

3.5. Multiplexed Screening of Peptides on Microcantilevers. To determine whether the microcantilever-based biosensor developed in this study could be used to screen combinatorial libraries of peptides, we investigated binding of *Salmonella* spp. to a random array of cantilevers coated with different recognition elements. An array of seven cantilevers was prepared on a single chip in which each cantilever was

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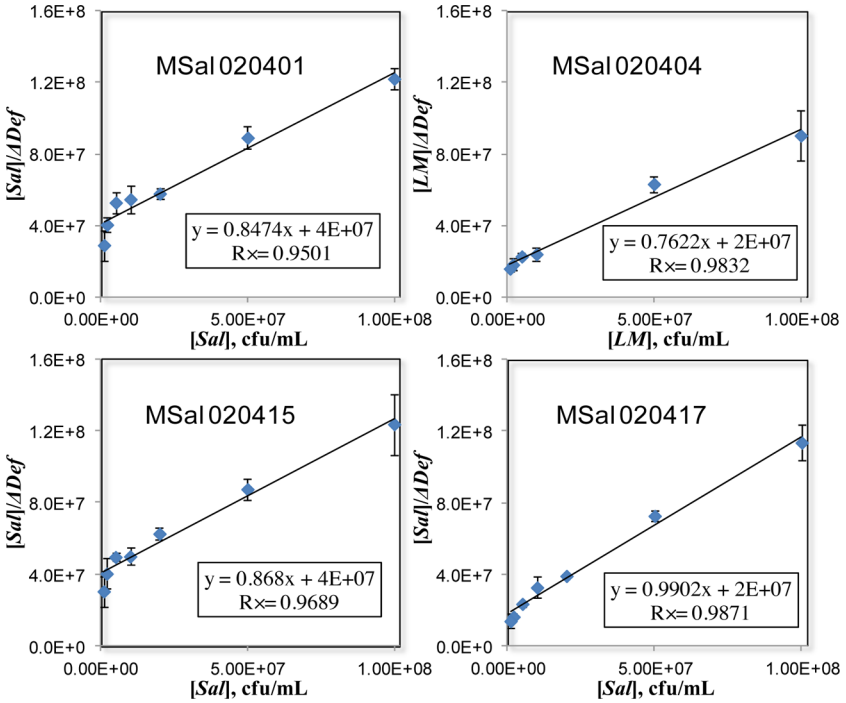

Figure 4. Data fitting plots to determine the binding affinity constant K_A. [Bacteria]/ΔDef values are plotted with respect to [Bacteria], and the equations for trend lines and R-squared values are reported in each graph. From this linear relationship, the slope and intercept of this plot were obtained, and binding affinity constant K_A was calculated using eq 3.

Table 2. Binding Affinity Constants $K_A \times 10^9$ (mL/cfu) to *Salmonella* spp. (*Sal*), *L. monocytogenes* (*LM*), and *E. coli* K12

constants K_A	*Sal*	*LM*	*E. coli*
MSal 020401	20.7	0.358	0.251
MSal 020404	7.33	42.9	0.335
MSal 020415	21.6	0.518	0.293
MSal 020417	55.4	4.41	0.180
antibody	33.6	5.28	0.288
alamethicin	25.3	27.1	26.5
PEG−SH	0.0689	0.0277	0.0411

functionalized with a different recognition element, as illustrated in Figure 5A. *Salmonella* spp. at a concentration of 1×10^7 cfu/mL was flowed past the chip. As shown in Figure 5B, the deflection of each cantilever was observed to be proportional to the binding affinity of the specific recognition element coated onto the cantilever as determined above, suggesting that the binding properties of the different recognition elements are not altered upon immobilization onto the array of microcantilevers. This simultaneous testing of multiple peptides on a single chip improves the efficiency of detection. These results illustrate the high potential of this microcantilever-based sensor for the screening of phage-derived peptides as well as other recognition elements.

3.6. Factors Governing Microcantilever-Based Sensing. The experiments and models we used to determine the binding affinity constant show that peptide MSal 020417 has better sensitivity and selectivity than anti-*Salmonella* antibody. In addition to the normalized deflection in Figure 2C, the cantilever deflections for the anti-*Salmonella* antibody (Figure 1B) and MSal 020417 (Figure 2D) provide a direct comparison for peptide selectivity. The difference between specific *Salmonella* binding and nonspecific binding (*E. coli* or *L. monocytogenes*) is obviously larger for MSal 020417 than for anti-*Salmonella* antibody. As a result, the microcantilever-based technique using the peptide MSal 020417 demonstrates high selectivity for *Salmonella* detection. Another important factor to consider is the grafting density of the recognition elements and its role in the cantilever signal. Differences in molecular size, electrostatic charge, and concentration may lead to differences in the number of recognition elements on the sensor surface. For conventional biosensing techniques, the transducer signal is directly correlated to the number of binding events, but the cantilever deflection is driven by changes in surface stress. Although the anti-*Salmonella* antibody has a smaller apparent surface density compared with MSal 020417 as a result of its larger size, it results in a larger nonspecific binding toward *E. coli* and *L. monocytogenes*, as shown in Figures 1B and 2D. This suggests that the affinity of the recognition element, rather than grafting density, drives cantilever deflection. Furthermore, the method employed in this study to immobilize the recognition elements leads to their random orientation, potentially blocking available for specific binding. Therefore, an oriented immobilization method can be explored to improve the detection sensitivity.[20,39,40]

4. CONCLUSION

We have developed a rapid and specific microcantilever-based technique for the detection of the pathogenic bacterium *Salmonella* by exploiting the binding affinities of *Salmonella*-specific recognition elements. In this study, four phage-derived peptides selected from the screening of a commercial phage display library were tested. The peptide MSal 020417 was found to have better binding affinity and specificity than a commercially available anti-*Salmonella* antibody. In addition to distinguishing *Salmonella* spp. from *L. monocytogenes* and *E. coli*, this microcantilever-based technique also enables distinguishing among eight *Salmonella* serovars. The binding properties of the

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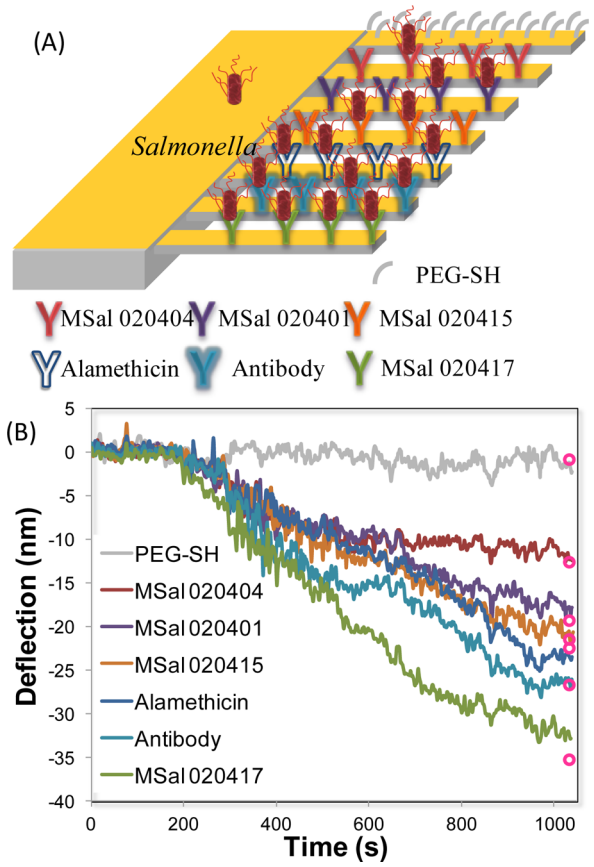

Figure 5. Multiplexed screening of recognition elements on microcantilevers. (A) Schematic representation of individual functionalization of cantilevers with various recognition elements on a single chip. (B) Multiplexed detection of *Salmonella* spp. using microcantilevers. The concentration of *Salmonella* spp. is 1×10^7 cfu/mL. Various recognition elements were immobilized on a single cantilever chip and tested simultaneously. The lines represent the deflection of cantilevers functionalized with different recognition elements as reported in the figure legend, and the hollow pink circles represent data obtained from experiments conducted using individual recognition elements.

various recognition elements to the different bacterial species were subsequently investigated using a Langmuir isotherm model, and the binding affinity constants K_A were calculated. The relative affinities of recognition elements evaluated with the Langmuir isotherm model were consistent with the experimental results. Furthermore, we show that immobilizing different peptides on an array of microcantilevers in a single chip allows for rapid screening of multiple peptides for the isolation of high affinity binders. We envision that by immobilizing a collection of peptides that present high selectivity for different bacterial pathogens, a multiplexed detection system could be generated to detect multiple pathogens simultaneously, largely improving the detection efficiency of the biosensor. In summary, multiplexed detection on microcantilevers can be used to reliably and efficiently detect pathogens, as well as to screen pathogen specific binders as recognition elements.

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (713) 348-6055. Fax: +1 (713) 348-5478. E-mail: biswal@rice.edu.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Velusamy, V.; Arshak, K.; Korostynska, O.; Oliwa, K.; Adley, C. *Biotechnology Advances* **2010**, *28* (2), 232−254.
(2) Lazcka, O.; Del Campo, F. J.; Munoz, F. X. *Biosens. Bioelectron.* **2007**, *22* (7), 1205−1217.
(3) Humphrey, T. *Nat. Rev. Microbiol.* **2004**, *2* (6), 504−509.
(4) Fratamico, P. M. *Mol. Cell. Probes* **2003**, *17* (5), 215−221.
(5) Abdel-Hamid, I.; Ivnitski, D.; Atanasov, P.; Wilkins, E. *Anal. Chim. Acta* **1999**, *399* (1−2), 99−108.
(6) Jenikova, G.; Pazlarova, J.; Demnerova, K. *Int. Microbiol.* **2000**, *3* (4), 225−229.
(7) Turner, A. P. F. *Science* **2000**, *290* (5495), 1315−1317.
(8) Cwirla, S. E.; Peters, E. A.; Barrett, R. W.; Dower, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87* (16), 6378−6382.
(9) Chames, P.; Van Regenmortel, M.; Weiss, E.; Baty, D. *Br. J. Pharmacol.* **2009**, *157* (2), 220−233.
(10) Piliarik, M.; Parova, L.; Homola, J. *Biosens. Bioelectron.* **2009**, *24* (5), 1399−1404.
(11) Chen, S.-H.; Wu, V. C. H.; Chuang, Y.-C.; Lin, C.-S. *J. Microbiol. Methods* **2008**, *73* (1), 7−17.
(12) Hoogenboom, H. R. *Nat. Biotechnol.* **2005**, *23* (9), 1105−1116.
(13) Bradbury, A. R. M.; Marks, J. D. *J. Immunol. Methods* **2004**, *290* (1−2), 29−49.
(14) Marks, J. D.; Hoogenboom, H. R.; Bonnert, T. P.; McCafferty, J.; Griffiths, A. D.; Winter, G. *J. Mol. Biol.* **1991**, *222* (3), 581−597.
(15) Feldhaus, M. J.; Siegel, R. W. *J. Immunol. Methods* **2004**, *290* (1−2), 69−80.
(16) Gannot, G.; Tangrea, M. A.; Gillespie, J. W.; Erickson, H. S.; Wallis, B. S.; Leakan, R. A.; Knezevic, V.; Hartmann, D. P.; Chuaqui, R. F.; Emmert-Buck, M. R. *J. Mol. Diagn.* **2005**, *7* (4), 427−436.
(17) Kitamatsu, M.; Futami, M.; Sisido, M. *Chem. Commun.* **2010**, *46* (5), 761−763.
(18) Park, I. S.; Kim, N. *Biosens. Bioelectron.* **1998**, *13* (10), 1091−1097.
(19) Minunni, M.; Mascini, M.; Carter, R. M.; Jacobs, M. B.; Lubrano, G. J.; Guilbault, G. G. *Anal. Chim. Acta* **1996**, *325* (3), 169−174.
(20) Torun, O.; Boyaci, I. H.; Temur, E.; Tamer, U. *Biosens. Bioelectron.* **2012**, *37* (1), 53−60.
(21) Mannoor, M. S.; Zhang, S.; Link, A. J.; McAlpine, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (45), 19207−19212.
(22) Biswal, S. L.; Raorane, D.; Chaiken, A.; Birecki, H.; Majumdar, A. *Anal. Chem.* **2006**, *78* (20), 7104−7109.
(23) Pinnaduwage, L. A.; Boiadjiev, V. I.; Hawk, J. E.; Gehl, A. C.; Fernando, G. W.; Wijewardhana, L. C. R. *Nanotechnology* **2008**, *19* (10), 105501.
(24) Morton, J.; Karoonuthaisiri, N.; Stewart, L. D.; Oplatowska, M.; Elliott, C. T.; Grant, I. R. *J. Appl. Microbiol.* **2013**, *115* (1), 271−281.

(25) McKendry, R.; Zhang, J. Y.; Arntz, Y.; Strunz, T.; Hegner, M.; Lang, H. P.; Baller, M. K.; Certa, U.; Meyer, E.; Guntherodt, H. J.; Gerber, C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (15), 9783−9788.
(26) Leitgeb, B.; Szekeres, A.; Manczinger, L.; Vagvolgyi, C.; Kredics, L. *Chem. Biodiversity* **2007**, *4* (6), 1027−1051.
(27) Velanki, S.; Kelly, S.; Thundat, T.; Blake, D. A.; Ji, H.-F. *Ultramicroscopy* **2007**, *107* (12), 1123−1128.
(28) Liu, K.-W.; Biswal, S. L. *Anal. Chem.* **2010**, *82* (18), 7527−7532.
(29) Wang, J.; AlMakhaita, M.; Biswal, S. L.; Segatori, L. *J. Biosens. Bioelectron.* **2012**, *3* (1), 115.
(30) Maekawa, T.; Kuriyama, R. *J. Cell Sci.* **1991**, *100*, 533−540.
(31) Bouvrette, P.; Luong, J. H. T. *Int. J. Food Microbiol.* **1995**, *27* (2−3), 129−137.
(32) Stoney, G. G. *Proc. R. Soc. London: Ser. A* **1909**, *82* (553), 172−175.
(33) Matsumoto, K.; Torimaru, A.; Ishitobi, S.; Sakai, T.; Ishikawa, H.; Toko, K.; Miura, N.; Imato, T. *Talanta* **2005**, *68* (2), 305−311.
(34) Sakai, G.; Saiki, T.; Uda, T.; Miura, N.; Yamazoe, N. *Sens. Actuators, B* **1997**, *42* (2), 89−94.
(35) Morton, J.; Karoonuthaisiri, N.; Charlermroj, R.; Stewart, L. D.; Elliott, C. T.; Grant, I. R. *PloS One* **2013**, *8* (9), e74312−e74312.
(36) Zasloff, M. *Nature* **2002**, *415* (6870), 389−395.
(37) Sakai, G.; Ogata, K.; Uda, T.; Miura, N.; Yamazoe, N. *Sens. Actuators, B* **1998**, *49* (1−2), 5−12.
(38) Sakai, G.; Nakata, S.; Uda, T.; Miura, N.; Yamazoe, N. *Electrochim. Acta* **1999**, *44* (21−22), 3849−3854.
(39) Jung, Y.; Jeong, J. Y.; Chung, B. H. *Analyst* **2008**, *133* (6), 697−701.
(40) Kausaite-Minkstimiene, A.; Ramanaviciene, A.; Kirlyte, J.; Ramanavicius, A. *Anal. Chem.* **2010**, *82* (15), 6401−6408.