Nanoparticle-based biosensing using interfacial electrokinetic transduction

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Abstract

We present a novel label-free electrokinetic method for detecting biomolecular binding on nanoparticles suspended in the vicinity of a laminar microfluidic interface. The sensor is based on the deflection of the liquid interface in an external AC electric field. Biomolecular binding on particles is shown to increase the interfacial electrical conductivity of the suspending electrolyte, which is sensitively transduced as a change in electrokinetic interfacial deflection. Using this approach, we detect the binding of biotin on streptavidin-functionalized nanoparticles at concentrations as low as 500 aM and perform detection of human IgG at clinically relevant concentrations (1.25-12 mg/mL) without labels. The interface response is only influenced by specific binding on nanoparticle binding sites and decreases when the particle concentration of reactive particles is reduced. Furthermore, we show that control experiments with both non-reactive particles and lack of a specific target analyte do not produce interface deflection. This work provides a promising method for quantifying bead-based binding kinetics for sensitive and specific biosensing in solution without labels.

Keywords

Biosensing, Electrokinetics, Microfluidics, Dielectrophoresis, Nanoparticles

1. Introduction

The ability to detect biomolecular targets without labels is important in clinical diagnostics, drug discovery, environmental monitoring, and biomedical research. Biosensors require three basic components: a substrate for specific binding to occur, a transducer to translate the binding event into a signal, and a detector to measure the transduced signal. Bead-based biosensors use nanoparticles with surface-immobilized receptors as a biosensing substrate for the detection of a wide variety of biomolecular targets including proteins¹, DNA, and bacteria.^{2,3} In comparison to traditional microarray technology, the high nanoparticle surface-to-volume ratio can lead to increased detection sensitivity and reduced assay time. Bead-based methods, for example, have been shown to increase the limit of detection (LOD) in SPR sensing⁴, fluorimetric assays⁵, and electrochemical amperic detection⁶, and have been used in a broad range of colorimetric³, enzymatic⁷, mechanical⁸, electrochemical⁹ and fluorimetric⁵ assays.

Nanoparticle-based biosensing is often accomplished using a heterogeneous support, where particles are adsorbed onto a solid surface¹⁰ or a sol-gel matrix¹¹, functionalized, and flushed with a fluid or gas containing the relevant target analyte. Because colloids must be assembled and functionalized, these surface-based methods typically require multiple rinsing/drying steps^{12,13}, and can suffer from functional group crowding¹⁴ and surface fouling.¹⁵

To reduce detection time and assay complexity, and to improve portability, biosensors can be integrated with microfluidics. With microfluidics, biomolecular binding can be poised to occur in solution phase at the fluid interface formed between two co-flowing laminar liquid streams. This sensing strategy is robust and has been successfully applied to detecting DNA hybridization¹⁶

and for protein immunoassays.¹⁷ Matthews et al, for example, reported a novel homogenous biosensor that used a laminar aqueous interface as a biorecognition substrate. Binding was initiated by driving two different fluids – one containing a target analyte and a second with complimentary receptor molecules – to flow side-by-side along a main microfluidic flow channel. Biomolecular binding occurred at the fluid interface as target and receptor molecules diffused between the two fluids. Using fluorescent microscopy, binding kinetics were quantified by measuring fluorescent intensity produced from bound product at varying distances down the microchannel length.¹⁸

Aqueous liquid interfaces are easy to create using “T-channel” or “Y-channel” microfluidic geometries.^{19,20} When driven to flow side-by-side at low Reynolds number, the two fluids produce a quasi-stable liquid interface where mass transport is driven strictly through diffusion.²¹ Biosensing by diffusion and binding at the fluid interface has several advantages over traditional binding on a solid substrate. First, because biomolecular binding occurs in solution phase, diffusion-limited binding occurs over much shorter timescales when compared to a surface. Second, the interface is continually replenished and renewed with molecular target and biorecognition receptors at any given position in the microchannel, and is therefore not subject to surface fouling and non-specific adsorption.

Despite the usefulness and advantages, current liquid interface-based microfluidic sensors require fluorescent labeling and fluorescent microscopy for signal transduction and detection.²² To overcome this limitation, we recently developed a novel label-free biosensor strategy using an electrokinetic method known as fluidic dielectrophoresis (fDEP).²³ In fDEP, an alternating

current (AC) electric field is driven across a microfluidics interface where one co-flowing fluid stream has a different electrical conductivity and dielectric constant than the adjacent stream. When an electric field is applied, the interface polarizes and electrohydrodynamically deflects across the microfluidic channel with a magnitude and direction that is sensitive to the electrical properties (e.g. conductivity and dielectric constant) at the liquid interface.²³ Exploiting this phenomena, we demonstrated that biomolecular binding influences the electrical conductivity at the liquid interface, and that binding can be dynamically detected by monitoring interfacial deflection at varying positions down the microchannel.²⁴ Biorecognition is transduced as a change in electrokinetically-induced interfacial deflection in a process we have described as interfacial electrokinetic transduction (IET).²⁴ This approach was used to detect model avidin:biotin biomolecular binding at avidin concentrations as low as 50 femtomolar (fM), enabling it to compete with methods like surface plasmon resonance (SPR) (3 nm to 1.5 fM)²⁵ and enzyme-linked immunosorbent assays (ELISAs) (100 fM and below)²⁶, which are the current standard of clinical sensitivity.

In this work, we extend the usefulness of IET to that of nanoparticle bead-based biosensing assays, and show that biomolecular binding on nanoscale particles influences the deflection of a microfluidic liquid interface. Specifically, our strategy uses a suspension of surface functionalized nanoparticles as a microfluidic substrate for biomolecular recognition. The particles are functionalized with either streptavidin or Protein A molecules, which serve as specific binding receptors for biotin or human IgG, respectively in solution. We demonstrate that specific binding on the nanoparticles produces a change in interfacial electrical conductivity, which can be detected by IET. This colloid-based biosensing strategy does not use labels,

amplified signaling, or methods for concentrating the analyte samples and is capable of detecting biotin concentrations as low as 500 aM and physiologically relevant human IgG at concentrations down to 1.25 mg/mL.

2. Materials and Methods

2.1 Device Fabrication

In this section we describe both the methods used fabricate the experimental devices and a theoretical basis for using fDEP as a transducer for nanoparticle-based biosensing. The experimental device employed a combination of microfluidic flow, electrokinetics, and colloid-based biomolecular surface chemistry. We created a liquid interface for binding by co-flowing two different fluid streams through microfluidic T-channel. One stream contained a suspension of functionalized nanoparticles at a known weight percent (wt%), while the other contained target analyte molecules. To deliver an electric field across the interface, we integrated an array of parallel point microelectrodes within the main flow channel (Figure 1a). The complete device, illustrated in Figure 1b, was fabricated using a combination of microfluidic soft lithography and wet etching techniques. To fabricate the microarray of electrodes, we first coated glass cover slides (50x30 mm, no. 1, Fisher Scientific) with a 20 nm layer of chromium, followed by a 30 nm layer of gold, using electron beam deposition. Positive photoresist (S1813, Shipley) was spun onto the gold-coated slides and exposed using a UV contact aligner with a positive mask (Fineline Imaging) before etching the gold and chrome layers with chemical etchants (Alfa Aesar). The electrodes were aligned symmetrically across the main flow channel axis, and spaced at 20 μm intervals, with pointed tips to focus the electric field at the liquid interface (Figure 1b). To fabricate the microchannel, we created a T-channel master mold by spin-coating

negative photoresist (SU-8 3050, Microchem Corp.) on a 4-inch silicon wafer (Silicon Inc.) and exposed to a UV light source through a negative photomask. The microfluidic channels were created by pouring PDMS at a 10:1 elastomer to curing agent ratio over the wafer mold. The device was baked at 85 °C for 30 minutes and the cured polymer was peeled off and cut to size. Finally, fluid ports were created with a 0.75 mm biopsy punch (Ted Pella, Inc) and the PDMS was bonded to the glass slide by exposure to oxygen plasma (Model 42A, Jetlight) for 1 minute and immediately aligned and sealed by eye under an inverted microscope. The completed device consisted of two fluidic inlet channels connected to a main microfluidic channel measuring 100 μm across and 35 μm high (Figure 1b). Fluid flow was driven into the device using an external pressure source at a constant flow rate of 5 $\mu\text{L}/\text{min}$.¹⁹ To create the electrical properties for interface deflection, it is necessary that one fluid stream have a greater dielectric constant than the neighboring fluid phase. For the binding experiments in this work, one fluid phase was composed of a 10x diluted phosphate buffered saline (PBS) solution and the adjacent phase was a 0.8 M solution of 6-aminohexanoic acid (AHA) (Sigma Aldrich). AHA is a water-soluble zwitterion intended to increase the dielectric constant of the aqueous solution.²⁷

2.2 Creating the Electrical Liquid Interface

To create a biosensing system at the liquid interface, we integrated a biomolecular recognition event between the two fluids, where the PBS stream contained a suspension of functionalized nanoparticles and the adjacent phase containing either the target analyte or a negative control. We used both biotin and human IgG, and streptavidin and Protein A-functionalized nanoparticles as biomolecular systems to test our particle-based method. Because of the strong binding affinity, biotin (Sigma Aldrich) and 100 nm streptavidin-coated silica nanospheres ($\zeta = -34.6$

mV) (Corpuscular Inc.) were first used as a model bioreaction and 100 nm carboxylated silica nanospheres ($\zeta = -34.3$ mV) (Corpuscular Inc.) were used as a non-reactive negative control. A 4mM biotin stock solution was made in 0.8 M AHA and subsequently diluted to experimental concentrations ranging between 500 aM and 16 μ M. The PBS stream contained the nanoparticle substrate: streptavidin-coated particles were triple washed and re-suspended in PBS solution and particle suspensions were maintained at 0.0375 wt% for all experiments unless specified otherwise. Human immunoglobulin G (IgG) (Sigma Aldrich) and 350 nm protein-A-coated silica nanospheres (Corpuscular Inc.) served as a more physiologically relevant reaction scheme. Human IgG was dissolved in deionized water and diluted to experimental concentrations (1.25, 3, 6 and 12 mg/mL). The receptor/binding solutions were driven side-by-side using a constant pressure source and the resulting fluid interface was imaged using confocal microscopy by labelling the AHA “red” with 10 ng/mL Alexa Fluor 594 and the adjacent PBS stream (containing either streptavidin, Protein A, or carboxylated nanoparticles) “green” with 10 ng/mL Alexa Fluor 488 (Figure 1c).

2.3 Fluidic Dielectrophoresis

When each fluid phase was driven into the main microchannel, a steep gradient in the electrical properties formed at the interface between the two fluid phases. The PBS stream had a high conductivity and a low permittivity ($\sigma = 0.35$ mS/cm, $\varepsilon = 78.2$), while the AHA stream had a low electrical conductivity and a high permittivity ($\sigma = 32$ μ S/cm, $\varepsilon = 110$). When an AC electric field was applied across the microfluidics channel, the fluid interface displaced in a direction perpendicular to the main flow field. The direction of this displacement is sensitive to the frequency (ω) of the electric field. At low AC field frequency ($\omega > \text{COF}$), the high conductive

PBS stream displaced into the low conductive AHA stream (Figure 2a), as the field oscillates slowly enough for mobile ions in solution to electromigrate to the interface. Because we engineered the fluid interface such that one phase had a greater electrical conductivity and the other a greater dielectric constant, the direction and magnitude of the interfacial displacement approached zero (Figure 2b) at a characteristic crossover frequency (COF). At high AC frequency ($\omega > \text{COF}$), however, the direction of the displacement reversed, and the high-dielectric stream displaced across the channel into the low-dielectric stream (Figure 2c) since polarization by ionic conduction does not have enough time to occur and dielectric polarization dominates the interfacial polarization. Confocal micrographs depicting the 3D structure of the interface are shown in Figure 2d. The interfacial stress as a function of frequency can be described using Maxwell-Wager polarization theory. The frequency-dependent expression for the interfacial fDEP force²³ is:

$$\langle F_{DEP} \rangle = \langle \sigma_i E \rangle = 1/2 \text{Re}[K(\omega)] \epsilon_0 E_0^2 \quad (1)$$

where $\text{Re}[K(\omega)]$ is the real (in phase) component of the Clausius–Mossotti (CM) factor which describes the degree to which the interface has polarized. The CM factor is sensitive to both differences in the conductivity and permittivity of each fluid stream (stream 1 and stream 2):

$$\text{Re}[K(\omega)] = ((\epsilon_2 - \epsilon_1) \tau^2 \omega^2) / ((\epsilon_2 + \epsilon_1)(\tau^2 \omega^2 + 1)) + ((\sigma_2 - \sigma_1) \tau^2 \omega^2) / ((\sigma_2 + \sigma_1) \tau^2 \omega^2 + 1). \quad (2)$$

where $\tau = ((\epsilon_2 + \epsilon_1) / (\sigma_2 + \sigma_1))$ is the charge relaxation timescale of the electrical liquid interface, which describes the characteristic timescale required for mobile ions to electromigrate to the interface. The frequency at which both dielectric and conductive charging mechanisms

balance (e.g. where $F_{DEP} = 0$) and no displacement occurs is referred to as the COF. This is determined by setting $\text{Re}[K(\omega)] = 0$,

$$\omega_{cof} = 1/(2\pi)[((\sigma_1 - \sigma_2)(\sigma_1 + \sigma_2))/((\epsilon_2 - \epsilon_1)(\epsilon_2 + \epsilon_1))]^{1/2}. \quad (3)$$

Equation 3 can be used to determine the COF for a microfluidic liquid interface as a function of the fluid electrical conductivity and permittivity. This expression illustrates the linear relationship between the interfacial electrical conductivity and the COF, as shown in Figure 2e.

Our nanoparticle biosensing scheme relies on the sensitivity of the COF to the electrical properties at the microfluidics interface. If a biomolecular reaction increases the electrical conductivity difference across the interface, for example, the COF will increase. As the reaction propagates down the channel length the COF will continue to increase until the biomolecular reaction saturates. In the next section, we show experimental results that demonstrate the ability to detect binding on nanoparticles at a liquid interface by monitoring the COF along the axial position length of a microchannel.

3. Results and Discussion

3.1 Maxwell-Wagner Polarization

In order to determine if our existing MW theory is appropriate to apply to a dilute colloidal suspension we first performed fDEP experiments to determine the interfacial COF as a function of electrolyte conductivity with a dilute colloidal suspension of 100 nm carboxylated silica particles in PBS buffer. To obtain an accurate COF reading at the interface before diffusive mixing occurred, we measured the COF near the entrance of the microfluidic T-junction ($x^* = 0.2$) where the interface was sharp and the COF was uninfluenced by diffusive mixing. The

resulting COF data is plotted against our theoretical model (equation 3) in Figure 2c for varying concentrations of colloid weight percent. The data show good agreement with the MW theory for both the pure liquid-liquid and liquid-colloid experiments. From Figure 2c, we therefore assume that the colloidal suspensions used in this work are dilute enough to be mathematically and electrically approximated as a pure fluid, and that the presence of particles does not require additional modifications to our existing polarization model to accurately predict the fDEP response of the fluid interface.

We next measured the net displacement of the interface at different electric field frequencies and interfacial electrical conductivities in order to determine how differences in electrical conductivity across the liquid interface influence the COF and magnitude in the interface deflection. To accomplish this, we adjusted the conductivity of the colloidal suspension while keeping the weight percent and adjacent stream conductivity constant. In Figure 2d we plot the experimental displacement for three different interfacial conductivities ($\Delta\sigma = 0.101, 0.268$ and 0.418 mS/cm). Each COF (labeled A, B, and C) are highlighted in Figure 2c. The data demonstrates that the magnitude of the interfacial displacement increases with interfacial conductivity at low field frequencies below the COF. However, the high frequency displacement remains unaffected since frequency regime is only influenced by dielectric differences between the two fluids.²³

3.2 Biotin-Streptavidin Binding

With the frequency response of a non-reacting interface known, we next sought to determine if specific biomolecular binding on nanoparticles influences this behavior. To detect specific

binding at the electrical interface, we flowed one stream with a target probe against an adjacent stream of analyte. We investigated a well-known model reaction: biotin and streptavidin. The biomolecular reaction between biotin and streptavidin are strongly associated with a very small dissociation constant, $K_d \sim 10^{-15} \text{ M}^{29}$, and therefore the reaction is rapid, nonreversible and serves as a good model system to experimentally validate our biosensing strategy. We used streptavidin conjugated nanoparticles as a substrate for binding. With a small diffusional coefficient ($10^{-8} \text{ cm}^2/\text{s}$)³⁰, the particles provided a well-defined region of active binding sites at the liquid-liquid interface for biotin, a smaller, quickly-diffusing molecule, to rapidly diffuse towards the nanocolloidal interface and bind. First, biotin in an excess concentration (16 μM) was co-flowed against a 0.0375 wt% suspension of streptavidin-silica nanoparticles. We applied an AC electric field across the interface and measured the COF at the inlet, which we denoted here as COF_{in} . Next, we performed subsequent COF measurements at varying positions down the electrode array and normalized the resulting response by COF_{in} . Shown in Figure 3, the biotin-streptavidin system produced an increase in the interfacial COF with axial position. The COF continued to increase down the axial length of the channel before reaching a plateau and declining, which we believe is indicative of a combination of binding saturation in the vicinity of the nanocolloidal-functionalized interface and diffusional smoothing between the two fluid streams.

We next performed a series of control experiments to validate our COF measurements. First, we performed the same COF experiments without biotin and observed the COF does not increase, but rather it decreased over the axial channel length (Figure 3a). We attribute this decrease to diffusional blending across the interface, where ion diffusion gradually reduces the

sharp differences in electrical properties and reduces the interfacial COF. As a second non-reactive negative control, we replaced the 100 nm streptavidin-coated silica nanospheres with carboxylated silica beads of the same size and wt%, and tested the interface response against the same excess concentration of biotin (16 μ M). The surface carboxyl groups (-COOH) are meant to emulate the nanocolloidal interface, while providing a non-reactive surface to observe any false positive signal from non-specific adsorption. Similar to the first negative control experiment, the COF again decreased (Figure 3a).

3.3 Limit of Detection and Varying Binding Sites

We then repeated these biosensing experiments with different biotin concentrations varying several order-of-magnitude ranging between 16 μ M and 500 aM (Figure 3b). The biosensor response, as determined by the interface COF, increased with increasing concentrations of biotin (Figure 3). Since the wt% for each experiment was fixed at 0.0375%, the number of available binding sites remained the same for each experiment. As is expected with a substrate-based sensor response, the interface was observed to saturate for the given particle wt% at a biotin concentration of 10 pM. Above this concentration the COF signal was not influenced by further increases in the biotin concentration. Using the 3-sigma method, we determined the experimental LOD for biotin to be 500 aM. Given the dimensions of the microfluidic device (100 μ m main channel width x 35 μ m channel height) and a total internal volume of 60 nL, this concentration translates to approximately 36 molecules of biotin in the device at any given time.

In a related experiment, we varied the weight percent of the nanoparticles from 0.005 – 0.0375 wt% in order to determine if the COF is influenced by the number of available binding sites at

the liquid interface. Depicted in Figure 4, the COF response decreases with decreasing nanoparticle wt%. For this experiment, we also decreased the interfacial conductivity from 0.35 mS/cm to 0.24 mS/cm. It is interesting to note that this reduction increased the contribution of the reaction relative to the baseline conductivity difference, and thus led to an increase the magnitude of the COF signal response. This increase can be observed by comparing the sensorgram data for 16 μ M biotin in Figure 3, where the maximum signal response is 1.09, to the case in Figure 4, where the maximum response increased to 1.19. These sensorgrams were compared against two different controls using carboxylated silica particles at both the low (0.005) and high (0.375) wt% concentrations. Shown in Figure 4, the COF is not influence by the presence of varying concentration of non-reactive nanoparticles, and only increases when binding sites are available for biotin.

3.4 Background Protein

We next performed a series of experiments to test the robustness and specificity of the biosensor. First, we introduced a background of 5 mg/ml bovine serum albumin (BSA) to the biotin stream. Shown in Figure 5, we observed that the presence of BSA reduced the COF signal, but it is still capable of detecting biotin. In moving toward detection of physiologically relevant targets, we then determined if we could detect the presence of human IgG in solution using a suspension of 350 nm silica nanoparticles coated with protein A. The affinity of protein A binding to human IgG is reported to be on the order of $K_d \sim 10^{-10} \text{ M}$ ³¹, five orders of magnitude smaller than biotin-streptavidin. In Figure 6, we were able to detect human IgG at concentrations as low as 1.25 mg/mL, which is comparable to relevant concentrations used in modern ELISA assays.³²

3.5 Human IgG-Protein A

Finally, we sought to use our polarization model to determine the electrical mechanism for why biomolecular binding increases the interface COF. In previous work, we demonstrated that biomolecular binding in solution without nanoparticles produces a local increase in electrical conductivity difference across the liquid interface.²⁴ This was demonstrated this by measuring the magnitude of the interfacial deflection for different analyte concentrations during binding. This method is possible because the displacement of the interface is solely dependent on differences in electrical conductivity and permittivity below and above the COF, respectively. Therefore, by measuring the interfacial motion at both low and high frequency we can determine how the electrical conductivity and the permittivity across the interface are influenced by the reaction. For reactions without nanoparticles, we showed that increased analyte concentration only produced a measurable change in the interfacial displacement at low field frequency (e.g. only influences electrical conductivity) and fDEP motion at high frequency above the COF was not influenced by binding.

To determine what interfacial electrical properties were influenced by binding on nanoparticles, we measured the magnitude of interfacial deflection at varying voltages (1-20 Vpp) and frequencies both below (500 kHz) and above (40 MHz) the COF for systems with and without a nanoparticle-based biomolecular reaction. In the presence of a biotin-streptavidin reaction, low frequency displacement was influenced by binding and no difference was observed at high frequency (Figure 7). Based on these experiments and our MW polarization model, biotin

binding on streptavidin nanoparticles increases the local electrical conductivity difference across the liquid interface which produces an increase in the interfacial COF. It is worth noting that our fDEP method is unable to determine the mechanism for this conductivity increase. We speculate that the negatively charged nanoparticles shed ions in their diffuse cloud during binding, which could account for the local increase in electrical conductivity. Future work will focus in understanding the physical mechanism surrounding this observed conductivity increase.

4. Conclusions

We have presented results that demonstrate the ability to perform label-free detection of a biotin and human IgG on nanoparticles in solution using fluidic dielectrophoresis. Using our method, we quantified the degree to which a microfluidic liquid interface deflected in an external AC electric field and showed that binding on particles in solution influences the electrical conductivity in the liquid phase at the interface. This electrical difference produced a measurable increase in the interface crossover frequency. Using this approach, we were able to detect biotin-streptavidin binding at low 500 aM concentration and human IgG at concentrations of 1.25 mg/mL. We experimentally demonstrated these biomolecular reactions produce a local increase in the electrical conductivity difference at the fluid interface. The interface only produced a increase in crossover frequency when specific binding sites were available and exhibited a saturation response when all binding sites were depleted from the fluid interface and the crossover frequency decreased when the number of available binding sites were decreased. The When compared to our previous work conducted in solution, the addition of functionalized nanoparticles increased the detection sensitivity for biotin by two orders of magnitude. Future work will involve developing this biosensing strategy for testing analytes in physiologically

relevant samples. Nanoparticles can very easily be functionalized with the relevant receptors to detect malaria, various cancers, and for other diagnostic exams that require femtomolar detection capabilities. Given the nature of the microfluidic flow device, our fDEP biosensor can also be used to dynamically monitor reaction propagation and reaction kinetics since we can obtain a complete reaction sensorgram by taking COF measurements at different points down the channel length.

Figure Captions

Figure 1. Microfluidic T-Channel

(a) Schematic of the microfluidic T-channel device with integrated electrodes. (b) Top-down confocal micrograph of the T-channel device with two co-flowing fluorescently labelled fluids. Scale bar 100 μm . (c) 3D confocal z-stack of the microfluidic-generated electrical liquid interface. Scale bar 50 μm .

Figure 2. fDEP Crossover Frequency (a) Top-down confocal images of the microfluidic channel, showing interfacial displacement for frequencies below the crossover, (b) at the crossover, (c) above the crossover. Scale bar 25 μm . (d) 3D confocal images showing the tilt of the electrical interface for frequencies below the crossover (left), at the crossover (center), and above the crossover (right). Scale bar 50 μm . (e) Crossover frequency plotted as a function of conductivity difference. The MW Theory line is plotted as Eq. 3 for fixed values of $\epsilon_1 = 78.2$ & $\epsilon_2 = 110$. Lettered boxes are selected from the 0.0375 wt% data. (f) Displacement of the electrical interface as a function of applied frequency. Each complete dataset's COF is highlighted in a lettered box in (d).

Figure 3. IET Bead-Based Biosensing Assay (a) A schematic illustration of the case where no reaction is present. Down the length of the channel, the crossover decreases. (b) In the case of a biomolecular reaction, the crossover increases as the reaction propagates down the channel. (c) Detection sensorgram showing positive detection in the presence of a reaction, with decreasing

control signals. (d) Limit of detection sensorgram showing the lowest biotin concentration detected with the colloid-assisted platform.

Figure 4. Streptavidin Limited Detection by Varying Particle wt%. Sensorgram illustrating the decrease in signal with decreasing weight percent of streptavidin-coated particles.

Figure 5. Biotin Detection in BSA Background. Sensorgram illustrating the ability of the fDEP biosensor to detect biotin-streptavidin binding in a background of 5 mg/mL BSA.

Figure 6. Detection of Human IgG-Protein A Binding. Detection sensorgram showing positive detection of human IgG with 350 nm silica nanoparticles coated with protein A.

Figure 7. Influence of Binding Reaction on Electrical Properties Measured by Interface Displacement. Displacement of the electrical interface versus applied voltage squared for both the low and high frequency case, in the case of a biomolecular reaction and a negative control. The divergence of the low frequency data suggests that the presence of a reaction alters the local conductivity at the electrical interface.

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Figure 1

Figure 2

Figure 3

Figure 4

- 0.0375 wt% Streptavidin-coated Silica vs 16 μ M Biotin
- 0.03 wt% Streptavidin-coated Silica vs 16 μ M Biotin
- ▲— 0.02 wt% Streptavidin-coated Silica vs 16 μ M Biotin
- ▼— 0.01 wt% Streptavidin-coated Silica vs 16 μ M Biotin
- ◆— 0.005 wt% Streptavidin-coated Silica vs 16 μ M Biotin
- 0.0375 wt% Streptavidin-coated Silica vs 0.8 M AHA
- 0.005% wt% Streptavidin-coated Silica vs 0.8 M AHA

Figure 5

Figure 6

Figure 7

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