A Universal Biomolecular Concentrator To Enhance Biomolecular Surface Binding Based on Acoustic NEMS Resonator

Abstact: In designing bioassay systems for low-abundance biomolecule detection, most research focuses on improving transduction mechanisms while ignoring the intrinsically fundamental limitations in solution: mass transfer and binding affinity. We demonstrate enhanced biomolecular surface binding using an acoustic nano-electromechanical system (NEMS) resonator, as an on-chip biomolecular concentrator which breaks both mass transfer and binding affinity limitations. As a result, a concentration factor of $10^3$ has been obtained for various biomolecules. The resultant enhanced surface binding between probes on the absorption surface and analytes in solution enables us to lower the limit of detection for representative proteins. We also integrated the biomolecular concentrator into an optoelectronic bioassay platform to demonstrate delivery of proteins from buffer/serum to the absorption surface. Since the manufacture of the resonator is CMOS-compatible, we expect it to be readily applied to further analysis of biomolecular interactions in molecular diagnostics.

Introduction

Highly sensitive detection of biomolecular interactions at ultralow concentration is crucial for continued progress in applications ranging from clinical diagnostics to drug discovery and fundamental research such as intracellular trafficking, cell signaling, neuronal impulse transmission, artificial implant technology, and gene regulatory dynamics. The recent trend focuses on exploring miniaturized analytical systems for scarce biomolecule detections which has advantages of reduced reagent consumption, high sensitivity, rapid detection, and multiplexed analysis, etc. Many micro-/nanoscale biosensors with novel transduction mechanisms have been developed to this end, including plasmonic enzyme-linked immunosorbent assay (ELISA), nanowire-based sensors, and micro-/nano-electromechanical sensors. Since miniaturization of sensors often increases their signal-to-noise ratio which is largely attributed to the high surface to volume ratio, micro-/nanoscale sensors have demonstrated the capability of specific biomolecule detections of only a few thousand (or even a few hundred) analyte molecules in the sample volume. Despite the impressive advances in signal transduction technology, the mass transfer and binding affinity limitations are fundamentally hindering the improvement of the limit of detection (LOD) of micro-/nanoscale biosensors. Most surface-based biosensors require analytes in solution to react with probes immobilized on a solid surface. Being heterogeneous, this process depends on numerous different parameters. In solution, the rate depends on the convection and diffusion of the biomolecules (mass transfer limitation). At the interface, the rate relies on the biomolecular interaction forces between the analytes and the probes (affinity limitation). To solve these issues, researchers have developed techniques to enhance the total flux of the solution or actively deliver the analyte molecules toward the sensor surface. Examples include electrokinetics-assisted binding that brings biomolecules toward the absorption surface by electrostatic fields, aiming to overcome the diffusion and binding barriers. Nevertheless, this technique is restricted to targets that are inherently charged, and solutions having low ionic strength, which limits its applicability to many practical assays. Other active methods include magnetically-assisted and optically-assisted binding, which require extra labeling steps or complicated setups which limit their throughput. Acoustic approaches have emerged as useful tools to manipulate microscale objects with many distinct advantages including simplicity, biocompatibility, and low power consumption. However, due to the competition between the acoustic radiation force and frictional force induced by Stokes' law, a critical radius exists below which the acoustic radiation force becomes too small to overwhelm the frictional or streaming forces in the medium, resulting in inefficient direct manipulation of biomolecules. Hydrodynamic approaches use designed micro-

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shown in Figure 1B, the model handles the variation in analyte concentrations of analytes in bulk solution \([A]_0\) and at concentration by dividing the solution into two compartments: absorption surface \([A]_s\). Fluidic chips to generate microvortices and demonstrate the direct trapping of biomolecules.\(^{34,35}\) However, such methods are limited by the use of closed microfluidic chips which cannot apply to other biosensing techniques. Consequently, universal, noninvasive, and highly efficient methods to directly manipulate and trap biomolecules are imperatively preferred to enhance biomolecular surface binding and realize highly sensitive bioassays.

In this work, we propose a novel molecular manipulation system to trap biomolecules in open space and enhance their surface binding using an on-chip designed acoustic nanoelectromechanical system (NEMS) resonator. The resonator works as a biomolecular concentrator to precisely control the hydrodynamic collection and accumulations of biomolecules into a three-dimensional (3D) virtual micropocket, thus fundamentally breaking both mass transfer and binding affinity limitations. This system is compatible to any biological solutions, and the trapping of biomolecules in open space allows the integration of the device with other biosensing techniques, thus achieving a real universal biomolecular concentrator and sensing system.

### RESULTS AND DISCUSSION

#### Theoretical Considerations and System Design

For surface-based biosensors, the two-compartment model has been widely used to describe the biomolecular surface binding process, such as the convection, diffusion, and reactions.\(^{36}\) As shown in Figure 1B, the model handles the variation in analyte concentration by dividing the solution into two compartments: concentrations of analytes in bulk solution \([A]_0\) and at absorption surface \([A]_s\). A diffusion layer where the flow velocity approaches zero exists between these two compartments. Theoretically, the two-compartment reaction can be described with eq 1:

\[
[A]_0 \xrightleftharpoons[k_{on}][k_{off}] [A] + [B] \xrightleftharpoons[k_{on}'][k_{off}'] [AB]
\]

where \(k_m\) is the diffusion or convection controlled rate constant, \(k_{on}\) and \(k_{off}\) are the association and dissociation rate constants for the biomolecular interactions, respectively, and \([B]\) and \([AB]\) represent the density of probes and absorbed analytes at the absorption surface, respectively. Because of zero flow in the diffusion layer, transfer of analytes from the bulk solution to the absorption surface over the diffusion layer occurs primarily by diffusion which normally takes a long period of time. Such a mass transfer limitation results in an unmatched analyte concentration between bulk solution and absorption surface \(([A]_s \ll [A]_0)\). Active stirring is generally recommended to increase \([A]_s\) by accelerating the mass transfer,\(^{37,38}\) yet the ability is inherently limited given that \([A]_s\) cannot exceed \([A]_0\) leading to a corresponding binding affinity limitation.

To break these two limitations, our system is designed by the integration of an acoustic NEMS resonator in an open liquid system to generate special hydrodynamic conditions which can trap and accumulate the biomolecules in a predicted virtual micropocket. The resonator is fabricated through a standard CMOS process (Supporting Information, Figure S1). Figure 1C,D shows the top-view scanning electron microscope (SEM) image and schematic cross-section structure of the resonator. It is composed of a freestanding aluminum nitride (AIN) piezoelectric nanoplate (450 nm) sandwiched by periodic molybdenum (Mo) electrodes as interdigital transducer (IDT) which is isolated from the silicon substrate with an air cavity to avoid dissipation of energy into the silicon substrate. After a power is applied to the electrodes, acoustic waves are generated in the piezoelectric nanoplate via the converse piezoelectric effect.\(^{39}\) When the resonator works in liquid, the acoustic streaming effect is generated because of the dissipation of acoustic energy into liquid, which is...
experimentally verified by the decrease of quality factor ($Q$) from 1175 to 56 (Supporting Information, Figure S2). Consequently, four symmetric counter-flowing Rankine microvortices (SCRMVs) are formed close to the resonator’s central region, and a virtual micropocket is further generated, where biomolecules are massively trapped and concentrated; hence, the concentration of the target biomolecules at the surface is larger than the bulk concentration ($[A]_s > [A]_0$), and the molecule diffusion is enhanced by the hydrodynamic flow (Figure 1E). Since the trapping of molecules is realized in the open space, and the position of the micropocket can be well predicted, this technique can be easily combined with other biosensors by locating the transducer at or close to the virtual micropocket to achieve a real universal biomolecular concentrator.

**Flow Profile.** A numerical simulation is performed to understand the resonant behaviors of the acoustic NEMS resonator and the hydrodynamic behaviors of the flow using the 3D finite element method (FEM). As illustrated in Figure 2A, after a power of 0.01 mW is applied on the resonator in air, acoustic waves confined in the piezoelectric nanoplate propagate along the transverse direction ($y$ axis) with the maximum vibration amplitude of 0.09 nm. When resonating in liquid, the resonator leads to the generation of SCRMVs, each of which is composed of a forced microvortex core surrounded by a free vortex zone, as shown in the normalized simulation results of the 2D flow profile in the $x$–$y$ plane that is 50 μm above the resonator surface ($z = 50 \mu m$) in Figure 2Bi. The forced microvortex core is rotational, and its flow velocity scales with the radius and decays to zero at the microvortex center, while the free microvortex zone is irrotational, and its flow velocity varies inversely with the radius to satisfy the boundary condition of no motion at infinity. The formation of the four SCRMVs results from the acoustic streaming which is generated by spatial attenuation of acoustic waves. In brief, acoustic waves attenuate and create a pressure gradient during the propagation along the $y$ axis, resulting in the formation of two fluid jets at both sides of the resonator. Because of the flow continuity, recirculated flows are correspondingly generated along the $x$ axis. Figure 2Bii shows the zoom-in 2D flow profile...
which consists of inlets along the $x$ axis and outlets along the $y$ axis at the center of the resonator. As a consequence of the superposition of component velocities of the four SCRMVs, a stagnation point is created at the junction of the four SCRMVs where the flow velocity approaches to zero. Ultimately, the primary result of this flow velocity distribution inside the rectangle region in Figure 2Bii is the generation of a virtual micropocket in the open space, as shown in Figure 2C.

It is worth mentioning that an azimuthal angle exists for SCRMVs. As revealed in Figure 2Di,ii, the fluid flows toward the bottom of the virtual micropocket along the $x$ axis and departs along the $y$ axis at an angle downward. This azimuthal angle originates from the fact that the mechanical vibration of the resonator simultaneously induces the propagations of attenuated acoustic waves along the longitudinal direction. Namely, the velocity of the fluid jets is a superposition of component velocities along both the transverse and longitudinal direction, therefore leading to the 3D azimuthal recirculation of the SCRMVs at the angle of $\theta$, as shown in Figure 2Diii.

We employed micro-PIV to experimentally visualize the SCRMVs. PS particles of 5 $\mu$m were used to track the motion of the microvortices by the actuation of the resonator (Supporting Information, Movie S1). Figure 2E shows the typical captured fluorescence image of PS particle motion which clearly shows four SCRMVs at a power of 4 mW (Supporting Information, Movie S2). Figure 2F shows the 2D flow profile and velocity of the SCRMVs at the $x$-$y$ plane analyzed by Diatrack 3.04 (Supporting Information, Movie S3). It is characterized by an obvious stagnation point which is surrounded by a high-velocity region with a maximum flow velocity at 220 $\mu$m/s.

It is also noted that, from Figure 2E, the fluorescence intensity of the vortex zone is remarkably enhanced compared to the background, indicating that the particles were dragged into the vortices and trapped inside. We further compared the size and the trapping efficiency of the vortex by applying different powers. The location and the size of the microvortices stay constant at different applied powers (Supporting Information, Figures S4 and S5). However, the number of the particles being trapped in the vortex increased by using higher power, as indicated by the fluorescence intensity results in Figure S4. This is due to the fact that the higher power will induce a stronger vortex and will generate larger drag forces which will in turn trap more particles in the vortex. This is also verified by the simulation. The amplitude of the resonator vibration at the resonant frequency increases with higher power. As a consequence, larger resonant amplitude will induce more vigorous microvortices via the acoustic streaming effect; thus, more particles are brought into the vortices zone. Since the volume and the location of the vortex zone can be predesigned with the device dimension, it will benefit the application to use such trapping for different applications. In addition, the power can be readily adjusted to tune the amount of the trapped particles or molecules, which will be described in the following section.

**Biomolecular Concentrator.** As reported by hydrodynamic trapping in microfluidics, biomolecules in vortex...
Enhanced immunoassays of human IgG using the acoustic NEMS resonator as biomolecular concentrator. (A) Cartoon showing the process of functionalization on the resonator surface. (B) Time-lapse fluorescence images of Cy3-labeled human IgG (i) with and (ii) without the actuation of the resonator in solution. Scale bar, 25 μm. (C) Extraction of time-lapse fluorescence intensity from part B. The data are calculated from the region of highest intensity of fluorescence signals (with an area of 5 μm × 30 μm at the center of the resonator). (D) Fluorescence intensity after buffer rinsing and drying. Scale bar, 10 μm. The inset shows the fluorescence images of each sample. (E) Time-lapse fluorescence intensity under the actuation of the resonator with the power changing from 0 to 4 mW. (F) Time-lapse fluorescence intensity under the actuation of the resonator with different Q value.

Figure 3B. Figure 3C,D quantifies the area and the average height of the concentration region, respectively. The active area reaches up to 2700 μm² with the actuation of the resonator for 15 min, approaching saturation. A similar trend is revealed in terms of the average height which shows an average saturated value around 80 nm with the maximum height as high as 260 nm. This height restriction is resulted by the azimuthal recirculation of the SCRMVs at an angle of θ as shown in Figure 3E. Namely, biomolecules move toward the resonator from inlets at an angle downward and tend to be collected at the bottom of the virtual micropocket. It should be noted that the position and the size of the virtual micropocket are rather repeatable by using the same power. Thus, this method can be directly applied to enhance protein detections by locating the probe-functionalized sensor substrate at this region which can be significantly beneficial from the markedly enhanced analyte concentration during biomolecular interaction assays where the response time and surface-absorbed proteins are inherently limited by diffusion and affinity, especially for measurement of biomolecules at ultralow concentrations. It is also noted that since the acoustic trapping is noninvasive, the enhanced molecular assay can be applied to any surface-based biosensors.

Enhanced Immunoassay. The clearly demonstrated protein concentration effect will markedly lower the LOD of the specific protein detection, such as in an immunoassay. To prove this, we performed the detection of the human immunoglobulin G (IgG) through specific antibody–antigen interactions using a resonator-enhanced immunoassay. Figure 4A depicts the configuration of the detection system. Antihuman IgGs were immobilized on the resonator surface through a PLL−PEG−biotin−SAV linker. The main reason that we employed PLL−PEG−biotin−SAV for anti-IgG functionalization on the resonator surface is to provide a uniformly high bioaffinity configuration of the detection system.
immobilization instead of direct conjugation of anti-IgG is to test the concentration effect on the same device by regenerating the sensor surface. Since the assemblies of the PLL−PEG−biotin−SAV on the resonator are driven by electrostatic interactions between the positively charged polymer and negatively charged surface, such assemblies can be regenerated easily by tuning the pH value of the buffer. Thus, the device can be reused many times which facilitated the comparison of the concentration effect. In addition, the PEG chains were grafted in the polymer to prevent nonspecific protein bindings. Different concentrations of Cy3-labeled human IgGs were then introduced into the solution chamber. After reaction with the immobilized anti-IgGs, the fluorescence intensity can be used to quantify the amount of the surface-absorbed proteins. The concentration effect is compared with and without the resonator actuation. As the trapped proteins were saturated after 15 min of actuation of the resonator in the DIPHM measurement, we kept all the actuation experiments no longer than 15 min. Figure 4B,C shows the time-lapse fluorescence images and corresponding fluorescence intensities by exposing various concentrations of the Cy3-labeled human IgG to antihuman IgG coated resonator. This clearly shows the enhancement of the fluorescence intensity with the resonator actuation. The fluorescence intensity at 50 pM with the resonator-induced concentration for 15 min is comparable to that at 5 μM without the resonator operation, indicating that the concentration factor A reaches up to $10^5$. As calculated from the fluorescence intensity of 50 pM IgG solutions (Supporting Information), the total number of IgG molecules in solution is $5.88 \times 10^8$, and the total number of concentrated molecules is $4.50 \times 10^8$; hence, the concentration efficiency for 50 pM IgG solution is $4.50 \times 10^8 / 5.88 \times 10^8 = 76\%$. Similarly, the total number of IgG molecules in 500 pM IgG solution is $5.88 \times 10^9$, and the total number of concentrated molecules is $1.33 \times 10^9$; hence, the concentration efficiency for 500 pM IgG solution is $1.33 \times 10^9 / 5.88 \times 10^9 = 23\%$. It is worth noting that the concentration factor and the total number of the molecules to saturate the trap may vary case-by-case considering the practical dependence on a series of conditions such as protein interactions, the concentration of the analytes, the viscosity of the solution, and the intrinsic properties of the protein molecules.

It is also worth mentioning that the fluorescence intensities from Figure 4C are derived from the analytes both absorbed at the resonator surface and collected in 3D space. To specifically quantify the proteins absorbed at the resonator surface, we removed the protein solutions after 15 min of incubation and actuation by the resonator. The devices were then thoroughly rinsed with buffer to remove the physical absorbed proteins. After drying, the fluorescence intensity of each concentration was recorded (Figure 4D). The results again clearly demonstrate the significantly enhanced amount of the surface-absorbed proteins by the resonator actuation. This indicates that the resonator plays a critical role in the enhancement of biorecognition events via the controlled hydrodynamic trapping. Namely, analytes are efficiently trapped, concentrated, and specifically bound to surface-
immobilized probes under the actuation of the resonator by breaking the mass transfer and binding affinity limitations.

As predicted in theory analysis and simulations, the hydrodynamic manipulations are related with the power applied to the device and the Q of the resonator. The power-dependent and Q-dependent characteristics of the resonator-induced concentration effect are characterized in detail by using the same antibody–antigen interactions. Figure 4E shows the results of power-dependent concentration effects (according to fluorescence intensity) with the power changing from 0 to 4 mW. No fluorescence is observed in the control experiment (0 mW) after 15 min of incubation, indicating that the analyte concentration is below the LOD of the fluorescence microscope. With the same incubation time and power increasing from 0.25 to 4 mW, the fluorescence intensity enhances significantly. The simulation analysis explicitly indicates that the amplitude of resonator vibration increases to 0.68 nm with the power growing up to 4 mW (Supporting Information, Figure S4). As a consequence, higher resonant amplitude will induce more vigorous microvortices, whereby more target molecules are brought into the vortices and finally trapped at the virtual micropocket. The power-dependent characteristics of the resonator-induced concentration effect render the resonator as a regulator to control the biomolecular interaction rate via adjusting the power, which plays a key role in enzymology and other biological research. Except for the power, the resonator with higher Q value is characterized by larger vibration amplitude and has a stronger streaming effect in solution, which will lead to a more efficient molecular concentration effect. As shown in Figure 4F, after incubation and actuation of the resonator for 15 min, the resulting fluorescence intensity for the resonator with Q = 41 is 3.7 times larger than that for the resonator with Q = 33.

**Optoelectronic Bioassay System.** A key advantage of the resonator-induced enhancement is that it can concentrate biomolecules at a 3D collection zone in an open space, which can directly benefit any type of surface-based biosensor by locating the transducer at the virtual micropocket. In such a case, the resonator is used as an active fluid delivery and molecular manipulation component. Such a combination will achieve a real universal biomolecular concentrator and sensing system.

To explore this assumption, we developed an optoelectronic bioassay system by integrating resonator actuation into a biolayer interferometry (BLI) biosensor for protein binding analysis (Supporting Information, Figure S5). The BLI is a label-free technique for measuring biomolecular interactions using a fiber-optic probe approach. Any changes in the number of molecules bound to the probe surface would induce a wavelength shift in the interference pattern between the incident and reflected light. Thus, it can provide real-time measurement for biomolecular surface absorptions. Our optoelectronic bioassay platform uses a resonator as an actuator to provide analyte accumulations at the BLI optical probe interface, thus enhancing the amount of the surface-absorbed molecules.

The BLI optical probe was functionalized with PLL–PEG–biotin; then, the resonator was integrated into the BLI system by locating the device directly below the optical probe. Different concentrations of SAV were applied into the system. Figure 5Ai shows the results of SAV bindings in HEPES buffer with and without resonator actuations. After a power of 4 mW is applied, the LOD of SAV detection extends to 50 fM, which is 1000-fold lower than the results without resonator enhancement (50 pM). The improvement of LOD proves the overcoming of the surface limitation due to the accumulation of SAV molecules around the optical probe surface. Binding enhancement factor, $B_B$, which is defined as the ratio of response with resonator to response without resonator, is calculated as well. As shown in the inset of Figure 5Aii, $B_B$ is as high as 145 at the concentration of 50 pM while it gradually decreases to 1.6 at the concentration of 5 nM. The decrease of $B_B$ with the increasing SAV concentration results from the depletion of limited binding sites (biotin) at the absorption surface which indicates the same saturation response for the two measurements process. In other words, the binding enhancement under the actuation of the resonator is more prominent for biomolecular interactions at extremely diluted conditions. We believe this method would be useful not only in clinic diagnosis but also in the affinity measurement of biomolecular interactions such as for drug screening where the protein binding is usually applied in buffer conditions.

The resonator was also used to concentrate PSA molecules during the PSA measurement. The BLI optical probe was functionalized with anti-PSA through a similar approach as shown in Figure 4A (Supporting Information, Figure S6). Figure 5B shows the results of PSA bindings in HEPES buffer with and without resonator actuations. After a power of 4 mW is applied, the LOD of PSA detection extends to 50 pM, which is 200-fold lower than the results without resonator enhancement (10 nM). As shown in the inset of Figure 5B, $B_B$ is 93 at the concentration of 10 nM while it decreases to 8 at the concentration of 50 nM. The results are consistent with the response for SAV measurement.

To further demonstrate the truly meaningful sensing enhancement of this optoelectronic bioassay system, we also conducted the protein detection in serum where background signals from nonspecific binding are considered to be a non-negligible factor to the sensing results. Alternatively, the PSA binding pairs are replaced by IgG binding pairs to prove the universality of the optoelectronic bioassay system for a diverse range of biomolecules. Figure 5C shows the measurement results of IgG in serum with and without resonator actuations. As revealed, the LOD of IgG measurement extends to 2 nM, which is 10-fold lower than the results without the resonator-induced concentration effect (20 nM), and $B_B$ reaches 20.6 at the concentration of 20 nM while it decreases to 9.6 at the concentration of 200 nM. The optoelectronic bioassay does not show very high sensitivity in serum since the hydrodynamic trapping is not selective, and other proteins or molecules will be concentrated by the acoustic devices as well. Thus, the nonspecific bindings will be strongly influenced their performance in serum. Further studies are definitely required to improve the binding enhancement in serum or other complicated conditions where clinical diagnoses are usually run. One solution could be using a prefiltration chip, where specific probe-functionalized micropillars or nanoparticles could be used to purify the serum samples.

The successfully demonstrated optoelectronic bioassays prove the practical feasibility of the integration of the device with other biosensing techniques, which is attributed to their merit of open-space trapping. Meanwhile, the results also indicate that the hydrodynamic trapping of biomolecules using the NEMS resonator is a noninvasive approach without denaturing their bioactivities, which is rather important to develop an enhanced biosensing platform.
CONCLUSION

Overall, we have demonstrated a universal approach to enhance biomolecular surface binding for biosensing applications in the open space with an acoustic NEMS resonator, which is featured by the concentration factor of 10^5. This approach bridges a major gap between signal transduction technology and the fluidic system in the investigation of biomolecular interactions. It offers several competitive advantages: First, it is a noninvasive and biocompatible approach which works for a diverse range of biomolecules, regardless of their physical and chemical properties. Second, the concentration process is highly efficient, requiring only a few minutes, which is beneficial for rapid biomarker detections. Third, the trapping of biomolecules in open space without microfluidic channels allows the combination of the device with many surface-based biosensing techniques (e.g., surface plasma resonance, quartz crystal microbalance, electrochemistry, and ELISA, etc.) to achieve a real universal biomolecular concentrator and sensing system. Fourthly, the concentration process is achieved using a simple, miniaturized, low-cost, CMOS-compatible device; thus, it can be readily applied to an established system for biomolecule analysis. Given the above advantages, our approach is valuable in the field of biomedical engineering such as molecular diagnostics and drug discoveries.

METHODS

Synthesis of PLL−PEG−Biotin. PLL was dissolved in 50 mM sodium carbonate buffer (pH = 8.5) at a concentration of 40 mg/mL. The solution was then filtered through a 220 nm pore syringe filter. NHS-PEG−biotin was added to the dissolved PLL solution under vigorous stirring. The reaction was allowed to proceed for 5 h under room temperature, followed by the dialysis against PBS at pH = 7.4 and deionized water for 24 h using a centrifugal filter device (molecular weight cutoff 8 kDa). The dialyzed solution was lyophilized for 24 h and stored in a −25 °C freezer.

Device Fabrication. The acoustic NEMS resonator was fabricated using a CMOS-compatible process (Supporting Information, Figure S1). It was started by etching an air cavity on silicon substrate by reactive ion etching, followed by deposition of phosphosilicate glass (PSG) using chemical vapor deposition (CVD). After that, the surface was planarized using chemical mechanical polish (CMP). Then, 200 nm Mo film was deposited and patterned as the bottom electrode. After that, T = 450 nm AIN film was employed as piezoelectric layer by RF reactive magnetron sputtering. Next, 200 nm Mo film was deposited and patterned as the top electrode. Then, AIN was etched by a combination of Cl₂-based plasma etching and potassium hydroxide wet etching. After the AIN etch, Au was then evaporated and patterned by lift-off, serving as electrical connection and pads. Finally, the silicon wafer was immersed in diluted hydrofluoric acid solution to release PSG in the cavity. Here, the top electrode was patterned with the same shape of the bottom electrode to form IDT. As a consequence, a 350 MHz resonator, with an aperture p = 15 μm, width w = 10 μm, length l = 150 μm, and number n = 12 IDT fingers was fabricated. The resonance characteristic is presented by Smith chart in air and liquid (Supporting Information, Figure S2). When working in solution, mechanical resonance generated from the resonator is partially coupled into liquid that can be seen by the shrink of Smith chart which is an indicator of energy losses in the system.

Flow Profile Quantification. We tracked the movement of 5 μm PS particles from which the flow profile and velocity are quantitatively analyzed. With the assumption that PS particles are small enough to have no significant effects on the flow profile, they are simply dragged along with behaviors similar to the flow profile. PS particles were tracked using a video camera (Olympus DP73, Tokyo, Japan) attached to an optical microscope (Olympus BX53, Tokyo, Japan), and their movements were analyzed with commercially available software, Diatrack 3.04.

Holographic Microscopy. Reflection DIPHM was utilized to obtain the 3D surface profile of analytes during the concentration (Supporting Information, Figure S3). The illumination source was a tunable diode laser at λ = 690 nm (Nanobase, Xperay-TL-STD, 639−697 nm) which was split into the object beam and the reference beam. The expanded object beam illuminated and reflected from the resonator surface to create the object wavefront, which interfered with the reference wavefront and formed the surface profile in 3D perspective images. Here, the resonator was used to concentrate 10 μg/mL SAV in 10 mM HEPES buffer with a power of 1 mW. The 3D profile was extracted from the phase shift between real-time images and initial images, which gave quantifiable information about the optical thickness of concentrated analytes.

Protein Concentration. The resonator was exposed to air plasma for 5 min to form a clean and negatively charged surface, and then immersed in the aqueous solution of PLL−PEG−biotin (1 mg/mL) at room temperature for 30 min, followed by rinsing with HEPES buffer (10 mM). SAV (200 nM) was then attached onto PLL−PEG−biotin linker via biotin−SAV binding for 30 min. Afterward, 100 μg/mL biotin−labeled antihuman IgGs were immobilized. After surface functionalization, the chip is wire-bound to a chip holder, and a PDMS channel is mounted on top of the device to facilitate the protein sensing experiments. All the analytes used in the experiments were dissolved in HEPES (pH = 7.4) buffer. Fluorescence intensity was recorded by fluorescence microscopy (Olympus BX53).

Measurement of PSA/IgG Using Optoelectronic Bioassay Platform. The resonator was integrated into an optoelectronic bioassay platform (Supporting Information, Figure S5). The fiber optic probe was first immersed in piranha solution for cleaning and generation of the negatively charged hydroxyl group. Then, 1 mg/mL PLL−PEG−biotin, 200 nM SAV, and 200 μg/mL biotin−labeled anti-PSA/anti-IgG were immobilized sequentially on the probe surface by immersion in their solution for 15 min (Supporting Information, Figure S6). After that, the fiber optic probe was precisely positioned around the stagnation point by a positioning stage, and a continuous set of PSA/IgG samples dissolved in buffer/serum were introduced.

Caution. Piranha solution reacts violently with organic solvents and should be handled with great care. For more information, please see http://cenblog.org/the-safety-zone/2015/01piranha-solution-explosions/.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssen-cs.8b00301.
Additional theoretical analysis, figures, materials, fabrication procedures, characterization data, and experimental results (PDF)

Movie S1: movement of 1 μm PS particles under the actuation of the acoustic NEMS resonator with a power of 4 mW (AVI)

Movie S2: movement of fluorescence 5 μm PS particles under the actuation of the acoustic NEMS resonator with a power of 4 mW (AVI)

Movie S3: movement of 5 μm PS particles under the actuation of the acoustic NEMS resonator with a power of 0.01 mW (AVI)

Movie S4: concentration of FITC-SAV molecules under the actuation of the acoustic NEMS resonator with a power of 4 mW (AVI)

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Notes
The authors declare no competing financial interest.

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