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Microcantilever-Based Label-Free Characterization of Temperature-Dependent Biomolecular Affinity Binding

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Abstract

This paper presents label-free characterization of temperature-dependent biomolecular affinity binding on solid surfaces using a microcantilever-based device. The device consists of a Parylene cantilever one side of which is coated with a gold film and functionalized with molecules as an affinity receptor to a target analyte. The cantilever is located in a poly(dimethylsiloxane) (PDMS) microfluidic chamber that is integrated with a transparent indium tin oxide (ITO) resistive temperature sensor on the underlying substrate. The ITO sensor allows for real-time measurements of the chamber temperature, as well as unobstructed optical access for reflection-based optical detection of the cantilever deflection. To test the temperature-dependent binding between the target and receptor, the temperature of the chamber is maintained at a constant setpoint, while a solution of unlabeled analyte molecules is continuously infused through the chamber. The measured cantilever deflection is used to determine the target-receptor binding characteristics. We demonstrate label-free characterization of temperature-dependent binding kinetics of the platelet-derived growth factor (PDGF) protein with an aptamer receptor. Affinity binding properties including the association and dissociation rate constants as well as equilibrium dissociation constant are obtained, and shown to exhibit significant dependencies on temperature.

Keywords

aptamer; affinity binding; microcantilever; label-free detection; temperature dependence

1. INTRODUCTION

Biomolecular affinity binding is of fundamental importance for a wide variety of biological processes in that conformational and chemical complementarity between the binding molecules strongly influences cellular signal transduction and expression [1]. Modern drug development and therapeutics have thus increasingly relied on biomolecular binding studies [2]; current experimental and computational screening of compounds for therapeutic drugs is often based on interrogation of ligand-receptor binding affinity [3]. Since the binding affinity commonly involves thermodynamic activity and is determined by the Gibbs energy during the biomolecular interaction [3], consideration of temperature effects is crucial in

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therapeutic ligand design. In addition, as *in-vivo* relevant therapeutic assays have become increasingly important, insight into the temperature-dependent nature of biomolecular affinity binding [4, 5] can be critical for understanding the mechanisms governing these interactions, such as the efficacy of drug molecules under thermally active stimulation [6]. Moreover, knowledge about the temperature dependence of ligand-receptor systems can assist in identifying therapeutic targets by exploring receptor dysfunction associated with metastatic cell proliferation, or receptor recovery leading to tissue repair [7]. One clinically significant example is the development of inhibitory ligands for platelet-derived growth factor (PDGF), a protein regarded as an ubiquitous mitogen and chemotactic factor [8] in angiogenesis. If the inhibitory ligands to PDGF are used *in vivo*, it is crucial to understand the temperature-dependence underlying PDGF-ligand interaction. Therefore, there is a strong need for a technique that can reliably characterize the effects of temperature on biomolecular affinity binding.

Affinity binding can occur with target and receptor molecules in solution, or with either molecule immobilized to solid surfaces. Solution-based affinity binding is commonly characterized using methods such as UV-absorption [8], differential and titration calorimetry [9], matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [10] and electrophoretic separation [11]. Surface-based affinity binding, which is widely used in affinity biosensors [12], can be investigated with methods such as protein arrays [13], immunoassays [14], and thermal-shift assays [15], which use fluorescent or radioactive labeling groups to signal binding events. Such labeling of target or receptor molecules is in general time-consuming and labor intensive, and is not capable of distinguishing signals from analytes in inactive and active forms [16]. More importantly, when used for temperature-dependent studies, fluorescent or radioactive labeling compounds can be temperature-dependent in their own right, or interfere with the binding under investigation.

Label-free methods, such as surface plasmon resonance (SPR) [17] and quartz crystal microbalance (QCM) [18], have also been used for characterization of affinity binding on solid surfaces. More recently, there has been increasing use of microcantilevers to study surface-based affinity binding [19], offering a simple, low-cost platform capable of highly sensitive, real-time detection of biomolecular interactions and conformational changes [20, 21]. Microcantilevers have been applied to studies of membrane protein-ligand [22], antibody-antigen [23], receptor-ligand [24], protein-protein [25], and protein-DNA [26] systems.

However, there has been a lack of studies that adequately characterize temperature-dependent thermodynamic properties of the binding interaction [27]. Currently, microcantilever-based characterization of biomolecular interaction at controlled temperatures is scarce, while the few existing examples [28] offer limited interpretation of affinity binding kinetics at relatively low temperature resolutions. This need can be potentially addressed by leveraging temperature-control microdevices. Conventional schemes, such as heating the entire physical space in which the devices are located [29] or placing devices on external temperature-controlled modules [30], are unsuitable for real-time biomolecular characterization as they typically involve heating of large thermal masses, inaccurate temperature sensing, and slow thermal response. Microfabricated thin-film temperature control elements integrated within microfluidic devices allow for efficient temperature-dependent investigations [31]; however, their application for characterizing biomolecular affinity binding has yet to be fully explored.

This paper presents label-free characterization of temperature-dependent biomolecular affinity binding on solid surfaces, using a microfluidic device integrating sensitive polymer cantilevers and on-chip temperature sensing. With *in-situ* temperature sensing enabled by an

integrated temperature sensor with unobstructed optical access, the device enables the elucidation of kinetic and equilibrium binding properties at well-controlled temperatures, and can be potentially extended to study transient thermal effects on biomolecular binding. Using the device, we demonstrate a label-free study of temperature-dependent affinity binding between PDGF and a complementary aptamer. Quantitative binding properties are obtained as a function of temperature, including the association and dissociation rate-constants (k_{on} and k_{off}) as well as equilibrium dissociation constant (K_d). These results offer previously unavailable insight into the temperature-dependence of the aptamer-PDGF affinity system.

2. MATERIALS AND METHOD

2.1. Temperature-Dependent Affinity Binding on a Microcantilever

Microcantilevers can transduce surface-based biochemical interactions into mechanical signals. In particular, when biomolecules immobilized on the cantilever surface undergo intermolecular reactions, their conformational change causes a change in the surface stress on the cantilever due to reduction decrease in free energy [28]. The deflection of the cantilever induced by the surface stress change provides a direct measure of the biomolecular interaction. For instance, the steady-state cantilever deflection can indicate the fraction of immobilized biomolecules that have bound to free analytes in solution, while the time to reach steady state reflects the rate constant of the specific binding. When the microcantilever is integrated with microheaters and temperature sensors, temperature-dependent properties of biomolecular interactions can be investigated at well-defined temperatures.

We exploit this concept and employ a cantilever-based microfluidic device (below) to investigate temperature-dependent ligand-receptor binding, using PDGF and a PDGF-specific aptamer as an example affinity system for demonstration. Aptamers are a class of synthetically developed ribonucleic acids (RNA) or deoxyribonucleic acids (DNA) that bind to specific target analytes with high specificity [1, 6]. This PDGF-specific aptamer is a 36t ligand containing a three-way helix junction secondary structure motif [8] and synthesized with a 3'-3'-linked thymidine nucleotide (designated by [3'T]) at the 3'-end and a thiolated 5'-end for attachment to gold surface via thiol chemistry (Figure 1). The aptamer-PDGF interaction is enabled by continuously introducing PDGF solution to the immobilized aptamer. Upon binding, conformational changes in the nucleic acid structure of the aptamer molecules subsequently impart a uniform surface stress on the cantilever, which causes microcantilever deflection (Figure 2). Thus, measurement of this deflection, e.g., using an optical method in which a light beam reflects off the cantilever, can be interpreted to reveal kinetic properties within the binding between aptamer and PDGF.

2.2. Device Design, Fabrication and Testing Setup

Our approach is based on a cantilever-based microfluidic chip coupled to a Peltier thermoelectric module (Figure 3). The chip mainly consists of three functional layers: a silicon chip featuring the Parylene microcantilever, a poly(dimethylsiloxane) (PDMS) spacer layer defining the microfluidic chamber, and a glass plate with an indium tin oxide (ITO) temperature sensor. The inlet and outlet tubings are embedded in the PDMS spacer layer to allow continuous flow during experiments. Temperature is controlled in closed loop by adjusting the voltage applied to the Peltier module (which allows both heating and cooling by thermoelectric effects [32]) according to the feedback from the *in-situ* ITO temperature sensor. Transparent ITO patterned glass is chosen for unobstructed optical detection of microcantilever deflection. Parylene is employed as a device material to exploit

benefits such as higher potential detection sensitivity due to its low Young's modulus and excellent chemical inertness.

The fabrication of the microcantilever chip began on an oxide-precoated silicon wafer. Multiple cantilevers were included in the chamber for redundancy. First, anchor cavities surrounding the cantilevers were defined and etched by KOH to a depth of approximately 20 μm (Figure 4a). Then the pits underneath the cantilevers were defined, followed by uniformly coating a 6- μm thick Parylene film via chemical vapor deposition (CVD) (Figure 4b). Cantilevers were then patterned by oxygen-plasma reactive ionic etching (RIE), and coated with a Cr/Au (5/45 nm) thin film via thermally evaporation and wet etching (Figure 4c). Subsequently, the pits defined above were completed by XeF_2 gas-phase etching to fully release the cantilevers (Figure 4d). In parallel, the ITO temperature sensor was patterned on an ITO-coated glass slide (Delta Technologies, CB-50IN) by wet etching using 25% HCl (Figure 4e) and passivated by a 1.5- μm thick SU-8 photoresist layer (Figure 4f). Also, a 250- μm thick PDMS spacer layer defining the microfluidic chamber was fabricated using a replica molding technique [33] (Figure 4g). Finally, the microcantilever chip, the PDMS spacer layer, and the ITO glass slide were bonded together in sequence with the interfaces treated with oxygen plasma and reinforced by gluing (Figure 4h). The inlet and outlet solution lines were coupled to the device by capillary tubings inserted into the PDMS spacer layer. The resulting chip package was attached to a Peltier module (Melcor) using a thermally conductive glue (Omega Engineering). Figure 5a shows a packaged device with 17 microcantilevers in a circular chamber and Figure 5b the micrograph of a representative single cantilever.

As shown in Figure 6, the experimental setup mainly consisted of electrical instruments for temperature control, and an optical lever for optical detection. The temperature inside the reaction chamber of the chip was maintained at a desired setpoint using the Peltier thermoelectric module driven by a DC power supply (Agilent E3631) and the ITO temperature sensor measured by a digital multimeter (Agilent 34410A), regulated under closed-loop proportional-integral-derivative (PID) control algorithm. During experiments, the two inlets were connected to two parallel solution lines driven by syringe pumps (New Era Pump Systems NE-1000) which allowed convenient switch between two different injected solutions. For optical detection, a home-built optical lever system was used, in which a laser beam from a diode laser generator was directed to the gold surface of a cantilever and reflected to a photosensitive detector (PSD, Coherent). The signal was amplified by a PSD amplifier (Photonics OT-301) and then measured by a nanovoltmeter (Agilent 34420A). The on-chip temperature control and optical signal acquisition were computer-automated and monitored by a LabVIEW-based program.

2.3. Materials and Experimental Procedure

An isoform of PDGF, PDGF-BB (Sigma Aldrich), was chosen as a model analyte in our experiments. PDGF-BB was prepared in PBSM buffer (10.1 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 137 mM NaCl, 2.7 mM KCl, and 1 mM MgCl_2 , pH 7.4). The PDGF-specific aptamer was obtained from IDT DNA and prepared in sterile water (Fisher Scientific). All solutions were degassed prior to introduction into the microfluidic chambers to avoid inducing air bubbles. Meanwhile, the microcantilever device was pre-cleaned with acetone, ethanol, and sterile water sequentially. To immobilize aptamer molecules on the cantilever surface, 3 μM aptamer solution was allowed to perfuse through the reaction chamber overnight at a constant flow rate of 0.5 $\mu\text{L}/\text{min}$ at 4 $^\circ\text{C}$. During the aptamer-PDGF association and dissociation experiments, PBSM buffer and 5 nM PDGF solutions respectively perfused through the reaction chamber via the device's two microfluidic inlets as follows. The chamber was initially flushed with PBSM buffer and then infused with PDGF solution, initiating the association between PDGF and the aptamer as detected via the

cantilever deflection. After the association reached equilibrium, the infusion was switched back to PBSM buffer to enable the dissociation process. Throughout the experiments, the flow rate of both PBSM buffer and PDGF solution was maintained at 10 $\mu\text{L}/\text{min}$, which was chosen to allow an acceptable mass transport rate at which PDGF molecules were accessed by the aptamer-functionalized surface [34], while limiting the flow-induced shear force on the molecules that could affect the binding activity [35]. After the measurement, the system was regenerated by removing any PDGF molecules that remained on the surface by 4 M urea and 15 mM EDTA and then rinsing with PBSM buffer. The baseline in response signal, i.e., the PSD output of the optical detection system with no occurrence of the aptamer-PDGF affinity binding, was measured with PBSM buffer alone flowing through the microchamber.

2.4. Monovalent Binding Kinetic Model

We consider a monovalent model for the equilibrium affinity binding between the immobilized receptor (of concentration $[R]$) and the target analyte (of concentration $[A]$) to form a complex (of concentration $[RA]$) [36]:

where k_{on} and k_{off} are the association and dissociation rate constants, respectively. The net rate of complex formation varies with time according to the following differential equation:

$$\frac{dy}{dt} = k_{\text{on}}[A](y_{\text{max}} - y) - k_{\text{off}}y \quad (2)$$

where y and y_{max} respectively represent the observed response signals respectively corresponding to the complex concentration $[RA]$ and saturation complex concentration $[RA]_{\text{max}}$ (i.e., the asymptotic value of $[RA]$ at infinite time) for a given concentration of injected analyte $[A]$.

In flow-through mode as used in the experiments, either the target analyte solution (for association) or pure buffer (for dissociation) is introduced continuously to the cantilever. At a sufficiently large flow rate, the analyte concentration $[A]$ can be assumed to be a constant c in the association process, or 0 in the dissociation process [36]. Eq. (2) thus reduces to the following equations, respectively, for the association and dissociation processes:

$$\frac{dy}{dt} = k_{\text{on}}c(y_{\text{max}} - y) - k_{\text{off}}y \quad (3)$$

$$\frac{dy}{dt} = -k_{\text{off}}y \quad (4)$$

Solving Eq. (3) (using initial condition $y = 0$ at $t = 0$) yields the time-dependent association response signal:

$$y = y_{\text{max}} \frac{c}{K_d + c} (1 - e^{-(k_{\text{on}}c + k_{\text{off}})t}) \quad (5)$$

where $K_d = \frac{k_{\text{off}}}{k_{\text{on}}}$ is the equilibrium dissociation constant.

For the dissociation process, solving a Eq. (4) yields

$$y = y_0 e^{-k_{\text{off}} t} \quad (6)$$

where y_0 is the initial signal at the beginning of the dissociation process ($t = 0$). Using Eqs. (5) and (6), the kinetic and equilibrium binding constants (k_{on} , k_{off} , and K_d) can be obtained from the time-resolved measurement signal y .

3. RESULTS AND DISCUSSION

3.1. Aptamer-PDGF binding and selectivity to PDGF

We first validated the specificity of PDGF binding in our device with three sets of control experiments. These include (1) introduction of PDGF solution into the reaction chamber with an aptamer-free cantilever surface, followed by PBSM buffer; (2) introduction of only PBSM buffer into the reaction chamber of an aptamer-functionalized cantilever; and (3) introduction of a lysozyme (egg white, Sigma Aldrich) solution to an aptamer-functionalized cantilever, followed by PBSM buffer. Here lysozyme, which has comparable molecular weight (14.3 kDa) to PDGF (25 kDa), was used as a non-binding protein for testing the aptamer specificity.

Figure 7 shows the signal traces of the control experiments, combining the phases of sample injection and PBSM buffer injection, compared with a representative trace of aptamer-PDGF binding undergoing the same phases. Throughout the experiments, this device showed a noise level of less than ± 2 mV and repeatability within 10%. In the absence of either aptamer or PDGF, no signal above noise level was detected upon the presence of biomolecules or buffer solution. More importantly, for aptamer-PDGF binding, there existed an exponential increase to a binding equilibrium corresponding to the introduction of PDGF molecules, and a relatively slow shift back to the original equilibrium upon PBSM buffer injection. Thus, the non-specific binding of PDGF to either surface of the cantilever was generally negligible compared with the affinity binding between aptamer and PDGF.

3.2. Characterization of temperature-dependent aptamer-PDGF binding

Temperature-dependent characterization of the aptamer-PDGF binding was performed by monitoring the association and dissociation processes at temperature varying from 19–37 °C. This temperature range is considered suitable for characterization of aptamer-PDGF interactions since aptamer-based therapeutics typically demand a physiologically relevant temperature up to 37 °C. With our experimental setup, the temperature inside the chamber, indicated by the ITO temperature sensor, was consistently controlled within ± 0.3 °C at setpoints in the range of 19–37 °C. The experimental data were then fitted to the monovalent binding kinetic model given in Eqs. (5) and (6) to obtain the temperature-dependent kinetic properties of the rate constants for association (k_{on}) and dissociation (k_{off}), and the equilibrium dissociation constant (K_d). For each temperature setpoint, we used the GraphPad Prism software [37] to fit the experimental data on the combined dissociation and association processes in a manner that ensures the baseline consistency.

Figure 8 shows the experimental signal (baseline subtracted) of aptamer-PDGF association and dissociation processes at controlled temperature setpoints of 19, 25, 31, and 37 °C, as well as the fitted curves to the monovalent binding kinetic model. These data showed a clear shift with temperature, and thus considerable temperature dependence within the aptamer-PDGF interaction. In particular, as the temperature increased from 19 to 37 °C, the characteristic time for the association process to reach equilibrium decreased from approximately 30 to 15 minutes, indicating that the rate of the aptamer-PDGF association process increased with temperature. In addition, the steady-state deflection of the cantilever

in equilibrium aptamer-PDGF binding also increased with temperature (Figure 8). This indicated a more significant surface stress change on the cantilever, which was primarily caused by a larger fraction of immobilized aptamer molecules that are bound to PDGF molecules. These results suggest that temperatures in the physiological range present an optimal condition for the binding of PDGF to aptamer, which is determined by a combination of aptamer tertiary conformation, molecular orientation, and binding energy, as well as the effect of generally increased Brownian motion at higher temperature.

There was a small apparent overshoot in the detected signal before the association process reached equilibrium (Figure 8). This could be attributed to the variation in the flow rates during the introduction of PBSM buffer and PDGF solution to the microchamber, which most likely were caused by the difference in the syringe pumps and access channels between PBSM buffer and PDGF solution injections. As the association process was triggered by switching the introduction of PBSM buffer to that of PDGF solution, the variation in flow rate induced a difference in the hydrodynamic force on the cantilever and in turn a slight overshoot in cantilever deflection. However, this did not significantly influence the values of binding parameters obtained from the model (Eqs. (5) and (6)) to the experimental data.

In the flow-through experiments, the mass transport of biomolecules occurred by convection and diffusion. To assess this effect on the aptamer-PDGF binding in our device, we first estimated the characteristic time for PDGF molecules accessed by the cantilever surface. The rate of molecular transport to the surface is given by the Onsager coefficient of mass transport [34]:

$$k_m \approx \sqrt[3]{\frac{D^2 u}{h^2 b l}} \quad (7)$$

where D is the diffusivity of sample biomolecules, u is the flow rate for sample introduction, h is the height of the reaction chamber, and b and l are the width and length of the cantilever. For the experimental data above, $h = 250 \mu\text{m}$, $b = 150 \mu\text{m}$, $l = 250 \mu\text{m}$, and $D \approx 10^{-10} \text{ m}^2/\text{s}$ [38]. Using $u = 10 \mu\text{L}/\text{min}$, $k_m = 5.4 \times 10^{-4} \text{ m}/\text{min}$, and the time scale for the analyte diffusion was estimated to be $h/k_m = 0.46 \text{ min}$. Compared with the apparent time scale of aptamer-PDGF association process (approximately 15–30 minutes), it is reasonable to assume that the aptamer-PDGF binding process was not limited by mass transport at this flow rate [34].

We further determined the kinetic and equilibrium binding properties by fitting the monovalent binding model (Eqs. (5) and (6)) to the experimental data. The temperature dependent kinetic binding rate constants are shown in Figure 9. As the temperature increased from 19 to 37 °C, k_{on} increased from 1.3×10^7 to $2.3 \times 10^7 (\text{M} \cdot \text{min})^{-1}$, while k_{off} decreased from 0.015 to 0.01 min^{-1} . Meanwhile, K_d , as shown in Figure 10, decreased from approximately $12 \times 10^{-10} \text{ M}$ to $5 \times 10^{-10} \text{ M}$ as the temperature changed from 19 to 37 °C. This indicates stronger aptamer-PDGF binding, i.e., a more favorable conformational change of the aptamer molecules for the affinity interaction as the temperature approached the physiological values, at which the aptamer was synthetically isolated [8]. Moreover, the decrease of K_d with temperature implies that the aptamer-PDGF system becomes more thermodynamically stable as the temperature increases, which typically involves negative Gibbs free energy [3]. These results are consistent with published data using conventional methods (e.g., UV-absorption [8]). The K_d values we obtained are higher than those obtained with the aptamer in solution (typically $\sim 10^{-10} \text{ M}$), which is likely attributable to two reasons. First, the surface-immobilized receptor has restricted conformational flexibility for the analyte to access the entire binding sites [39], and thus may not retain its full solution-based activity [40]. In addition, avidity, in which the binding of analyte molecules

with receptor molecules is synergistically stabilized by entropic effects [36], is known to have a noticeable contribution to high-affinity binding systems [41]. In our experiments, the presence of the solid surface may have led to reduced avidity effects because of less efficient diffusion [42] and limited clustering of analyte and receptor molecules [36].

4. CONCLUSION

This paper presents label-free characterization of temperature-dependent biomolecular affinity binding on solid surfaces using a microcantilever-based device integrating on-chip temperature sensing. The device consists of a Parylene cantilever one side of which is coated with a thin gold film and functionalized with molecules of an affinity receptor to a target analyte. The cantilever is located in a PDMS microfluidic chamber which is integrated with a transparent ITO thin-film resistive temperature sensor on a glass slide. The ITO sensor allows for real-time measurements of the temperature inside the chamber with unobstructed optical access for reflection-based optical detection of the cantilever deflection. The device is situated on a Peltier thermoelectric module, which, in conjunction with the integrated ITO sensor, is used to control the chamber temperature based on a closed-loop PID algorithm. To test the temperature-dependent binding between the target and receptor, the temperature of the chamber is maintained at a constant setpoint, while the analyte solution is continuously infused through the chamber. The measured cantilever deflection is used to determine the thermodynamic properties associated with the target-receptor binding according to a monovalent binding kinetic model.

We studied the temperature-dependent affinity binding between PDGF, a protein regarded as an ubiquitous mitogen and chemotactic factor in angiogenesis, and an affinity aptamer. We first verified the detection specificity using this device and then systematically characterized the aptamer-PDGF association and dissociation processes with the chamber temperature controlled in the range of 19–37 °C. Quantitative binding properties were obtained, indicating strong temperature dependence of the binding of PDGF to the aptamer. As the temperature increased from 19 to 37 °C, the association rate constant increased from 1.3×10^7 to 2.3×10^7 (M·min)⁻¹, while dissociation rate constant decreased from 0.015 to 0.01 min⁻¹. This corresponds to a decrease of the equilibrium dissociation constant from approximately 12×10^{-10} M to 5×10^{-10} M. These results provide a starting point for label-free characterization of temperature-dependent biomolecular interactions, and can potentially be used for the screening and optimization of inhibiting ligands of PDGF and other target molecules.

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Figure 1.
The nucleic acid structure of the PDGF-specific aptamer.

Figure 2.
Principle of the microcantilever-based detection of aptamer-PDGF binding.

Figure 3. Design schematic of the cantilever-based microfluidic device for characterization of temperature-dependent aptamer-PDGF binding.

(a) Define and wet etch cavities for cantilever anchoring.

(b) Define the pit underneath the cantilever and coat a uniform Parylene film.

(c) Pattern the cantilever using RIE and coat it with a thin Cr/Au layer.

(d) Release the cantilever by XeF_2 etching.

(e) Pattern the temperature sensor on ITO glass using wet etching.

(f) Passivate with a thin SU-8 layer.

(g) Fabricate PDMS spacer layer.

(h) Bond cantilever chip, ITO glass, and PDMS spacer.

Figure 4.
Fabrication process of the microcantilever device.

Figure 5. Fabricated microcantilever device. (a) Image of a packaged device. (b) Micrograph of a representative single cantilever.

Figure 6. Experimental setup for the temperature control, optical detection and signal acquisition in the cantilever-based microfluidic device.

Figure 7. Binding specificity demonstrated by association and dissociation signal traces of control experiments in the absence of either aptamer or PDGF, compared with a representative aptamer-PDGF binding trace (all traces intentionally plotted with an offset of 5 mV for clarity).

Figure 8.

The experimental data of association (left) and dissociation (right) processes at 19, 25, 31, and 37 °C and the fitted curves to the monovalent binding kinetic model.

Figure 9.

Association rate constant (k_{on}) and dissociation rate constant (k_{off}) at controlled temperatures of 19, 25, 31, and 37 °C.

Figure 10.
Equilibrium dissociation constant (K_d) at controlled temperatures of 19, 25, 31, and 37 $^{\circ}C$.