Electronic Supplementary Information

Multiplexed Evaluation of Capture Agent Binding Kinetics Using Arrays of Silicon Photonic Microring Resonators

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Experimental Details

Materials

3-N-((6-(N'-Isopropylidene-hydrazino))nicotinamide)propyltriethyoxysilane (HyNic silane) and succinimidyl 4-formylbenzoate (S-4FB) were purchased from SoluLink (San Diego, CA). Aniline was obtained from Acros Organics (Geel, Belgium), and proteinase K was purchased from USB (Cleveland, OH). Mouse monoclonal anti-(human thrombin) (Cat. # AHT-5020) and human α-thrombin (Cat. # HCT-0020) were purchased from Haematologic Technologies Inc. (Essex Junction, VT). HPLC purified thrombin-binding aptamer and a control DNA sequence were purchased from Integrated DNA Technology (Coralville, IA). The full sequences are as follows.

Thrombin-binding aptamer: 5′–NH_2–$(CH_2)_{12}$–TTTTTGGTTGGTGTGGTTGG-3′

Control sequence: 5′–NH_2–$(CH_2)_{12}$–GGTAGTACAGCATATTCGAAAGTGTATAA GATT-3′

Vivaspin 500 columns (5 kDa MW cutoff) were purchased from Sartorius (Aubugne, France). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and used as received.

All buffers were made with purified water (ELGA PURELAB filtration system; Lane End, UK), and the pH was adjusted with 1 M HCl or 1 M NaOH. PBS was reconstituted from Dulbecco's phosphate buffered saline packets and adjusted to either pH 6.0 or 7.4. Glycine buffer consists of 10 mM glycine and 160 mM NaCl adjusted to pH 2.2. A blocking solution containing 2 % (w/v) BSA was prepared by dissolving bovine serum albumin in PBS (pH 7.4) and it was degassed under vacuum before being flowed across the sensor surface. Tween-PBS buffer was prepared by adding 0.05% (v/v) Tween 20 in PBS (pH 7.4). Tris buffer consists of 50 mM Tris-HCl, 100 mM KCl, and 5 mM $CaCl_2$ adjusted to pH 7.4, and proteinase K (0.1 mg/ml) solution was prepared using Tris buffer.

Sensor Substrates and Instrumentation

Microring resonator sensor chips and the instrumentation for resonance wavelength determination were acquired from Genalyte, Inc. (San Diego, CA) and have been described in detail previously.[1, 2] In brief, the 6 x 6 mm chips have an array of 32 individually addressable microrings with adjacent waveguides, and a tunable beam from a diode laser with a center

frequency of 1560 nm is coupled into the waveguides via grating couplers. Resonance wavelengths, are determined as a minimum in output coupler intensity since light is strongly coupled into the microring under the condition:

$$m\lambda = 2\pi r n_{eff}$$

where *m* is an integer, λ is the wavelength of light, *r* is the radius of the cavity, and n_{eff} is the effective refractive index of the optical mode. Biomolecular binding events at the microring surface modulate n_{eff}, causing a shift to longer resonance wavelengths and providing the transduction modality. Sensor chips are loaded into a custom Teflon cell with microfluidic channels defined by a Mylar gasket (fabricated by RMS Laser; El Cajon, CA), which allow four separate solutions to be directed to different regions of the sensor array. Solutions are introduced to the chip at controlled flow rates via negative pressure applied by syringe pumps (11 Plus syringe pump, Harvard Apparatus; Holliston, MA).

Surface Functionalization and Capture Agent Attachment

Sensor chips were cleaned with piranha solution (3:1 H_2SO_4:30%H_2O_2) for 1 or 2 minutes to remove any organic contaminants prior to functionalization, followed by rinsing with copious amounts of distilled (DI) water and dried under nitrogen. Microring substrates were then loaded into the described custom cell with two-channel microfluidics defined by a Mylar gasket, and functionalized by exposure to 1 mg/mL HyNic silane solution in 95% ethanol and 5% DMF at 5 μL/min for 30 min, followed by rinsing with 100% ethanol. Then the chip was loaded into a four-channel Mylar gasket and a corresponding Teflon cell for multiplxed functionalization.

A solution of anti-(human thrombin) was buffer exchanged in PBS (pH 7.4) using Vivaspin 500 spin columns three times. A 5-fold molar excess of S-4FB solution (0.4 mg/mL in DMF) was added to each solution. After 3 hours of incubation, unreacted S-4FB was removed by buffer-exchanging into PBS (pH 6.0) using Vivaspin 500 spin columns. A conjugation solution was prepared with 50 μg/mL 4FB-modified antibody with 100 mM aniline as a nucleophilic catalyst. TBA and control sequences were also separately buffer exchanged in PBS (pH 7.4) as described above to remove any residual ammonium acetate present in the sample. A 10-fold molar excess of S-4FB solution was added to each solution and incubated overnight in PBS (pH 6.0). After removing unreacted S-4FB using Vivaspin 500 spin columns, conjugation solutions were prepared by diluting the DNA solutions to 300 μL (about 20-30 ng/μL).

Each solution of 4FB-modified thrombin-binding aptamer, control sequence, and antibody were introduced into each microfluidic channel and flowed over HyNic-modified microring resonators at a rate of 5 μL/min for 60 min, followed by rinsing with buffer; PBS (pH 6.0) for DNA sequences and PBS (pH 6.0) containing 100 mM aniline for antibody. Aniline was added to the antibody conjugation solution as a nucleophilic catalyst that improves the amount of covalently bound antibody.[3] To further remove any residual antibody, antibody channel was exposed to a low pH glycine buffer (pH = 2.2) for 2 min. After capture agent immobilization, the four-channel Mylar gasket and the Teflon cell were switched back to two-channel microfluidics. The remaining unmodified microrings and the antibody immobilized microring surfaces, which were in the same microfluidic channel at this point, were blocked with 2 % (w/v) BSA in PBS (pH 7.4) at least for two hours in order to reduce non-specific protein adsorption in subsequent antigen detection experiments.

Antigen Detection

A solution of human α-thrombin was buffer exchanged to PBS (pH 7.4) using Vivaspin 500 spin columns. Working solutions containing 0.5 nM, 2 nM, 5 nM, 10 nM, and 50 nM of human α-thrombin in Tween-PBS were prepared by successive dilution of a 1 μM stock solution in PBS (pH 7.4). These solutions were then flowed over the functionalized sensor chip at 30 μL/min for 10 min as antigen association was monitored, followed by a Tween-PBS rinse for 20 min to observe antigen dissociation. To disrupt the remaining antigen-capture agent interactions and regenerate the sensor surface, glycine buffer was introduced to the antibody channel for two minutes and 0.1 mg/ml proteinase K solution in Tris buffer was flowed over the aptamer immobilized microrings for an hour. A solution of elastase was prepared in the same way as human α-thrombin.

Data Processing

Sensor microring data was corrected for any non-specific binding and a drift related to thermal and instrumental fluctuations by referencing to control microrings, which were either modified with random DNA control sequence or blocked with BSA. Offline, residual slope as well as offset of the baseline were corrected, and data aligned temporally at the point when antibody or antigen was introduced.

To obtain the values of association rate constant (k_a) and the dissociation rate constant (k_d), the association curves were fitted to a 1:1 binding model: [4, 5]

$$\Delta pm(t) = \frac{k_a C \Delta pm_{max}}{k_a C + k_d}\left(1 - e^{-(k_a C + k_d)t}\right) \quad (1)$$

where $R(t)$ is the relative peak shift, ΔR_{max} is the maximum signal obtained when all surface binding sites are occupied, and C is the protein solution concentration. Using the Langmuir adsorption isotherm, Equation 1 can be simplified to:

$$R(t) = \Delta R_{max}\theta(1 - e^{-\gamma t}) \quad (2)$$

where θ is the fraction of total surface coverage and:

$$\gamma = k_a C + k_d \quad (3)$$

Values of k_a and k_d are determined from the slope and y-intercept by plotting γ as a function of C and a binding affinity constant, K_D, is defined as the ratio of the rate constants ($K_D = k_a/k_d$). The k_d values were also determined independently from the dissociation curves by fitting to a simple exponential decay function:

$$\Delta pm(t) = \Delta pm_{max}(e^{-k_d t}) \quad (4)$$

where R_{sat} is the maximum peak shift at each concentration. The derivative of Equation 2, evaluated at $t = 0$, gives initial slopes of the association curves. Initial slope values determined from the fitting parameters were plotted as a function of concentration to construct a calibration curve and to evaluate unknown sample concentration (Figure S4).

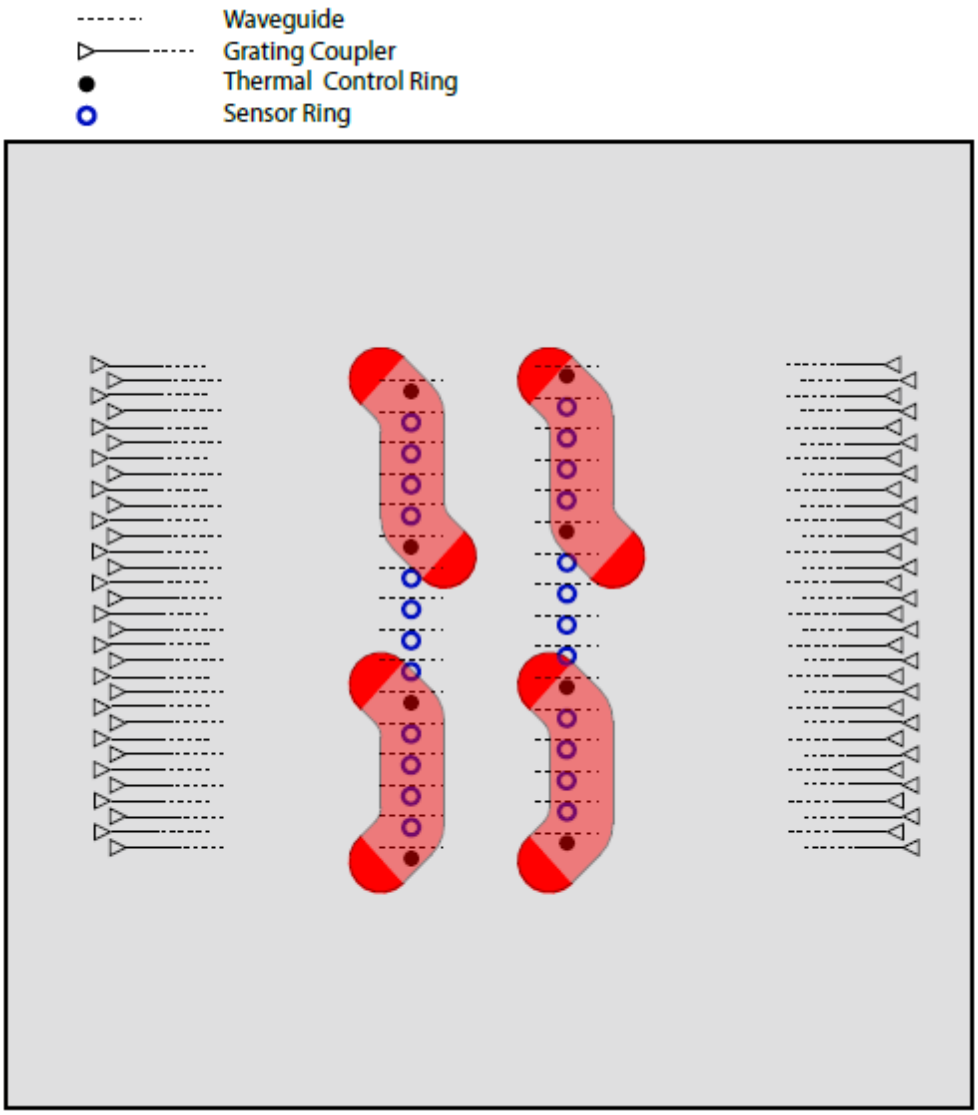

Figure S1. Schematic showing the four channel microfluidics (red) projected on the microchip surface. Inlet and outlet portions are shown in a darker red.

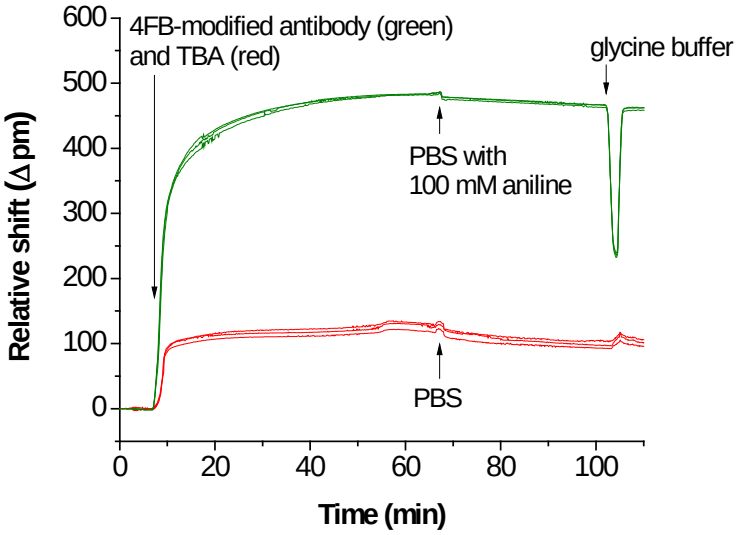

Figure S2. Real-time shifts in resonance wavelength from 6 microrings (3 each) upon covalent immobilization of 4FB-modified capture agents; anti-thrombin antibody (green) and thrombin-binding aptamer (red).

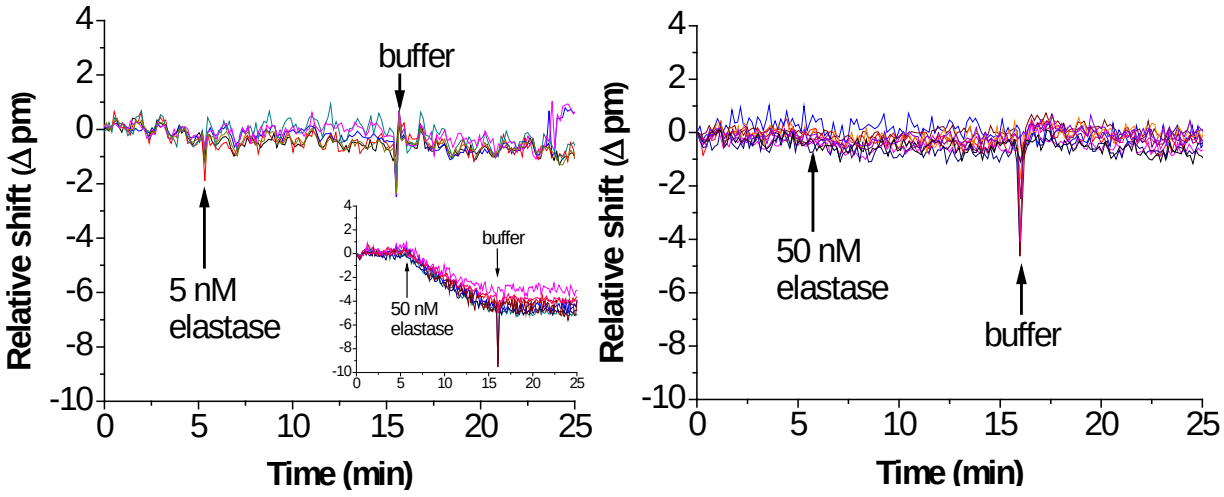

Figure S3. Real-time monitoring of resonance wavelength shifts of (a) antibody functionalized microrings (n=6 or 7) upon 10 min exposure to 5 nM and 50 nM (inset) elastase, and (b) TBA functionalized microrings (n=10) upon 10 min exposure to 50 nM elastase.

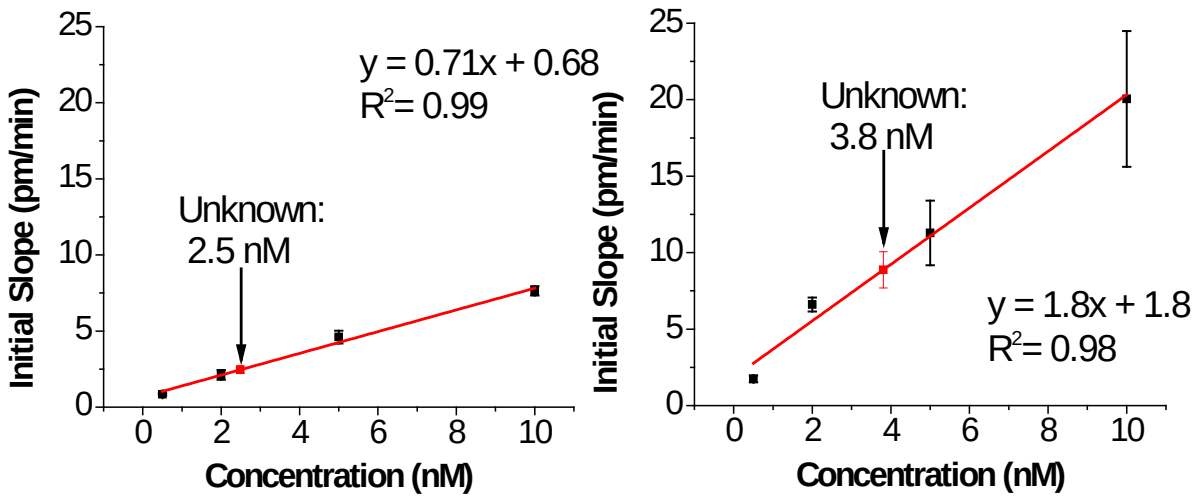

Figure S4. Overlay of the unknown solution on calibration plots for thrombin as determined by the initial slope method for microrings functionalized with (a) an anti-thrombin antibody and (b) the thrombin-binding aptamer. Error bars represent ± 1standard deviation of initial slope values determined from three microrings simultaneously measuring each concentration.

References

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[2] A. L. Washburn, L. C. Gunn, and R. C. Bailey, *Anal. Chem.*, 2009, **81**, 9499.
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[5] G. J. Wegner, A. W. Wark, H. J. Lee, E. Codner, T. Saeki, S. P. Fang, and R. M. Corn, *Anal. Chem.*, 2004, **76**, 5677.