Supporting Information for:

Quantitative, Label-Free Detection of Five Protein Biomarkers Using Multiplexed Arrays of Silicon Photonic Microring Resonators

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Experimental Details

Succinimidyl 4-formylbenzoate (S-4FB), and the silane 3-N-((6-(N'-Isopropylidene-hydrazino))nicotinamide)propyltriethyoxysilane (HyNic Silane), were purchased from SoluLink (San Diego, CA). Aniline was obtained from Acros Organics (Geel, Belgium). Monoclonal mouse anti-human CEA (Cat# M37401M), human CEA (Cat# A32030H), monoclonal mouse anti-human PSA (Cat #M86506M), human PSA (Cat# H6M07-323), monoclonal mouse anti-human AFP (Cat #H45610M), and human AFP (Cat# A81510H) were purchased from Meridian Life Science, Inc. (Saco, ME). Recombinant human IL-8 (Cat# 208-IL/CF) and monoclonal mouse anti-human IL-8 (Cat# MAB208) were obtained from R&D Systems (Minneapolis, MN). Monoclonal anti-human TNF-α (Cat# 16-7348), recombinant human TNF-α (Cat # 34-8329), and mouse IgG isotype control (Cat # 16-4714-85) were obtained from eBioscience (San Diego, CA). Zeba spin filter columns were purchased from Pierce (Rockford, IL). Silicone elastomer kit (RTV 615 2-Part Addition Cure Clear Silicone) was obtained from Momentive Performance Materials (Albany, NY). PBS was reconstituted from Dulbecco's phosphate buffered saline packets purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were obtained from Sigma-Aldrich and used as received.

All buffers were made with purified water (ELGA PURELAB filtration system; Lane End, UK), and the pH was adjusted as necessary with 1 M HCl or 1 M NaOH. Acetate buffer consisted of 50 mM sodium acetate and 150 mM sodium chloride adjusted to pH 6.0. Glycine buffer was 10 mM glycine and 160 mM NaCl adjusted to pH 2.2. BSA-PBS buffer was made by dissolving solid bovine serum albumin (BSA) in PBS (pH 7.4) to a final concentration of 0.1 mg/mL. For blocking, 2% BSA (w/v) in PBS was used. All solutions were degassed under vacuum before being flowed across the sensor surface.

Microring resonator array substrates and the instrumentation for analyzing the microring resonance frequencies were acquired from Genalyte, Inc. (San Diego, CA) and have been previously described.[1, 2] Briefly, 6 × 6 mm substrates hold sixty-four 30-μm diameter microrings with adjacent linear access waveguides that have input and output diffractive grating couplers at each end to independently measure the optical cavity spectrum of each microring. The entire wafer was spin-coated with a commercially-available perfluoro (alkenyl vinyl ether) copolymer (Asahi Glass Company) and annular openings were created over the active sensing rings via photolithography and reactive ion etching. Up to thirty-two microring sensors can be monitored simultaneously, with eight of the sensors not exposed to solution (covered by the fluoropolymer cladding) and used exclusively to compensate for thermal drift. The instrumentation uses a tunable, external cavity diode laser (center frequency 1560 nm) to rapidly scan the chip surface and couple light into the waveguides via grating couplers. Resonance wavelengths are determined as the wavelength at which the out-coupled light that has passed by the microring is at a minimum.

For all steps except antibody functionalization, sensor chips were loaded into a custom cell with microfluidic flow channels defined by a Mylar gasket that we have described previously.[2] Flow rates were maintained at 30 μL/min throughout the sensing experiments. For antibody functionalization, a 6-channel PDMS device was molded over a SU-8 template fabricated on a silicon wafer using standard photolithographic techniques. The PDMS device was created by mixing the base and curing agent in a 10:1 ratio and allowing the mixture to completely cure at 80 °C. Figure S-1 contains an illustration of how both the microfluidic device and the one-channel Mylar gasket overlay the microring sensor array.
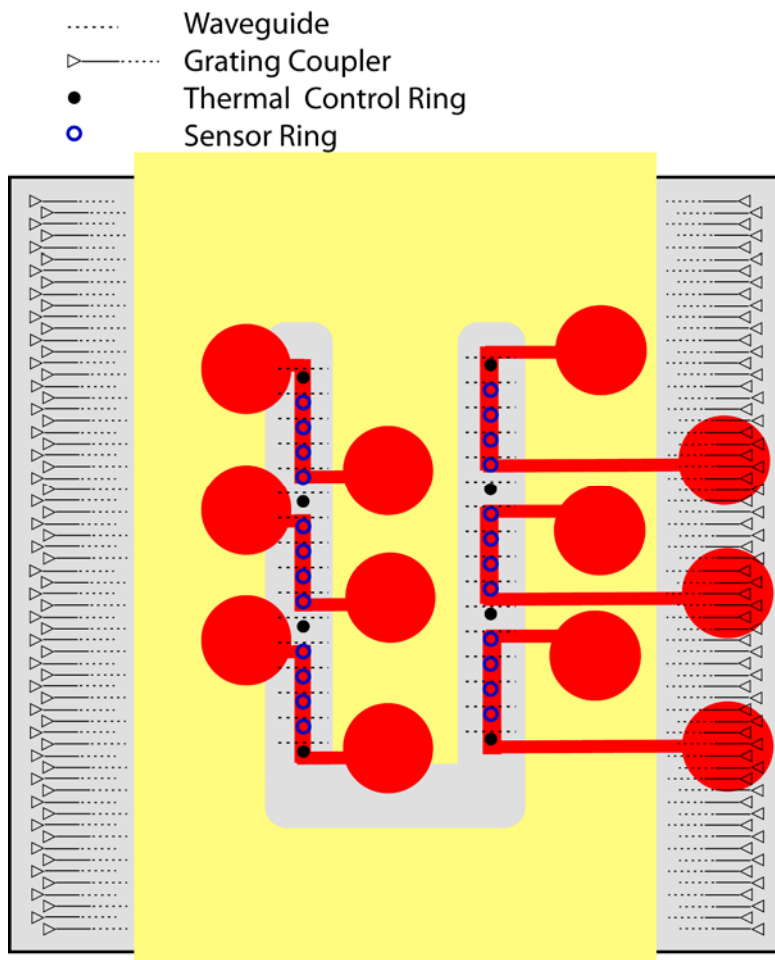
Figure S-1. Schematic showing the layout of microrings and grating couplers on the microchip surface. Projections of the 6 PDMS microchannels used to differentially functionalize microrings with antibodies are shown in red. The PDMS microfluidic device was used exclusively for the directed antibody functionalization step. The placement of the single-channel Mylar gasket for subsequent detection experiments is shown in yellow.

Before functionalizing the microring surfaces, sensor chips are first cleaned by a 30-second immersion in piranha solution[3] (3:1 H_2SO_4:30% H_2O_2) followed by copious rinsing with water and drying with nitrogen gas. To add a HyNic moiety to the surface, the sensor chip is exposed to a solution of 0.5 mg/mL HyNic Silane in 98% EtOH and 2% DMF for 10 minutes and then rinsed with 100% EtOH. Covalent attachment of antibodies is achieved by reacting the antibody (1 mg/mL) with a 5-fold molar excess of S-4FB (dissolved first in DMF and diluted in PBS to less than 5% DMF) for 2 h at room temperature. Excess S-4FB was removed by spin filtration with Zeba spin filter columns, which also allowed buffer exchange into acetate buffer. The antibodies are then covalently attached in a spatially-controlled fashion via microfluidic channels. Each 4FB-modified antibody was flowed through a different channel to functionalize sets of four rings, each with a different antibody: anti-PSA, anti-AFP, anti-CEA, anti-IL-8, anti-TNF-α, and mouse IgG isotype control. Aniline (100 mM) is added to each antibody solution to catalyze hydrazone bond formation[4] and enable the reaction to proceed in 20 minutes. A glycine buffer rinse is then used to remove any noncovalently bound antibody. A final blocking step is accomplished by overnight soaking in a 2% solution (w/v) of BSA in PBS.

Antigen calibration standards were prepared by diluting stock solutions of the antigens (0.1 mg/mL or greater) in BSA-PBS to concentrations below 1 ug/mL. Unknowns for the blind analysis were prepared in a similar manner. The concentrations of antigens in each of the mixtures are given in Table S-1. Air bubbles present while solution #8 was being analyzed resulted in several outliers, so the data from that run was discarded; however, because each of the concentrations was assayed in triplicate, quantitative analysis was still achieved. To generate an array of variable, but non-repeating concentrations, we consulted a website containing 5 × 5

Sudoku puzzles (http://www.sudoku-download.net) and assigned a different antigen concentration to each numerical value.

Table S-1. Concentration of antigen in ng/mL according to calibration solution number

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PSA	150	20	10	50	100	50	150	20	10	100	100	20	10	50	150
IL-8	10	150	100	20	50	10	100	50	150	20	20	150	50	10	100
AFP	100	10	50	150	20	20	50	150	100	10	150	10	100	20	50
CEA	20	50	150	100	10	100	20	10	50	150	50	100	20	150	10
TNF	50	100	20	10	150	150	10	100	20	50	10	50	150	100	20

Curve Fitting Methods

To calculate initial slopes for PSA, IL-8, and AFP curves, an exponential function was fit to the association curves using the equation:

$$S(t) = A\left(1 - e^{-B(t-t_0)}\right)$$
(S-1)

where $S(t)$ is the relative shift as a function of time (t), t_0 is the starting time, and A and B are fitting parameters. The variables A, B, and t_0 were fit to each binding curve independently, and all parameters were allowed to vary. Curve fitting was done using the nonlinear curve fit routine in the software package OriginPro 8. The initial slope was determined by evaluating the first derivative function of equation S-1 at $t = t_0$. As described previously,[2] for an antigen binding to a surface with first-order kinetics, the initial slope will always be proportional to the concentration of antigen. As a result, plotting the initial slope of the response curve versus concentration generates a linear calibration curve over a wide dynamic range. In addition, using real-time measurements, determining the initial slope provides greater precision on the measurement timescales used in these experiments.

Quantitation data for all 20 antigen-specific, antibody-modified microring sensors
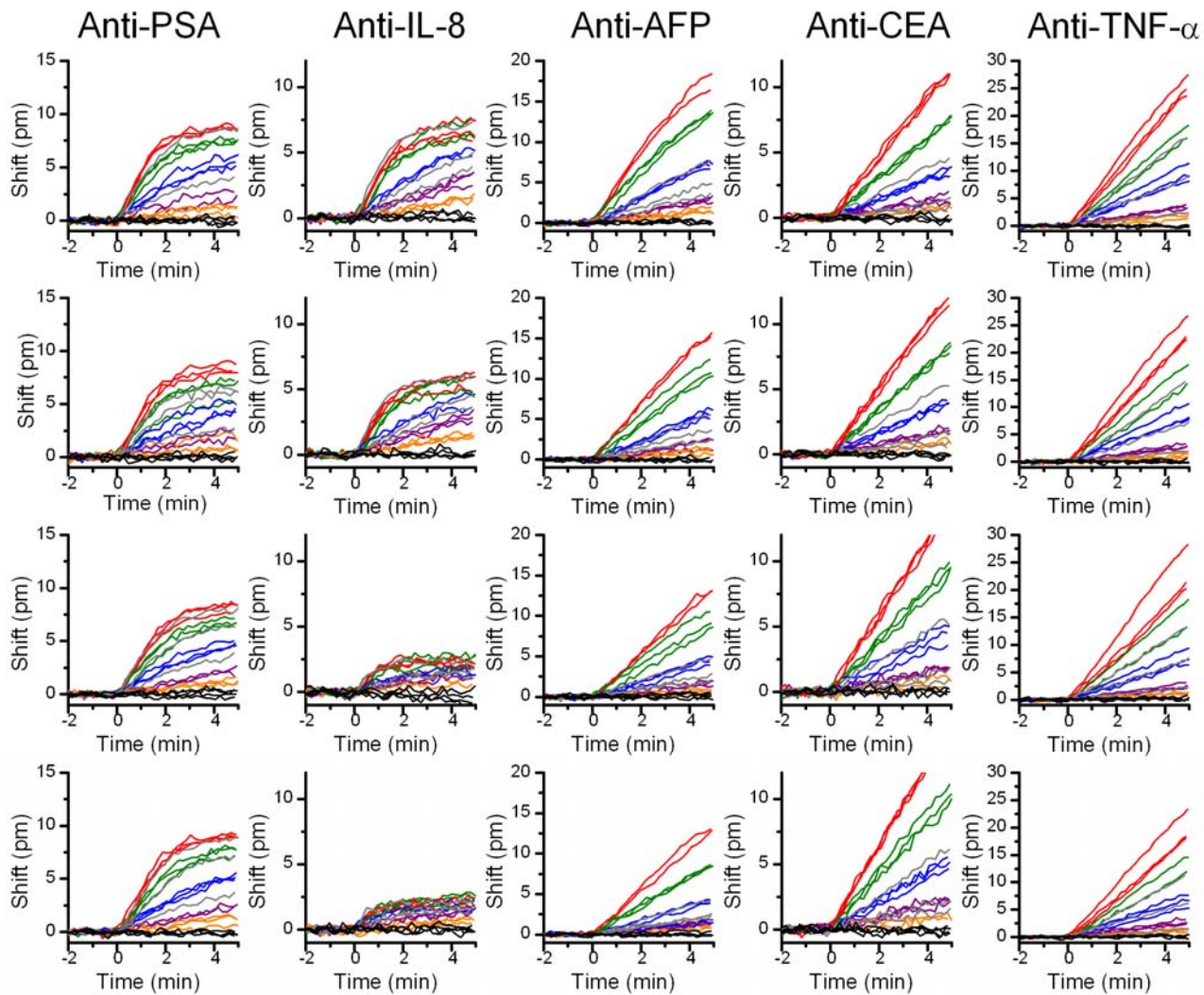

Figure S-2. Real-time response curves for all sensors used in the experiment. The plots in the top row are the same as those shown in Figure 2. The graphs within each column represent data taken from separate microrings functionalized with the same antibody. For the response curves, the colored lines represent the following concentrations of antigen: red, 150 ng/mL; green, 100 ng/mL; blue, 50 ng/mL; purple, 20 ng/mL; orange, 10 ng/mL; black, 0 ng/mL; gray, unknown solutions.
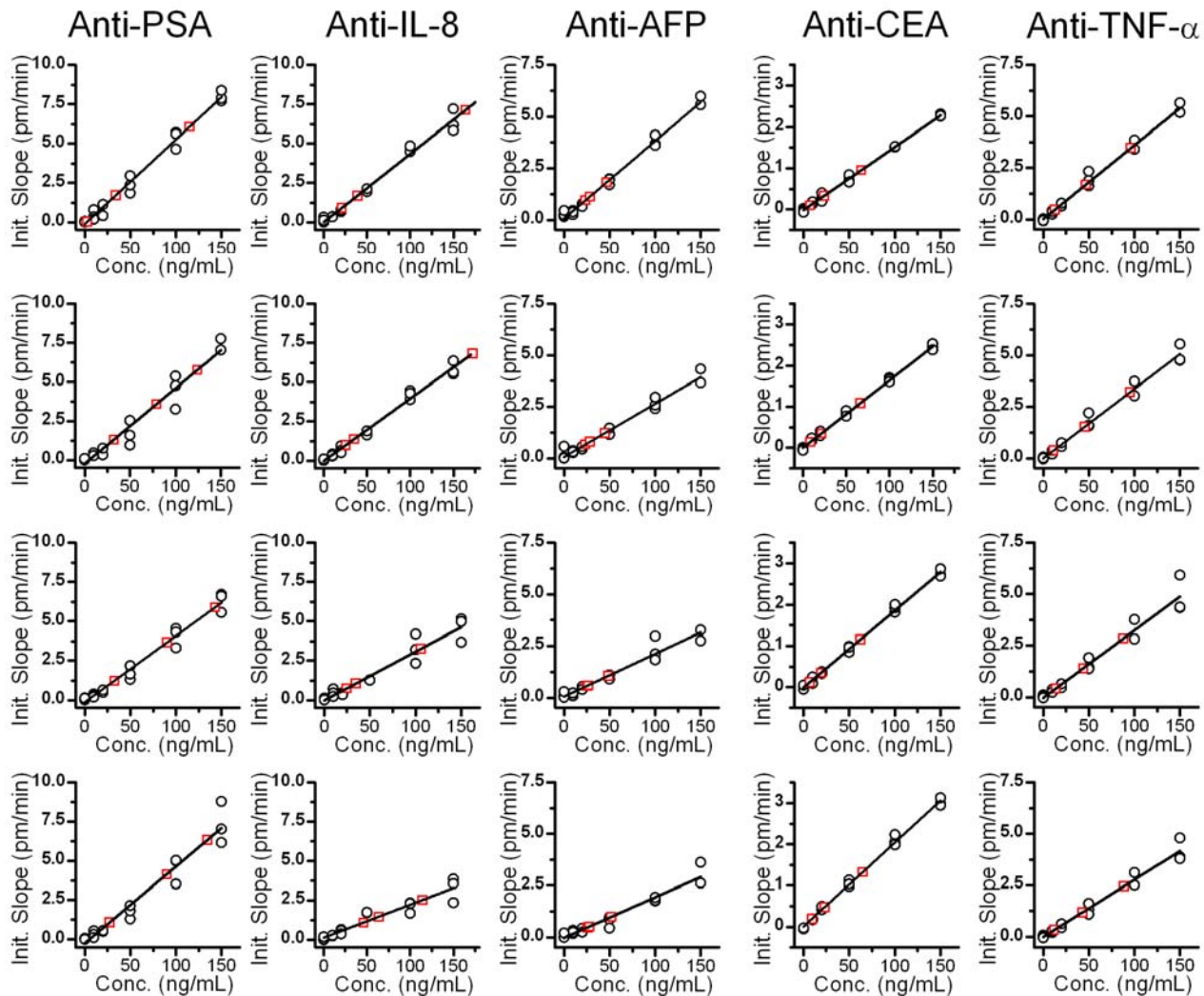
Figure S-3. Calibration curves generated from the real-time response curves in Figure S-2. The relative position of a calibration curve in the figure corresponds to the graph in the identical position in Figure S-2. For these graphs, black circles are calibration points and red squares are unknowns.

Unknown Concentration Values and Error Analysis.

Table S-2 below shows the actual values for the evaluation of unknowns as given in Figure 3. The first column gives the concentration as prepared by the researcher who created the unknowns. The second column lists the concentrations as determined from the calibration curves in Figure S-3. The uncertainty is then given as the 95% confidence interval based on four independent measurements, and the percent error is the ratio of the prepared concentration and the difference between the prepared and measured concentrations.

Table S-2. Values for unknown determinations: as-prepared concentration values, measured values, the associated uncertainty, and % error.

	Antigen	Prepared Concentration (ng/mL)	Measured Concentration (ng/mL)	Uncertainty (95% C.I.)	% error
Unknown A	PSA	32.4	31.5	8.3	2.7
	IL-8	146.4	138.9	53.7	5.1
	AFP	21.6	24.6	8.6	13.7
	CEA	10.8	8.0	3.0	25.8
	TNF-α	52.1	44.8	7.1	13.9
Unknown B	PSA	130.7	129.2	19.3	1.2
	IL-8	31.5	29.3	18.0	7.1
	AFP	57.5	48.1	8.5	16.3
	CEA	69.5	64.2	2.9	7.7
	TNF-α	15.8	12.5	7.2	20.9
Unknown C	PSA	86.6	86.4	17.3	0.2
	IL-8	47.1	43.0	22.1	8.6
	AFP	32.0	27.9	8.5	12.9
	CEA	24.5	21.6	3.0	11.6
	TNF-α	103.6	91.9	7.2	11.3

Supporting Information References

(1)   Iqbal, M.; Gleeson, M.; Tybor, F.; Spaugh, B.; Gunn, W.; Hochberg, M.; Baehr-Jones, T.; Bailey, R. C.; Gunn, L. C. *IEEE Journal of Selected Topics in Quantum Electronics* **2009**, in press.

(2)   Washburn, A. L.; Gunn, L. C.; Bailey, R. C. *Anal. Chem.* **2009**, *81*, 9499-9506.

(3)   ***Caution!*** *Piranha solutions are extraordinarily dangerous, reacting explosively with trace quantities of organics*

(4)   Dirksen, A.; Dawson, P. E. *Bioconjugate Chem.* **2008**, *19*, 2543-2548.