Langmuir

Article

pubs.acs.org/Langmuir

Coffee Ring Aptasensor for Rapid Protein Detection

Jessica T. Wen,[†,‡] Chih-Ming Ho,[§] and Peter B. Lillehoj*[,‡]

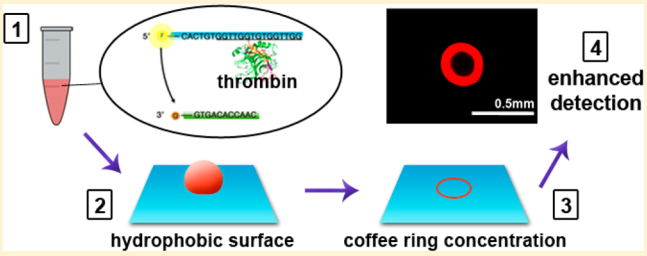
[†]Department of Bioengineering, University of California, Riverside, California 92521, United States

[‡]Department of Mechanical Engineering, Michigan State University, East Lansing, Michigan 48824, United States

[§]Mechanical and Aerospace Engineering Department, University of California, Los Angeles, California 90095, United States

Supporting Information

ABSTRACT: We introduce a new biosensing platform for rapid protein detection that combines one of the simplest methods for biomolecular concentration, coffee ring formation, with a sensitive aptamer-based optical detection scheme. In this approach, aptamer beacons are utilized for signal transduction where a fluorescence signal is emitted in the presence of the target molecule. Signal amplification is achieved by concentrating aptamer-target complexes within liquid droplets, resulting in the formation of coffee ring "spots". Surfaces with various chemical coatings were utilized to investigate the correlation among surface hydrophobicity, concentration efficiency, and signal amplification. On the basis of our results, we found that the increase in the coffee ring diameter with larger droplet volumes is independent of surface hydrophobicity. Furthermore, we show that highly hydrophobic surfaces produce enhanced particle concentration via coffee ring formation, resulting in signal intensities 6-fold greater than those on hydrophilic surfaces. To validate this biosensing platform for the detection of clinical samples, we detected α-thrombin in human serum and 4-fold-diluted whole blood. Coffee ring spots from serum and blood produced detection signals up to 40 times larger than those from samples in liquid droplets. Additionally, this biosensor exhibits a lower limit of detection of 2 ng/mL (54 pM) in serum, and 4 ng/mL (105 pM) in blood. On the basis of its simplicity and high performance, this platform demonstrates immense potential as an inexpensive diagnostic tool for the detection of disease biomarkers, particularly for use in developing countries that lack the resources and facilities required for conventional biodetection practices.



INTRODUCTION

The detection of biomolecules (proteins, DNA, and RNA) provides valuable information for a broad range of applications including clinical diagnosis, water and environmental analysis, food safety, and biodefense.[1] Current gold standard technologies for the detection of proteins heavily rely on antibody-based immunoassays such as enzyme-linked immunosorbent assay (ELISA), Western blot, and lateral flow. However, these methods generally involve timely sample processing, expensive laboratory equipment, and highly trained technicians.[2] In recent years, the global health and biomedical communities have identified an urgent need for low-cost, simple diagnostic tests for use in developing countries, where the burden of infectious disease is the highest.[3] Recently, aptamers have been widely utilized in biosensors because of their high binding affinities, versatile design strategies, and adaptability for multiple sensing platforms and schemes.[4] Various approaches have been explored in developing aptamer-based sensors for thrombin detection, including those based on electrochemistry,[5−9] electrochemiluminescence,[10−12] piezoelectric transduction,[13] surface-enhanced resonance Raman scattering,[14,15] and optical detection.[16−19] In particular, aptamer-based optical sensors offer several attractive characteristics including high sensitivity and the ability to be used for label-free in vitro detection.[20,21] In this approach, aptamers generally adopt two or more conformations where one allows for binding to a target molecule. When combined with a reporter−quencher pair, changes in aptamer conformation are detected by the emission of a fluorescence signal.[22] Similar to molecular beacons, aptamer beacons can employ a stem-loop structure where the reporter and quencher molecules are positioned at each end of the stem. In this configuration, the fluorescence signal from the reporter is absorbed by the quencher because of their adjacent proximity. The loop sequence contains a binding site for a target molecule, and in its presence, the aptamer undergoes a conformational change causing the loop structure to break apart, separating the reporter and quencher. As a result, the reporter is no longer quenched and a fluorescence signal is emitted. In this work, we adopt another generalized design scheme for aptamer beacons that utilizes an aptamer labeled with a reporter and a complementary oligonucleotide containing a quencher.[23,24] In the presence of the target, the binding equilibrium is shifted, causing the oligonucleotide to dissociate from the aptamer, allowing the reporter to fluoresce.

Received: January 17, 2013
Revised: March 27, 2013
Published: March 29, 2013

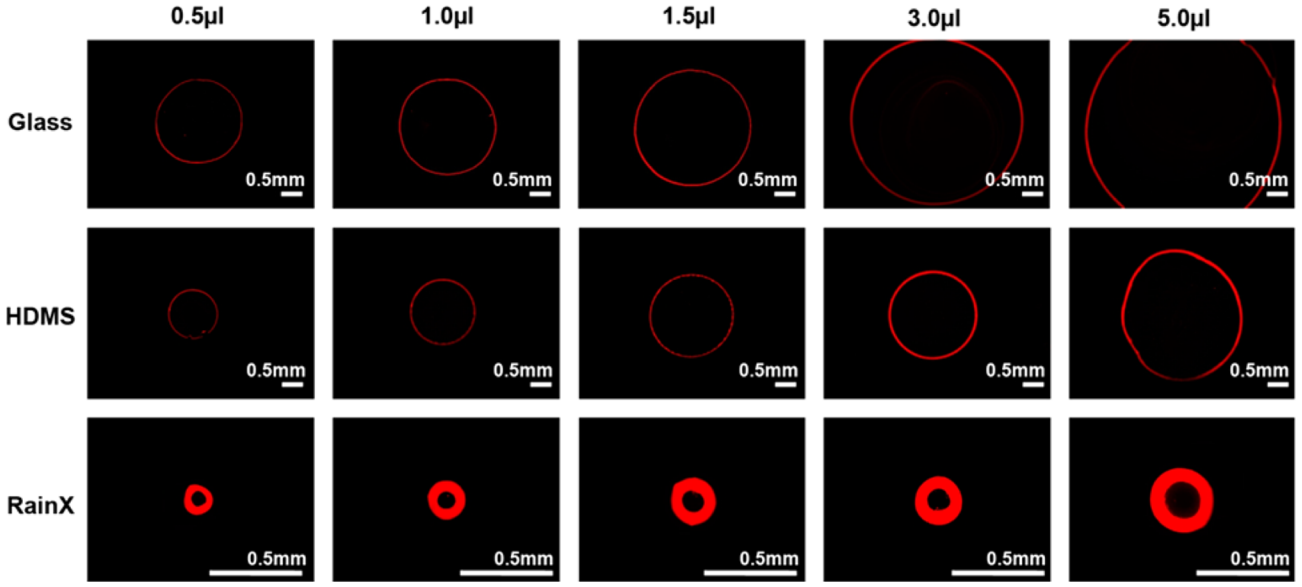


Figure 1. Fluorescent images of coffee ring formations on glass and HDMS- and RainX-treated surfaces. Hydrophobic surfaces concentrate particles more effectively than hydrophilic surfaces, resulting in smaller ring diameters. Glass and HDMS surfaces were imaged at 2× magnification, and RainX-treated surfaces were imaged at 10× magnification.

We combine this sensing scheme with an extremely simple method for biomolecular concentration based on the coffee ring effect. This everyday phenomenon occurs when a droplet of colloid solution evaporates on a solid surface, resulting in the formation of a coffee ring spot.[25] The underlying mechanisms that explain this effect are contact line pinning and capillary-induced flow, which drives suspended particles edgeward, concentrating them at the droplet perimeter.[26−29] Recent studies have employed this principle for concentrating nanoparticles to create 3D microstructures[30,31] and for biomolecular separation.[32,33] In this work, we employ the coffee ring effect to concentrate aptamer−thrombin complexes within liquid droplets for subsequent signal amplification. Using this approach, we are able to achieve significant signal enhancement of our optical-based biosensor without the use of additional equipment or labor-intensive sample amplification, thus offering a simple and inexpensive diagnostic platform requiring minimal economic and technological resources.

EXPERIMENTAL METHODS

Materials and Reagents. Colloid solutions of fluorescently dyed (excitation/emission: 580/605 nm) polystyrene microspheres with a stock concentration of 2% solid and a particle diameter of 0.02 μm were from Invitrogen (Carlsbad, CA). Solutions were diluted in purified deionized (DI) water (Barnstead EASYpure) with a resistivity of ∼18.3 MΩ·cm. Precleaned microscope glass slides were from Fischer Scientific (Pittsburgh, PA). HDMS (hexamethyldisilazane) was from the Nanoelectronics Research Facility at UCLA, and RainX was from a local vendor. Antithrombin aptamer beacon sequences used in this study were AlexaFluor647-5′ CACTGTGGTTGGTGTGGT-TGG-3′, labeled as FLUORapt, and 5′-CAACCACAGTG-3′-3BHQ2, labeled as BHQ2apt. AlexaFluor647 has excitation/emission wavelengths of 650/670 nm and exhibits exceptional photostability. All oligomers were from Integrated DNA Technologies (Coralville, IA) and human α-thrombin was from Haematologic Technologies, Inc. (Essex Junction, VT). Human serum and whole blood were from Bioreclamation, LLC (Westbury, NY). Proteins and aptamers were stored at −20 °C prior to experimentation.

Preparation of Surface Coatings and Surface Characterization. Three different surfaces were utilized in this study: glass, HDMS-treated glass, and RainX-treated glass. For HDMS surface treatment, glass slides were cleaned in isopropanol, rinsed in DI water, and dried at 150 °C for 5 min. The slides were exposed to saturated HDMS vapor for 30 min. RainX is a synthetic, hydrophobic surface treatment that is commonly used on automobile windshields. RainX surface treatment was carried out by dispensing RainX solution onto glass slides and repetitively polishing the surface until all residual liquid was removed. Samples were immediately treated prior to contact angle (CA) and coffee ring characterization measurements to minimize variations in surface properties that could result from prolonged storage. All three surfaces were characterized by performing static CA measurements using a First Ten Angstroms FTA4000 contact angle measurement system (Portsmouth, VA). DI water droplets of 5 μL were dispensed onto the surfaces, and images were captured and analyzed using FTA software.

Coffee Ring Formation and Characterization. Coffee ring formations of colloidal solutions were created as previously described by Wong et al.[33] Briefly, colloid solutions were diluted from stock concentration to a volume fraction of ∼0.0001%. Droplets of 0.5, 1, 1.5, 3, and 5 μL were dispensed onto the three different surfaces using a pipet and allowed to dry under standard atmospheric conditions (temperature of ∼25 °C and relative humidity of ∼46%). Coffee ring formations were imaged under a green fluorescent filter (555 nm) using a Nikon Eclipse TE2000 inverted microscope. Coffee ring formations on glass and HDMS surfaces were imaged using a 2× objective, and RainX surfaces were imaged using a 10× objective. Images were captured using a Photometric (Tucson, AZ) CoolSNAP CCD camera, and fluorescence signals were analyzed using RS Image software.

Aptamer Preparation and Thrombin Detection. Assays were performed to detect α-thrombin at serial concentrations from 1 to 500 ng/mL. Antithrombin aptamer beacons were generated by mixing FLUORapt and BHQ2apt together in Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA), heating the mixture to 90 °C for 3 min, and cooling to 25 °C at 0.2 °C/s. Aptamer preparation was carried out using an MJ Research thermocycler. α-Thrombin was diluted in either signaling buffer (20 mM Tris pH 8.3, 5 mM KCl, 1 mM $MgCl_2$), serum, or 4-fold-diluted whole blood (in DI water), mixed with the aptamer beacons (diluted to 1 μg/mL), and incubated for 30 min at 25, 31, 37, and 43 °C. Following incubation, 1 μL droplets of the aptamer−thrombin mixture were dispensed onto RainX-treated glass slides, dried, and analyzed using fluorescence microscopy. Droplets of unconcentrated aptamer−thrombin mixtures were imaged immediately after being dispensed onto the substrate. Each measurement was

dx.doi.org/10.1021/la400224a | Langmuir 2013, 29, 8440−8446

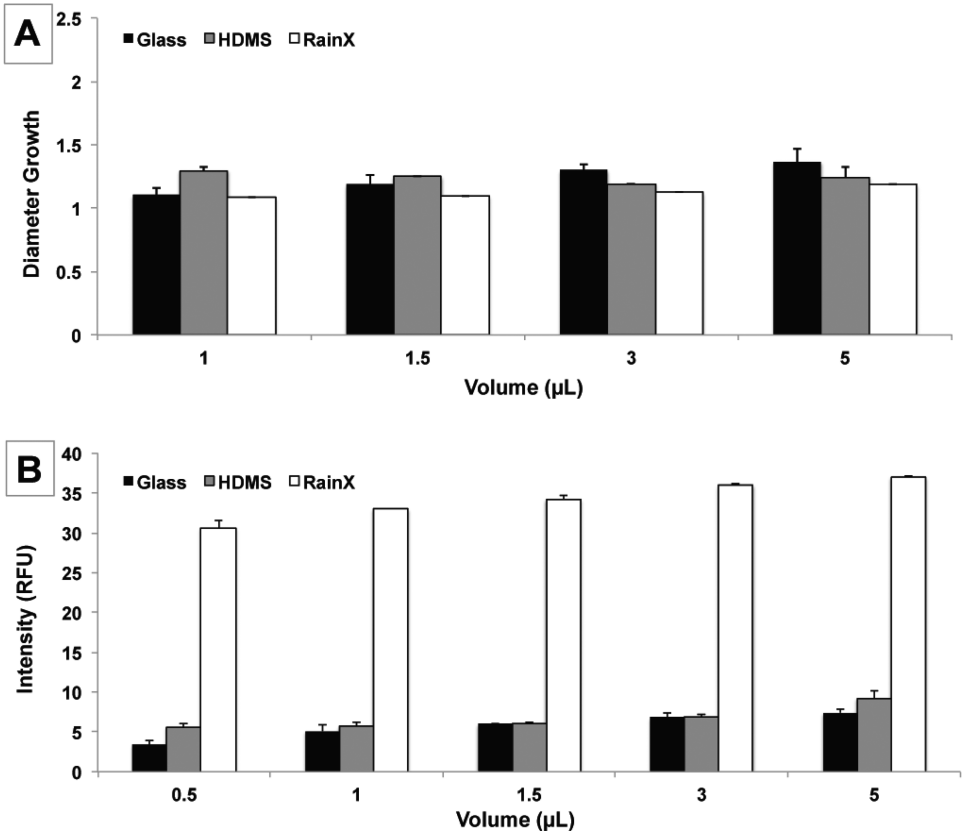


Figure 2. (A) Growth of coffee ring diameter with increasing droplet volumes. Data sets are normalized to the ring diameter at 0.5 μL. (B) Fluorescence signals of coffee rings from increasing droplet volumes on various surfaces. Each bar represents the mean ± standard deviation (SD) of three individual measurements.

taken within 5 s, and negligible photobleaching was observed. All fluorescence intensity measurements were performed at ~25 °C using a Nikon Eclipse TE2000 inverted microscope. Measurements in signaling buffer were imaged using a 10× objective, and measurements in serum and whole blood were imaged using a 4× objective. All detection assays were imaged under a red fluorescent filter (647 nm), which exhibits negligible autofluorescence of blood and serum.

RESULTS AND DISCUSSION

Surface Hydrophobicity Influences Coffee Ring Formation. Coffee ring spots were formed using colloid solutions of fluorescent microbeads (at a concentration of 200 μg/mL) on three different surfaces: glass, HDMS-treated glass, and RainX-treated glass. Each of these surfaces exhibits varying degrees of surface hydrophobicity: glass is hydrophilic (CA ≈ 26 ± 3°), HDMS-treated glass is mildly hydrophobic (CA ≈ 65 ± 4°), and RainX-treated glass is hydrophobic (CA ≈ 96 ± 3°). Droplets having volumes of 0.5 to 5 μL were used to investigate the correlation between surface hydrophobicity and coffee ring diameter. As shown in Figure 1, more hydrophobic surfaces produce smaller ring diameters for all droplet volumes. The primary underlying mechanism that describes this response is the liquid-to-surface contact area and the corresponding pinning perimeter.[34] For example, a droplet on a hydrophobic surface (RainX-treated glass) has a smaller contact area than the same droplet on a hydrophilic surface (glass). Consequently, the contact line perimeter (i.e., where the droplet is pinned to the surface) is condensed and produces a smaller coffee ring. One of the characteristics of coffee ring formation that is particularly well-suited for biosensing applications is its ability to form compact ring structures from droplets of various types of liquids such as buffer, serum, and blood. This is an important feature, especially when utilizing this sensor for the detection of clinical samples.

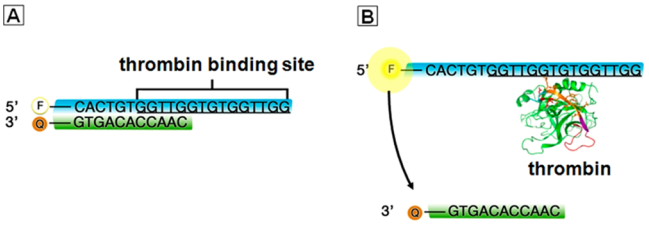
Quantitative analysis to compare the growth behavior of coffee rings was performed by normalizing the data to the ring diameter at 0.5 μL (Figure 2A). From this plot, we show that the ring diameter growth (from a smaller to a larger droplet) is comparable on all surfaces and thus independent of the surface hydrophobicity. Measurements were also performed using serum and blood (Figure S1 in Supporting Information), which exhibited similar drying behaviors as in buffer. The equivalence in diameter growth can be explained by the hydrodynamics of coffee ring formation (i.e., contact line pinning). The ring diameter is determined by the droplet volume and contact line pinning perimeter, which is dictated by the CA. By fixing the CA, the ring diameter becomes a function of the droplet volume. We also show that coffee rings on hydrophobic surfaces become wider as the perimeter is decreased, which results in enhanced particle concentration. Quantitative analysis of coffee ring concentration on different surfaces was performed by measuring the fluorescence signals, as the peak intensity per unit area, of coffee ring spots (Figure 2B). RainX surfaces produced the largest signal intensities, up to 6-fold greater, compared to those on hydrophilic surfaces at all droplet volumes. In addition to providing optimal signal amplification, the RainX surface produces coffee ring spots that are confined within a narrow field of view, even for varying droplet volumes. This characteristic is particularly favorable for optical-based biosensing systems, which would require only a

dx.doi.org/10.1021/la400224a | Langmuir 2013, 29, 8440−8446

fixed magnification objective, enabling facile integration with inexpensive, portable detectors.[35,36] It should also be noted that the generation of coffee ring spots results in relatively uniform structures with minimal geometric variability, allowing for enhanced repeatability, which is a highly desirable attribute for analytical measurements.

Temperature Characterization for Optimal Aptamer Binding Activity. Studies were performed to determine the optimal temperature for aptamer binding to obtain the highest detection signal. Coffee ring spots containing aptamer−protein complexes were generated in signaling buffer at various temperatures, and the resulting fluorescence signal was measured. We utilize an aptamer beacon consisting of a fluorophore-tagged antithrombin aptamer and a secondary oligonucleotide tagged with a quencher. In its initial conformation, the aptamer and oligonucleotide are hybridized such that the fluorophore and quencher are adjacent to one another, inhibiting the beacon from fluorescing (Scheme 1A).

Scheme 1. Illustration of the Aptamer Beacon Sensing Scheme[a]



[a](A) In its initial state, the anti-thrombin aptamer is hybridized with a secondary oligonucleotide. Because of the proximity of the reporter and quencher molecules, no fluorescence is emitted. (B) In the presence of thrombin, the oligonucleotide is dissociated from the aptamer, allowing the reporter to fluoresce.

The aptamer is designed to bind preferentially to thrombin; therefore, when thrombin is present, a shift in binding equilibrium causes the protein to bind to the aptamer and the oligonucleotide to dissociate (Scheme 1B). As a result, the fluorophore is no longer quenched, resulting in fluorescence emission. Using this design strategy, the concentration of thrombin was determined by quantifying the peak fluorescence intensity of coffee ring spots.

The optimal conditions for aptamer binding is dependent on several factors, including the internal chemical structure of the aptamer, and external factors such as the composition of the sample media and the aptamer binding temperature.[20,37] Because this sensor was utilized for the detection of clinical samples where the composition cannot be controlled, we focused our efforts on optimizing the aptamer binding temperature. RainX surfaces were exclusively used for the remaining studies in this work, owing to their ability to produce the greatest level of signal amplification and highest detection intensities. Figure 3 presents quantitative data of signal intensity, as signal-to-background ratios (SBRs), of coffee ring spots at various temperatures ranging from 25 to 43 °C and various protein concentrations ranging from 1 to 500 ng/mL. From this plot, it can be observed that highest detection signal is obtained at 37 °C throughout the entire range of protein concentrations. These results match closely with those previously described by Hall et al.[23] At temperatures <37 °C, the detection signals are substantially lower than those at 37 °C, particularly from 1 to 30 ng/mL. Although this data demonstrates that lower temperatures minimize the aptamer binding activity, it also shows that the detection signals do not increase correspondingly with temperatures above 37 °C. Specifically, temperatures beyond this threshold result in diminished aptamer binding activity and reduced aptamer kinetics (e.g., protein recognition, folding, and conformational changes).

Performance of the Coffee Ring Biosensor. The detection of α-thrombin in human serum and 4-fold-diluted whole blood was performed to demonstrate the applicability of this platform for the detection of clinical samples. Unconcentrated proteins in droplets of serum and blood were also measured to compare the improvement in amplification realized using coffee ring concentration. Figure 4 shows quantitative data of peak signal intensities, as SBRs, in serum (Figure 4A) and blood (Figure 4B). From these plots, we see that the detection signals of coffee ring spots are substantially higher (~10−40×) than those from samples of liquid droplets. In particular, the fluorescence signals of unconcentrated droplet

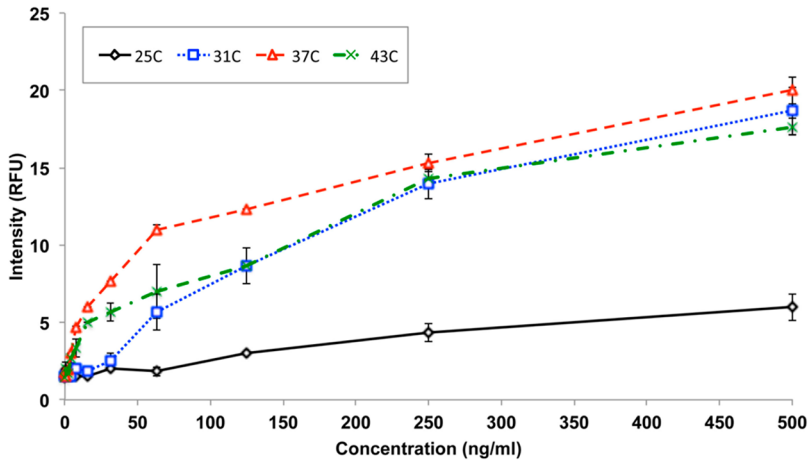


Figure 3. Influence of temperature on aptamer binding activity and subsequent protein detection. α-Thrombin was detected in buffer at 25, 31, 37, and 43 °C using coffee ring formations on RainX surfaces. These temperatures are those at which the aptamer solutions were prepared and the droplets were dried. The data is plotted in relative fluorescence units (RFU) and represents the signal-to-background ratio (SBR) of each measurement. Each data point represents the mean ± SD of three individual measurements.

dx.doi.org/10.1021/la400224a | Langmuir 2013, 29, 8440−8446

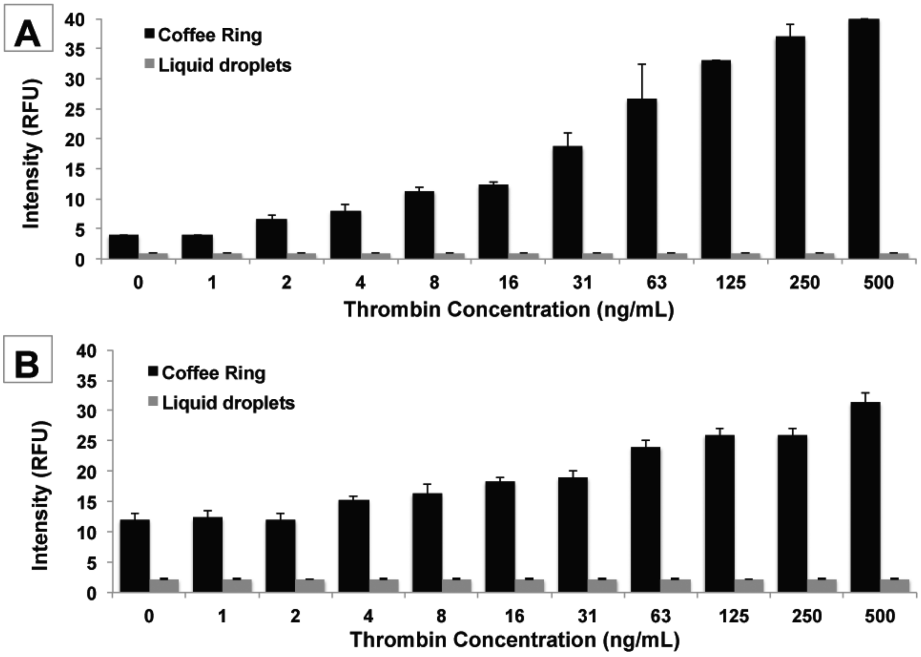


Figure 4. Detection of α-thrombin in (A) human serum and (B) 4-fold-diluted whole blood from coffee ring spots and liquid droplets. Aptamer binding was performed at 37 °C, and coffee rings were generated on RainX surfaces. The data is plotted in RFU and represents the SBR of each measurement. Each bar represents the mean ± SD of three individual measurements.

samples were undetectable even at the highest tested protein concentration, 500 ng/mL, in both serum and blood. In contrast, the formation of coffee ring spots enabled the aptamer–protein complexes to be substantially concentrated, resulting in enhanced signal amplification (Figure S2 in Supporting Information). This biosensor exhibits a limit of detection of 2 ng/mL (54 pM) in serum and 4 ng/mL (105 pM) in blood, which is comparable to previously reported thrombin aptasensors,[38] and in the range of many clinically relevant biomarkers, making this biosensor suitable for various diagnostic applications.[39] By comparing this data with that of detection in buffer (Figure 3), we see that slightly enhanced performance is achieved in serum and whole blood. Because the aptamer activity and binding performance are affected by the sample composition, we speculate that the enhanced aptamer activity in these clinical samples is a result of the additional components (biomolecules, electrolytes, etc.) that are not present in buffer. Previous studies have shown that nucleic acids are influenced by interactions with other molecules (i.e., intracellular proteins) and ions, affecting their folding structure and binding activity.[40−43] On the basis of these reports, we deduce that the stability of our aptamer–thrombin complexes is improved in blood and serum where the separation of the aptamer from its quenching oligonucleotide is enhanced, resulting in amplified fluorescence signals even at lower thrombin concentrations.

Detection in whole blood resulted in fluorescence intensities that were slightly lower than those in serum. Because whole blood contains other components (blood cells and fibrinogen) that are not present in serum, they can obstruct the aptamer binding activity, thereby minimizing the fluorescence signal. Additionally, measurements in whole blood required longer imaging (0.55 s) than those performed in serum (0.02 s), which we attribute to the presence of blood cells. Specifically, the fluorescence emission is absorbed and obstructed by the blood cells, which results in lower detection signals relative to those in serum. We also observed that the formation of coffee rings in serum and whole blood required slightly shorter incubation times compared to those generated in buffer. This can be attributed to the varying compositions of serum and blood, which contain higher concentrations of solutes compared to the buffer solution. Although measurements in serum and blood required several slight modifications to the experimental protocol, the coffee ring effect was still effective at concentrating aptamer–thrombin complexes for enhanced signal amplification, resulting in higher detection signals compared to those of samples in liquid droplets.

Further data analysis was performed by plotting the detection intensities in a log–log format to study the biosensor performance over a broad concentration range. As shown in Figure 5, measurements in blood exhibit a linear correlation over the entire range of tested concentrations from 1 to 500 ng/mL. In particular, the data exhibits a linear relationship in a log–log format that can be fitted to the power law $y = ax^n$ where the intensity, y, is proportional to the concentration, x, by an exponential factor n. We determined n by fitting linear regression lines to the data, which is shown in the plot. Within the tested concentration range, we obtained a value of 0.37 for n with a correlation coefficient of 0.97. Taking into account the previous results presented in this work, we demonstrate that this coffee ring biosensor is highly effective in concentrating proteins for signal amplification, which offers substantially enhanced signal intensity. When combined with a sensitive aptamer-sensing scheme, this platform provides a simple low-cost[35,36,44] method for biomolecular detection, which offers several main advantages over conventional laboratory-based assays that often require costly equipment and labor-intensive processes for sample amplification and high-sensitivity measurements.

dx.doi.org/10.1021/la400224a | *Langmuir* 2013, 29, 8440−8446

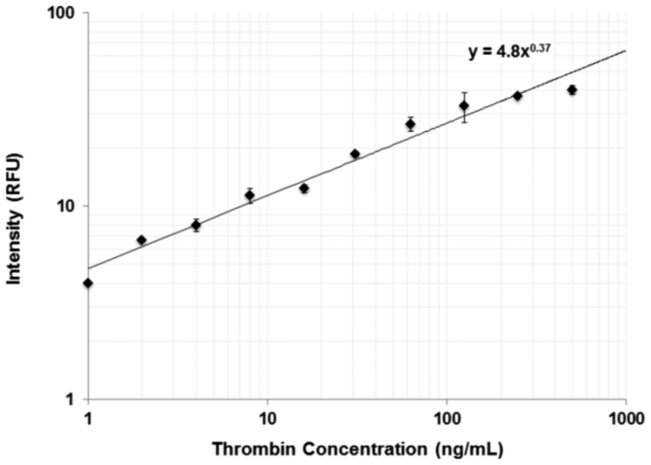


Figure 5. Log–log plot of signal intensity vs α-thrombin concentration in 4-fold-diluted whole blood. The data exhibits a linear correlation between 1 to 500 ng/mL that can be fitted to a power law relationship. Fitting linear regression lines to the data resulted in an exponential factor, n, of 0.37 with a correlation coefficient of 0.97. Each data point represents the mean ± SD of three individual measurements.

CONCLUSIONS

We report a new coffee ring aptasensor for biomolecular detection based on an inexpensive, highly sensitive biosensing platform. This technology utilizes an extremely simple signal amplification strategy based on coffee ring concentration. Substrates with varying surface properties were investigated, which revealed that highly hydrophobic surfaces resulted in concentration levels that were up to 6-fold greater compared to those of hydrophilic surfaces. Additionally, hydrophobic surfaces confine coffee ring diameters within a narrow field of view, facilitating integration with portable, low-cost detection units. Coupled with a sensitive aptamer beacon sensing scheme, we utilized this platform for the detection of clinically relevant proteins. In particular, α-thrombin was used as a model system that could be detected at concentrations down to 2 ng/mL (54 pM) in serum and 4 ng/mL (105 pM) in 4-fold-diluted whole blood. Moreover, coffee ring-concentrated aptamer–thrombin complexes produced fluorescence signals up to 40 times larger compared to those in liquid droplets. In summary, this biosensing platform offers a simple, cost-effective detection technology, forgoing the need for labor-intensive sample amplification and expensive high-sensitivity sensors. Furthermore, we envision that this platform can be employed for diagnostic testing in resource-limited settings and developing countries to minimize the burden of infectious diseases, such as human immunodeficiency virus (HIV), malaria, and tuberculosis, thereby improving global healthcare.

ASSOCIATED CONTENT

Supporting Information

Characterization of the coffee ring growth of serum and blood. Bright field and fluorescent images of coffee rings from serum and blood droplets. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: lillehoj@egr.msu.edu. Phone: (517) 432-2976. Fax: (517) 353-1750.

Author Contributions

This manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Author Contributions

J.T.W. and P.B.L. contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Bill and Melinda Gates Foundation (grant no. 442561-HC-80086), the NSF Center for Scalable and Integrated Nanomanufacturing (grant no. CMMI-0751621), and the NIH Center for Cell Control (grant no. PN2 EY018228). J.T.W. acknowledges support from the Center for Scalable and Integrated NanoManufacturing (SINAM). We thank Dr. T.-S. Wong, Dr. F. Wei, and Dr. N. Li for their discussion and advice on various aspects of this work.

REFERENCES

(1) Torres-Chavolla, E.; Alocilja, E. C. Aptasensors for detection of microbial and viral pathogens. *Biosens. Bioelectron.* **2009**, *24*, 3175–3182.
(2) Mabey, D.; Peeling, R. W.; Ustianowski, A.; Perkins, M. D. Tropical infectious diseases: diagnostics for the developing world. *Nat. Rev. Microbiol.* **2004**, *2*, 231–204.
(3) Yager, P.; Domingo, G. J.; Gerdes, J. Point-of-care diagnostics for global health. *Annu. Rev. Biomed. Eng.* **2008**, *10*, 107–144.
(4) Jayasena, S. D. Aptamers: an emerging class of molecules that rival antibodies in diagnostics. *Clin. Chem.* **1999**, *45*, 1628–1650.
(5) Ikebukuro, K.; Kiyohara, C.; Sode, K. Novel electrochemical sensor system for protein using the aptamers in sandwich manner. *Biosens. Bioelectron.* **2005**, *20*, 2168–2172.
(6) Bang, G. S.; Cho, S.; Kim, B.-G. A novel electrochemical detection method for aptamer biosensors. *Biosens. Bioelectron.* **2005**, *21*, 863–870.
(7) Floch, F. L.; Ho, H. A.; Leclerc, M. Label-free electrochemical detection of protein based on a ferrocene-bearing cationic polythiophene and aptamer. *Anal. Chem.* **2006**, *78*, 4727–4731.
(8) Du, Y.; Li, B.; Wei, H.; Wang, Y.; Wang, E. Multifunctional label-free electrochemical biosensor based on an integrated aptamer. *Anal. Chem.* **2008**, *80*, 5110–5117.
(9) Rahman, A.; Son, J. I.; Won, M.-S.; Shim, Y.-B. Gold nanoparticles doped conducting polymer nanorod electrodes: ferrocene catalyzed aptamer-based thrombin immunosensor. *Anal. Chem.* **2009**, *81*, 6604–6611.
(10) Wang, X.; Dong, P.; Yun, W.; Xu, Y.; He, P.; Fang, Y. A solid-state electrochemiluminescence biosensing switch for detection of thrombin based on ferrocene-labeled molecular beacon aptamer. *Biosens. Bioelectron.* **2009**, *24*, 3288–3292.
(11) Lin, Z.; Chen, L.; Zhu, X.; Qiu, B.; Chen, G. Signal-on electrochemiluminescence biosensor for thrombin based on target-induced conjunction of split aptamer fragments. *Chem. Commun.* **2010**, *46*, 5563–5565.
(12) Wang, J.; Shan, Y.; Zhao, W.-W.; Xu, J.-J.; Chen, H.-Y. Gold nanoparticle enhanced electrochemiluminescence of CdS thin films for ultrasensitive thrombin detection. *Anal. Chem.* **2011**, *83*, 4004–4011.
(13) Bini, A.; Minunni, M.; Tombelli, S.; Centi, S.; Mascini, M. Analytical performances of aptamer-based sensing for thrombin detection. *Anal. Chem.* **2007**, *79*, 3016–3019.
(14) Cho, H.; Baker, B. R.; Wachsmann-Hogiu, S.; Pagba, C. V.; Laurence, T. A.; Lane, S. M.; Lee, L. P.; Tok, J.B.-H. Aptamer-based SERRS sensor for thrombin detection. *Nano Lett.* **2008**, *8*, 4386–4390.

(15) Hu, J.; Zheng, P.-C.; Jiang, J.-H.; Shen, G.-L.; Yu, R.-Q.; Liu, G.-K. Electrostatic interaction based approach to thrombin detection by surface-enhanced Raman spectroscopy. *Anal. Chem.* **2009**, *81*, 87−93.
(16) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. Aptamer-functionalized Au nanoparticles for the amplified optical detection of thrombin. *J. Am. Chem. Soc.* **2004**, *126*, 11768−11769.
(17) Li, B.; Wei, H.; Dong, S. Sensitive detection of protein by an aptamer-based label-free fluorescing molecular switch. *Chem. Commun.* **2007**, *1*, 73−75.
(18) Li, N.; Ho, C.-M. Aptamer-based optical probes with separated molecular recognition and signal transduction modules. *J. Am. Chem. Soc.* **2008**, *130*, 2380−2381.
(19) Li, H.; Zhang, Y.; Wu, T.; Liu, S.; Wang, L.; Sun, X. Carbon nanospheres for fluorescent biomolecular detection. *J. Mater. Chem.* **2011**, *21*, 4663−4668.
(20) Song, S.; Wang, L.; Li, J.; Zhao, J.; Fan, C. Aptamer-based biosensors. *Trends Anal. Chem.* **2008**, *27*, 108−117.
(21) Cho, E. J.; Lee, J.-W.; Ellington, A. D. Applications of aptamers as sensors. *Annu. Rev. Anal. Chem.* **2009**, *2*, 241−264.
(22) Hamaguchi, N.; Ellington, A.; Stanton, M. Aptamer beacons for the direct detection of proteins. *Anal. Biochem.* **2001**, *294*, 126−131.
(23) Hall, B.; Cater, S.; Levy, M.; Ellington, A. D. Kinetic optimization of a protein-responsive aptamer beacon. *Biotechnol. Bioeng.* **2009**, *103*, 1049−1059.
(24) Levy, M.; Cater, S. F.; Ellington, A. D. Quantum-dot aptamer beacons for the detection of proteins. *ChemBioChem* **2005**, *6*, 2163−2166.
(25) Deegan, R. D.; Bakajin, O.; Dupont, T. F.; Huber, G.; Nagel, S. R.; Witten, T. A. Capillary flow as the cause of ring stains from dried liquid drops. *Nature* **1997**, *389*, 827−829.
(26) Shmuylovich, L.; Shen, A. Q.; Stone, H. A. Surface morphology of drying latex films: multiple ring formation. *Langmuir* **2002**, *18*, 3441−3445.
(27) Jung, J.-Y.; Kim, Y. W.; Yoo, J. Y. Behavior of particles in an evaporating didisperse colloid droplet on a hydrophilic surface. *Anal. Chem.* **2009**, *81*, 8256−825.
(28) Shen, X.; Ho, C.-H.; Wong, T.-K. Minimal size of coffee ring structure. *J. Phys. Chem.* **2010**, *114*, 5269−5274.
(29) Larson, R. G. Re-shaping the coffee ring. *Angew. Chem., Int. Ed.* **2012**, *51*, 2−5.
(30) Ming, T.; Kou, X.; Chen, H.; Wang, T.; Tam, H.-L.; Cheah, K.-W.; Chen, J.-Y.; Wang, J. Ordered gold nanostructure assemblies formed by droplet evaporation. *Angew. Chem.* **2008**, *120*, 9831−9836.
(31) Choi, S.; Stassi, S.; Pisano, A. P.; Zohdi, T. I. Coffee-ring effect-based three dimensional patterning of micro/nanoparticle assembly with a single droplet. *Langmuir* **2010**, *26*, 11690−11698.
(32) Jung, J.-Y.; Kwak, H.-Y. Separation of microparticles and biological cells inside an evaporating droplet using dielectrophoresis. *Anal. Chem.* **2007**, *79*, 5087−5092.
(33) Wong, T.-S.; Chen, T.-H.; Shen, X.; Ho, C.-M. Nano-chromatography driven by the coffee ring effect. *Anal. Chem.* **2011**, *83*, 1871−1873.
(34) Shin, D. H.; Lee, S. H.; Jung, J.-Y.; Yoo, J. Y. Evaporating characteristics of sessile droplet on hydrophobic and hydrophilic surfaces. *Microelectron. Eng.* **2009**, *86*, 1350−1353.
(35) Miller, A. R.; Davis, G. L.; Oden, M.; Razavi, M. R.; Fateh, A.; Ghazanfari, M.; Abdolrahimi, F.; Poorazar, S.; Sakhaie, F.; Olsen, R. J.; Bahrmand, A. R.; Pierce, M. C.; Graviss, E. A.; Richards-Kortum, R. Portable, battery-operated, low-cost, bright field and fluorescence microscope. *PLoS ONE* **2010**, *5*, e11890.
(36) Zhu, H.; Mavandadi, S.; Coskun, A. F.; Yaglidere, O.; Ozcan, A. Optofluidic fluorescent imaging cytometry on a cell-phone. *Anal. Chem.* **2011**, *83*, 6641−6647.
(37) Stoltenburg, R.; Reinemann, C.; Strehlitz, B. SELEX–a (r)evolutionary method to generate high-affinity nucleic acid ligands. *Biomol. Eng.* **2007**, *24*, 381−403.
(38) Mir, M.; Vreeke, M.; Katakis, I. Different strategies to develop an electrochemical thrombin aptasensor. *Electrochem. Commun.* **2006**, *8*, 505−511.
(39) Rifai, N.; Gillette, M. A.; Carr, S. A. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat. Biotechnol.* **2006**, *24*, 971−983.
(40) Nagatoishi, S.; Tanaka, Y.; Tsumoto, K. Circular dichroism spectra demonstrate formation of the thrombin-binding DNA aptamer G-quadruplex under stabilizing-cation-deficient conditions. *Biochem. Biophys. Res. Commun.* **2007**, *352*, 812−817.
(41) Vairamani, M.; Gross, M. L. G-quadruplex formation of thrombin-binding aptamer detected by electrospray ionization mass spectrometry. *J. Am. Chem. Soc.* **2003**, *125*, 42−43.
(42) Paeschke, K.; Simonsson, T.; Postberg, J.; Rhodes, D.; Lipps, H. J. Telomere end-binding proteins control the formation of G-quadruplex DNA structure in vivo. *Nat. Struct. Biol.* **2005**, *12*, 847−854.
(43) Rupcich, N.; Chiuman, W.; Nutiu, R.; Mei, S.; Flora, K.; Li, Y.; Brennan, J. Quenching of fluorophore-labeled DNA oligonucleotides by divalent metal ions: implications for selection, design, and applications of signaling aptamers and signaling deoxyribozymes. *J. Am. Chem. Soc.* **2006**, *128*, 780−790.
(44) Betsch, D. F.; Blais, B. S. A programmable $25 thermal cycler for PCR. *Biochem. Mol. Biol. Educ.* **2003**, *31*, 113−114.