Langmuir

Letter

pubs.acs.org/Langmuir

On-Chip Synthesis of RNA Aptamer Microarrays for Multiplexed Protein Biosensing with SPR Imaging Measurements

Yulin Chen,[†,∥] Kohei Nakamoto,[‡,§,∥] Osamu Niwa,[‡,§] and Robert M. Corn*[,†]

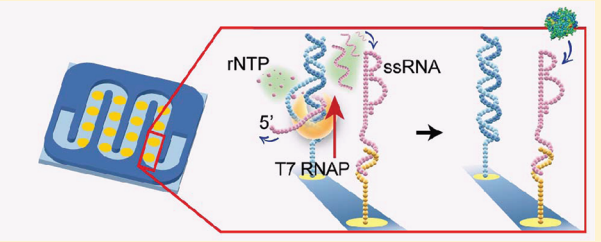
[†]Department of Chemistry, University of California—Irvine, Irvine, California 92697, United States

[‡]National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, 305-8566, Japan

[§]Institute of Material Science, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8573, Japan

Supporting Information

ABSTRACT: Microarrays of RNA aptamers are fabricated in a one-step, multiplexed enzymatic synthesis on gold thin films in a microfluidic format and then employed in the detection of protein biomarkers with surface plasmon resonance imaging (SPRI) measurements. Single-stranded RNA (ssRNA) oligonucleotides are transcribed on-chip from double-stranded DNA (dsDNA) templates attached to microarray elements (denoted as generator elements) by the surface transcription reaction of T7 RNA polymerase. As they are synthesized, the ssRNA oligonucleotides diffuse in the microfluidic channel and are quickly captured by hybridization adsorption onto adjacent single-stranded DNA (ssDNA) microarray elements (denoted as detector elements) that contain a sequence complementary to 5′-end of the ssRNA. The RNA aptamers attached to these detector elements are subsequently used in SPRI measurements for the bioaffinity detection of protein biomarkers. The microfluidic generator-detector element format permits the simultaneous fabrication of multiple ssRNA oligonucleotides with different capture sequences that can hybridize simultaneously to distinct detector elements and thus create a multiplexed aptamer microarray. In an initial set of demonstration experiments, SPRI measurements are used to monitor the bioaffinity adsorption of human thrombin (hTh) and vascular endothelial growth factor (VEGF) proteins onto RNA aptamer microarrays fabricated in situ with this on-chip RNA polymerase synthesis methodology. Additional SPRI measurements of the hydrolysis and desorption of the surface-bound ssRNA aptamers with a surface RNase H are used to verify the capture of ssRNA with RNA−DNA surface hybridization onto the detector elements. The on-chip RNA synthesis described here is an elegant, one-step multiplexed methodology for the rapid and contamination-free fabrication of RNA aptamer microarrays for protein biosensing with SPRI.



INTRODUCTION

The use of nucleic acid aptamer microarrays in conjunction with surface plasmon resonance imaging (SPRI) measurements is emerging as a rapid and robust alternative method to multiplexed antibody screening for the detection and quantitation of protein biomarkers in biological samples.[1−3] Aptamer microarrays possess several potential advantages over antibody microarrays in terms of their long-term stability, ease of fabrication, and reduced nonspecific adsorption.[3−6] SPRI measurements employing both DNA and RNA aptamer microarrays for protein biosensing have been reported previously.[1,2,7,8]

While a number of excellent high fidelity attachment methodologies are currently available for the fabrication of robust single-stranded DNA (ssDNA) microarrays on gold thin films for SPRI measurements,[9−12] the fabrication of single-stranded RNA (ssRNA) aptamer microarrays to SPRI measurements typically requires more care. Biotinylation or thiol-modification of ssRNA aptamers for surface attachment can often lead to cross-reactions of the oligonucleotides; often an alternative process that uses unmodified ssRNA and an enzymatic reaction to either create a biotin-type tag[13] or directly attach the ssRNA to 5′-phosphorylated ssDNA microarrays with surface ligation reaction is employed.[14,15] Moreover, these procedures for ssRNA aptamer microarrays must take place in a scrupulously clean lab environment because ssRNA is very susceptible to hydrolysis by trace amounts of RNase A. These additional fabrication and handling requirements have limited the application of ssRNA aptamer microarrays in SPRI biosensing.

In this paper, we demonstrate a simple, on-chip enzymatic synthesis process for the in situ fabrication of ssRNA microarrays in microfluidic format for the detection of protein biomarkers with SPRI. This multiplexed on-chip synthesis employs the surface enzymatic reaction of T7 RNA polymerase with double stranded DNA (dsDNA) templates that encode

Received: February 14, 2012
Revised: March 23, 2012
Published: March 29, 2012

   dx.doi.org/10.1021/la300656c | *Langmuir* 2012, 28, 8281−8285

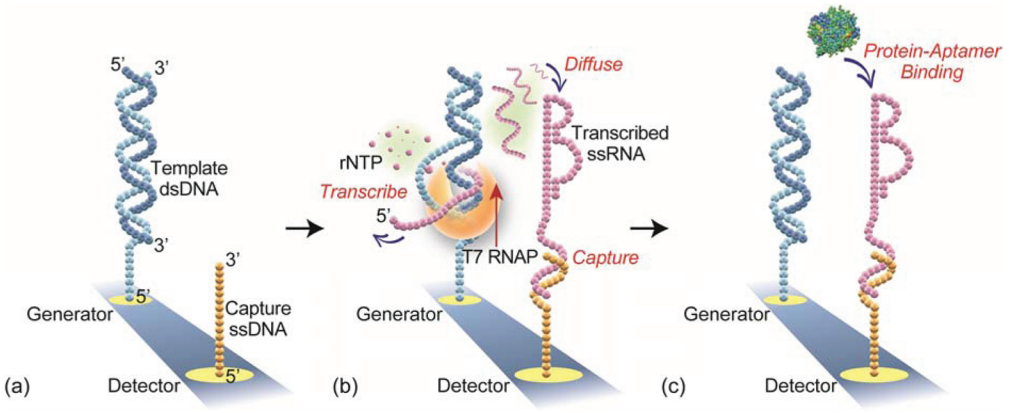


Figure 1. Schematic diagram of on-chip synthesis of RNA aptamer microarrays. (a) On the generator elements, surface promoter DNA was covalently attached to the gold surface and then hybridized with a template DNA. (b) The mixture solution of T7 RNA polymerase and rNTPs was injected into the microfluidic channel. RNA was transcribed with T7 RNA polymerase on the generator spot. Transcribed RNA was diffused to the adjacent detector elements and captured by bound ssDNA. (c) Injected protein was detected by RNA aptamer on the detector elements.

both an RNA aptamer and a unique capture tag sequence. The surface-transcribed ssRNA are captured by adjacent complementary ssDNA elements to form the RNA aptamer microarray. We have recently employed a similar on-chip RNA synthesis and capture methodology for the amplified SPRI detection of ssDNA at femtomolar concentrations.[16] The use of an on-chip RNA transcription methodology greatly reduces the risk of RNA degradation by removing any ex situ handling steps, and eliminates the need for purification of the synthesized ssRNA product.

EXPERIMENTAL SECTION

DNA Microarray Fabrication. SF-10 (Schott Glass) substrates were used to create the SPRI microarrays. First, a hydrophobic layer was formed by spin-coating CYTOP (Asahi Glass) at 5000 rpm on the glass substrate, followed by baking in oven at 70 °C for 50 min and then 190 °C for 1 h. The 16 elements (1 mm diameter) were fabricated by physical vapor deposition of chromium (1 nm) and gold (45 nm) utilizing a shadow mask. The details are described elsewhere.[17] The slides were then immersed in 1 mM MUAM (11-amino-1-undecanethiol hydrochloride, Dojindo) overnight, and 2 mg/mL of poly(L-glutamic acid) (pGlu, Sigma) was allowed to react for 1 h. Amino-modified DNA (0.5 μL, 250 μM) was immobilized onto the microarray elements using EDC/NHSS coupling reaction as described previously.[18]

On-Chip Transcription. The on-chip transcription process was performed on the SPRI instrument (GWC Technologies) that contains a temperature control system as described previously.[16] T7 RNA polymerase (RNAP) was purchased from Promega (RiboMax). First, the template dsDNA duplex on the generator element is formed by applying 1 μM complementary sequence (T1 or T2 in 1X PBS; all DNA sequences are available in the Supporting Information) for 10 min. PBS (1×) contains 137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, and is adjusted to pH 7.4. A solution of T7 RNAP (1.875 mM rNTP, 200−300 U/mL RNAP, and 1 U/μL RNase inhibitor) in the transcription buffer (5× transcription buffer was purchased from Promega and diluted to 1×) was injected into the SPRI flow cell (50 μL). The temperature was raised to 37 °C for 2 h for the transcription process. After transcription, the SPRI system was cooled down to room temperature and rinsed with buffer.

Protein Binding Assays. Human thrombin (hTh) and VEGF (165 amino acid form) were purchased from Sigma. Thrombin RNA aptamer was originally selected with affinity to bovine thrombin but can also bind to human thrombin.[19,20] In this paper, two nucleotides were added at the end of the hTh aptamer sequence to increase the folding stability. All hTh and VEGF binding assays were performed in 1× PBS.

RNase H Surface Hydrolysis Measurements. After the on-chip transcription process, RNase H (500 μL of 0.1 U/μL; Takara Bio) in Tris buffer (50 mM Tris-HCl, 300 mM KCl, 10 mM $MgCl_2$, and 10 mM DTT, adjust to pH 7.8) was applied to the chip for 5 min at room temperature.[20] The real-time SPRI measurement of RNase H surface hydrolysis was shown in the Supporting Information (Figure S1).

RESULTS AND DISCUSSION

Our method for creating an RNA aptamer microarray via on-chip RNA transcription followed by RNA−DNA hybridization capture is shown pictorially in Figure 1. As in our previous work,[16] we employ a generator element-detector element configuration confined in a microfluidic channel (Figure 1a). The generator elements contain the dsDNA template for a ssRNA that consists of two parts, an ssRNA aptamer sequence and a 18-base capture tag on the 5′-end (all DNA and RNA sequences are listed in the Supporting Information). The detector elements contain an ssDNA sequence complementary to a capture tag on the ssRNA. The process of array fabrication is shown in Figure 1b: T7 RNA polymerase and ribonucleotide triphosphates (rNTPs) are introduced into the microfluidic cell. The RNA polymerase adsorbs onto the dsDNA template attached to the generator element and creates multiple copies of the ssRNA sequence. These ssRNA oligonucleotides rapidly diffuse to an adjacent detector element (the distance between elements is ca. 1 mm) where they adsorb by hybridization ("hybridization-adsorption") onto the complementary ssDNA sequence to form an RNA aptamer microarray element where the RNA aptamer is attached to the surface via the DNA−RNA heteroduplex. Each RNA aptamer can be encoded with a different capture sequence to attach to a different detector element, and thus, in one step the surface RNA polymerase reaction can form a multiplexed RNA aptamer microarray. This microarray can be used immediately for SPRI protein biosensing by rinsing the microfluidic cell with a buffer solution and then introducing a sample solution with the target proteins (Figure 1c). Since the entire process (in vitro transcription, array fabrication, and SPRI protein adsorption detection) occurs in a single, low-volume microfluidic flow cell, the opportunity for contamination is greatly reduced, and the implementation of RNA aptamer microarrays is greatly simplified.

A first application of these transcription-based microarrays to SPRI thrombin biosensing is shown in Figure 2. Human

dx.doi.org/10.1021/la300656c | Langmuir 2012, 28, 8281−8285

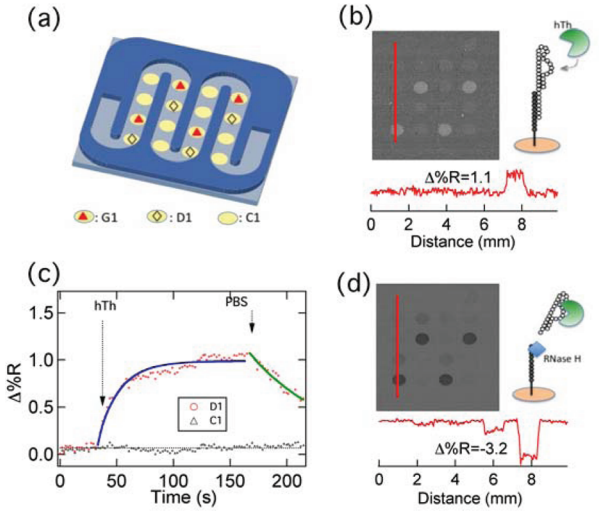


Figure 2. (a) Schematic of chip patterning on the microarray. Generator, detector, and control elements are immobilized with sequences G1, D1, and C1, respectively. (b) SPRI difference image after injecting 25 nM human thrombin (hTh) and corresponding line profile taken from the array image containing G1, D1, and C1 elements. (c) Normalized real-time SPRI kinetic curves for the detection of 25 nM hTh on D1 and C1 elements. (d) SPRI difference image by injecting 500 μL, 0.1 U/μL RNase H solution and corresponding line profile taken from the difference image. RNA at the DNA–RNA duplex was degraded by RNase H and protein–RNA aptamer complex was removed from the detector element.

thrombin (hTh) is a 37 kDa serine protease that catalyzes many coagulation-related reactions and has been used as a biomarker for various coagulation-related and cardiovascular diseases.[21,22] A number of both DNA and RNA aptamers have been identified for hTh; in these experiments, we employ the 31-base RNA aptamer that binds to the fibrinogen binding site.[19,20] A three-component, 16-element ssDNA microarray is fabricated in a 50 μL microfluidic SPRI cell as shown in Figure 2a. The DNA microarray elements are created by attaching amino-modified DNA to an electrostatically adsorbed poly-L-glutamic acid monolayer via an NHSS-EDC coupling chemistry described elsewhere.[18] Three different types of array elements (generator elements (sequence G1), detector elements (sequence D1), and control elements (sequence C1)) are fabricated in the spatial pattern shown in Figure 2a. The D1 and C1 sequences are ssDNA oligonucleotides, whereas the G1 and T1 sequence form a dsDNA 81-mer that contains both the RNA aptamer sequence and a capture sequence complementary to D1. On-chip in vitro transcription is initiated by exposing the microarray to a solution containing T7 RNA polymerase and rNTPs for 2 h at 37 °C. The transcribed ssRNA aptamers diffuse from the generator elements and are captured by the adjacent detector elements as described in Figure 1. After microarray fabrication, the SPRI microfluidic chip is cooled to room temperature, flushed clear with a PBS buffer solution and then a solution containing 25 nM hTh (total volume of 1 mL) is flowed through the SPRI chip. Figure 2b shows a SPRI difference image obtained after exposure of the chip for 120 s; the four detector elements for hTh are clearly visible in the Figure 2b. A line profile obtained from the image (red line) is also shown in Figure 2b, indicating that the detector elements exhibit an increased signal of $\Delta\%R = 1.07 \pm 0.09\%$. This value is comparable to that reported previously[20] in other SPRI measurements. The real-time microarray image data can also be processed to create real-time binding curves for the D1 and C1 elements as shown in Figure 2c. The solid lines are fits of the SPRI data to adsorption/desorption kinetic equations[23] from which we determine the rate constants k_{on} and k_{off}. The ratio of these two constants yields a Langmuir adsorption coefficient K_{ads} of 1.5×10^8 M^{-1}. An adsorption kinetics curve is observed for hTh adsorption onto element D1 with the limiting value of 1.0%. The kinetics data also shows negligible adsorption onto the control element C1, indicating the suppression of nonspecific protein adsorption.

As an additional demonstration of the presence of an RNA aptamer microarray in this experiment, the microfluidic cell was flushed with 500 μL of an RNase H solution and the SPR image in Figure 2d was obtained. As seen in previously experiments,[14,24] RNase H will very efficiently hydrolyze any DNA–RNA heteroduplexes on the microarray element, yielding a loss in the differential reflectivity SPR image. As seen in Figure 2d, $\Delta\%R$ for the detector elements decreased by the significant amount of $-3.2 \pm 0.2\%$, whereas very little loss was observed on the control elements. A little bit of reflectivity loss was observed due to removal of some RNA on the generator elements, but this did not interfere with the quantitation of the SPRI data (indeed, this is the value of the generator-detector dual element strategy). The loss of SPRI signal from the detector elements was larger than the signal increase during the previous hTh adsorption step, indicating that both the RNA and the hTh have been removed from the surface. From the amount of loss in the SPRI signal and previous SPRI measurements,[24] we can estimate that the amount of RNA on each detector element was approximately 10 femtomoles. Since the RNase H enzyme will not hydrolyze DNA (neither ssDNA nor dsDNA), the RNase H hydrolysis reaction has restored the generator and detector elements on the SPRI biochip to their original state. After the RNase H process, the biochip in principle is able to create new RNA aptamer microarrays by reapplying a new solution of T7 RNA polymerase and rNTPs onto the surface.[25]

In a second experiment, a two-component RNA aptamer microarray was transcribed and utilized in SPRI protein biosensing measurements in order to demonstrate the multiplexing capabilities of this fabrication methodology. This experiment employed a four-component, sixteen element microarray (as depicted in Figure 3a) that consisted of generator elements that contained a mixture of two dsDNA sequences (50% of G1-T1 duplex and 50% of G2-T2 duplex), two different detector elements (with sequences D1 and D2 attached) and one control element (sequence C2). The sequence G1 is the same as that used in the first experiment and encodes the hTh aptamer; the sequence G2 encodes a second RNA aptamer for vascular endothelial growth factor (VEGF). VEGF is a 46 kDa cell signaling protein whose overexpression is used a serum cancer biomarker, and the 29-mer RNA aptamer has been selected by previous researchers.[26] As in the first experiment, T7 RNA polymerase and rNTPs were introduced into the microfluidic cell for 2 h at 37 °C. The hTh and VEGF RNA aptamers were simultaneously transcribed, diffused, and then specifically attached by hybridization adsorption onto two different detector spots labeled D1 and D2, respectively. The SPRI difference images shown in Figure 3a and b were obtained after the sequential introduction of 40 nM of VEGF and 25 nM of hTh to the microfluidic cell (see the Supporting Information for details), and clearly indicate that that the microarray can be used to identify and quantitate

dx.doi.org/10.1021/la300656c | Langmuir 2012, 28, 8281–8285

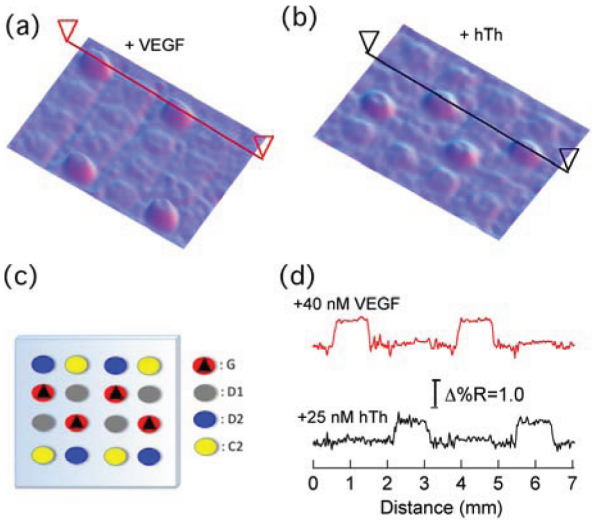


Figure 3. SPRI difference images after sequential injection of (a) 40 nM VEGF and (b) 25 nM hTh for 10 min. (c) Schematic of surface patterning of the microarray for multiplexed protein detection. Generator, detector element for VEGF and hTh, and control elements are represented as G (a mixture of sequences G1 and G2), D1, D2, and C2. (d) Line profiles of VEGF (red) and hTh (black) obtained by the corresponding difference images shown in (a) and (b). Line profiles for VEGF contains D2 and C2, and that for hTh contains G and D1, confirming very little nonspecific binding.

these two proteins simultaneously. Figure 3d plots in two line profiles obtained from the images; both RNA aptamer array elements showed an increase in differential reflectivity of approximately 1% when exposed to the corresponding protein target. A small amount of nonspecific adsorption (<0.2%) was also observed on the control elements of this microarray.

In summary, we have demonstrated an elegant one-step, on-chip synthesis methodology for the fabrication of RNA aptamer microarrays from DNA microarrays that uses a multiplexed surface-based RNA transcription reaction followed by hybridization adsorption capture in a microfluidic format. This rapid on-chip methodology has a number of significant advantages: (i) the integrated on-chip approach provides a simple method for generating multiple RNA aptamers simultaneously without the need for further purification or modification, and the microfluidic format greatly reduces the risks of RNase A contamination from the external environmental sources; (ii) only a very small amount of each RNA aptamer is needed (on the order of 10 fmol), but it is sufficient in the microfluidic format for biochip fabrication; (iii) the use of different capture sequences for the different RNA aptamers means that the aptamer microarray is formed from a single solution without the need for separate reaction compartments or spotting procedures; (iv) since each dsDNA on the generator element can transcribe multiple copies of ssRNA, the number of generator elements does not need to scale with the number of distinct aptamer sequences. In the future, in addition to SPRI detection, we will combine our new SPR phase imaging[27,28] techniques with this fabrication methodology for ultrasensitive protein biosensing with RNA aptamer microarrays.

■ ASSOCIATED CONTENT

Supporting Information

Table showing DNA sequences, SPRI data, and additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: rcorn@uci.edu.

Author Contributions

‖These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by a research fellowship of the Japan Society for the Promotion of Science for Young Scientists (No. 21-3300), and grants from the National Institute of Health (2RO1 GM059622), and the University of California, Cancer Research Coordinating Committee (CRCC).

■ REFERENCES

(1) Iliuk, A. B.; Hu, L.; Tao, W. A. Aptamer in bioanalytical applications. *Anal. Chem.* **2011**, *83*, 4440−4452.
(2) Tombelli, S.; Minunni, M.; Luzi, E.; Mascini, M. Aptamer-based biosensors for the detection of HIV-1 Tat protein. *Bioelectrochemistry* **2005**, *67*, 135−141.
(3) Bunka, D. H. J.; Stockley, P. G. Aptamers come of age - at last. *Nat. Rev. Microbiol.* **2006**, *4*, 588−596.
(4) Rowe, W.; Platt, M.; Day, P. J. R. Advances and perspectives in aptamer arrays. *Integr. Biol.* **2009**, *1*, 53−58.
(5) Nimjee, S. M.; Rusconi, C. P.; Sullenger, B. A. Aptamers: an emerging class of therapeutics. *Annu. Rev. Med.* **2005**, *56*, 555−583.
(6) Liu, J.; Cao, Z.; Lu, Y. Functional nucleic acid sensors. *Chem. Rev.* **2009**, *109*, 1948−1998.
(7) Potty, A. S. R.; Kourentzi, K.; Fang, H.; Jackson, G. W.; Zhang, X.; Legge, G. B.; Willson, R. C. Biophysical characterization of DNA aptamer interactions with vascular endothelial growth factor. *Biopolymers* **2009**, *91*, 145−156.
(8) de-los-Santos-Álvarez, N.; Lobo-Castanon, M. J.; Miranda-Ordieres, A. J.; Tunon-Blanco, P. SPR sensing of small molecules with modified RNA aptamers: Detection of neomycin B. *Biosens. Bioelectron.* **2009**, *24*, 2547−2553.
(9) Thiel, A. J.; Frutos, A. G.; Jordan, C. E.; Corn, R. M.; Smith, L. M. In situ surface plasmon resonance imaging detection of DNA hybridization to oligonucleotide arrays on gold surfaces. *Anal. Chem.* **1997**, *69*, 4948−4956.
(10) Lee, S. J.; Youn, B.-S.; Park, J. W.; Niazi, J. H.; Kim, Y. S.; Gu, M. B. sDNA aptamer-based surface plasmon resonance biosensor for the detection of retinol binding protein 4 for the early diagnosis of type 2 diabetes. *Anal. Chem.* **2008**, *80*, 2867−2873.
(11) Phillips, K. S.; Wilkop, T.; Wu, J.-J.; Al-Kaysi, R. O.; Cheng, Q. Surface plasmon resonance imaging analysis of protein-receptor binding in supported membrane arrays on gold substrates with calcinated silicate films. *J. Am. Chem. Soc.* **2006**, *128*, 9590−9591.
(12) Wolf, L. K.; Fullenkamp, D. E.; Georgiadis, R. M. Quantitative angle-resolved SPR Imaging of DNA−DNA and DNA−drug kinetics. *J. Am. Chem. Soc.* **2005**, *127*, 17453−17459.
(13) Srisawat, C.; Engelke, D. R. RNA affinity tags for purification of RNAs and ribonucleoprotein complexes. *Methods* **2002**, *26*, 156−161.
(14) Li, Y.; Lee, H. J.; Corn, R. M. Fabrication and characterization of RNA aptamer microarrays for the study of protein-aptamer interactions with SPR imaging. *Nucleic Acids Res.* **2006**, *34*, 6416−6424.
(15) Li, Y. A.; Wark, A. W.; Lee, H. J.; Corn, R. M. Single-nucleotide polymorphism genotyping by nanoparticle-enhanced surface plasmon

resonance imaging measurements of surface ligation reactions. *Anal. Chem.* **2006**, *78*, 3158−3164.
(16) Sendroiu, I. E.; Gifford, L. K.; Luptak, A.; Corn, R. M. Ultrasensitive DNA microarray biosensing via in situ RNA transcription-based amplification and nanoparticle-enhanced SPR imaging. *J. Am. Chem. Soc.* **2011**, *133*, 4271−4273.
(17) Goodrich, T. T.; Wark, A. W.; Corn, R. M.; Lee, H. J. Surface plasmon resonance imaging measurements of protein interactions with biopolymer microarrays. In *Methods in Molecular Biology*; Nedelkov, D., Nelson, R. W., Eds.; Humana Press: New York, 2006; Vol. *328*, pp 113−130.
(18) Chen, Y. L.; Nguyen, A.; Niu, L. F.; Corn, R. M. Fabrication of DNA microarrays with poly(L-glutamic acid) monolayers on gold substrates for SPR imaging measurements. *Langmuir* **2009**, *25*, 5054−5060.
(19) Liu, X.; Cao, G.; Ding, H.; Zhang, D.; Yang, G.; Liu, N.; Fan, M.; Shen, B.; Shao, N. Screening of functional antidotes of RNA aptamers against bovine thrombin. *FEBS Lett.* **2004**, *562*, 125−128.
(20) Li, Y.; Lee, H. J.; Corn, R. M. Detection of protein biomarkers using RNA aptamer microarrays and enzymatically amplified surface plasmon resonance imaging. *Anal. Chem.* **2007**, *79*, 1082−1088.
(21) Yang, X. H.; Wang, L.; Wang, K. M.; Tan, W. H.; Tang, H. X.; Meng, X. X.; Guo, Q. P. Novel protein detection method based on proximity-dependent polymerase reaction and aptamers. *Chin. Sci. Bull.* **2008**, *53*, 204−208.
(22) Huang, Y. C.; Ge, B. X.; Sen, D.; Yu, H. Z. Immobilized DNA switches as electronic sensors for picomolar detection of plasma proteins. *J. Am. Chem. Soc.* **2008**, *130*, 8023−8029.
(23) Jensen, K. K.; Ørum, H.; Nielsen, P. E.; Nordén, B. Kinetics for hybridization of peptide nucleic acids (PNA) with DNA and RNA studied with the BIAcore technique. *Biochemistry* **1997**, *36*, 5072−5077.
(24) Seefeld, T. H.; Zhou, W.-J.; Corn, R. M. Rapid microarray detection of DNA and proteins in microliter volumes with surface plasmon resonance imaging measurements. *Langmuir* **2011**, *27*, 6534−6540.
(25) Lee, H. J.; Wark, A. W.; Li, Y.; Corn, R. M. Fabricating RNA microarrays with RNA-DNA surface ligation chemistry. *Anal. Chem.* **2005**, *77*, 7832−7837.
(26) Jellinek, D.; Green, L. S.; Bell, C.; Janjic, N. Inhibition of receptor binding by high-affinity RNA ligands to vascular endothelial growth factor. *Biochemistry* **1994**, *33*, 10450−10456.
(27) Halpern, A. R.; Chen, Y.; Corn, R. M.; Kim, D. Surface plasmon resonance phase imaging measurements of patterned monolayers and DNA adsorption onto microarrays. *Anal. Chem.* **2011**, *83*, 2801−2806.
(28) Zhou, W.-J.; Halpern, A. R.; Seefeld, T. H.; Corn, R. M. Near infrared surface plasmon resonance phase imaging and nanoparticle-enhanced surface plasmon resonance phase imaging for ultrasensitive protein and DNA biosensing with oligonucleotide and aptamer microarrays. *Anal. Chem.* **2012**, *84*, 440−445.