A large-area hemispherical perforated bead microarray for monitoring bead based aptamer and target protein interaction

Jong Seob Choi, Sunwoong Bae, Kyung Hoon Kim, and Tae Seok Seo^{a)}

*Department of Chemical and Biomolecular Engineering (BK21 program) and
Institute for the BioCentury, Korea Advanced Institute of Science and Technology (KAIST),
291 Daehak-ro, Yuseong-gu, Daejeon 305-701, South Korea*

(Received 24 October 2014; accepted 26 November 2014; published online 9 December 2014)

Herein, we present a large-area 3D hemispherical perforated microwell structure for a bead based bioassay. Such a unique microstructure enables us to perform the rapid and stable localization of the beads at the single bead level and the facile manipulation of the bead capture and retrieval with high speed and efficiency. The fabrication process mainly consisted of three steps: the convex micropatterned nickel (Ni) mold production from the concave micropatterned silicon (Si) wafer, hot embossing on the polymer matrix to generate the concave micropatterned acrylate sheet, and reactive ion etching to make the bottom holes. The large-area hemispherical perforated micropatterned acrylate sheet was sandwiched between two polydimethylsiloxane (PDMS) microchannel layers. The bead solution was injected and recovered in the top PDMS microchannel, while the bottom PDMS microchannel was connected with control lines to exert the hydrodynamic force in order to alter the flow direction of the bead solution for the bead capture and release operation. The streptavidin-coated microbead capture was achieved with almost 100% yield within 1 min, and all the beads were retrieved in 10 s. Lysozyme or thrombin binding aptamer labelled microbeads were trapped on the proposed bead microarray, and the *in situ* fluorescence signal of the bead array was monitored after aptamer-target protein interaction. The protein-aptamer conjugated microbeads were recovered, and the aptamer was isolated for matrix assisted laser desorption/ionization time-of-flight mass spectrometry analysis to confirm the identity of the aptamer. © 2014 AIP Publishing LLC.

[<http://dx.doi.org/10.1063/1.4903939>]

I. INTRODUCTION

Advantages of sensitivity, simplicity, low cost, reliability, and reproducibility allow the nano- and micro-bead based assay to be popular in the biochemical analysis. Numerous genetic, proteomic, and cellular applications including DNA hybridization and amplification, immunoassay and protein-protein interaction, and cell isolation and proliferation have been reported using the bead chemistry.^{1–5} However, the bulk solution phase bead reaction retains intrinsic limitations in terms of lack of automation, low-throughput capacity, and bead aggregation issues. Meanwhile, the microfluidic device platform can be operated without human interference, screen the biochemical assay in a high-throughput manner by miniaturization and integration, and analyze the biological event even at a single bead level. Thus, the combination of the bead bioassay with the microfluidic technology can overcome the limitations of the conventional bead based bioassay, while maximizing the merits of the bead chemistry.^{6–10} To this end, Tan *et al.* proposed an integrated microdevice for trap-and-release of single microbeads. They used hydrodynamic resistance between the microchannel and the microtrapping site for single bead

^{a)} Author to whom correspondence should be addressed. Electronic mail: seots@kaist.ac.kr

transportation and immobilization, and optical-based microbubble generation technique for retrieval of single microbeads.¹¹ Henley *et al.* showed a microwell structure, which was fabricated by focused ion beam, and then, applied it for constructing the bead array to perform a sandwich immunoassay to analyze cytokines on the captured beads.¹² Sivagnanam *et al.* reported *in situ* micropatterning of streptavidin-coated microbeads inside a microchannel using electrostatic self-assembly technique and used it for microbead based immunoassay to detect IgG antigen.¹³ Burger *et al.* presented a centrifugal microdevice for improving the microbead trap efficiency by implementing V-cup barriers in the channel.¹⁴ Barbee *et al.* employed electric force to localize a negatively charged single bead into the individual microwell with high density.¹⁵ Tekin *et al.* used the silane-treated microdot arrays to immobilize antibody-functionalized small paramagnetic beads, and then loaded the antibody-coated large beads with trinitro-9-fluorenone (TNF)- α as a target antigen. Thus, the antibody-antigen-antibody immuno-complexes were recognized by detecting the small and large bead pairs.¹⁶

Although the previous microbead array formats are adequate for sensitive biochemical analysis in a high-throughput manner without cross-contamination between beads, most of their designs were restricted to microwell structure.^{8–18} Such a microwell structure mainly depends on the passive immobilization of the beads into each microwell, which is time-consuming, and suffers from the bead aggregate formation, leading to the non-uniform distribution of single beads in each chamber. In addition, the recovery of the immobilized beads is very difficult using the microwell array. To fully utilize the high-throughput and rapid screening capability of the single bead array and to perform the downstream biochemical analysis after bead-based bioassay, an advanced microdevice would be necessary which is capable of a large scale bead capture and release in more systematic and manageable way.

In this study, we propose novel hemispherical microarray structure, which has a large hole on the top and a small hole at the bottom. On the contrary to the conventional microwell format, which can only immobilize the beads in the wells, our unique microarray design enables us to manipulate the beads by hydrodynamic force with ease, so that the bead capture and release in a large scale can be realized with high speed and efficiency. We further demonstrated the usefulness of the large area single bead array for monitoring the aptamer and target protein interaction. The bead trap, the *in situ* fluorescence detection of aptamer-target protein complexes on the beads, retrieval of the bioassay beads, and characterization of the aptamer could be sequentially executed.

II. EXPERIMENTAL

A. Materials and methods

A positive photoresist (PR) (S1818) and MFTM-CD-26 developer were purchased from Rohm and Haas Electronic Materials Limited Liability Company (USA). A $\langle 100 \rangle$ Si wafer was obtained from iTASCO (Korea). An isotropic wet etching solution was prepared from a mixture of hydrofluoric acid (DC chemical, 50%), nitric acid (Junsei, 70%), and acetic acid (SAMCHUN, 99.5%). 3-Aminopropyltriethoxysilane (APTES), 3-glycidoxypopyl trimethoxysilane (GPTMS), lysozyme, human thrombin, bovine serum albumin (BSA), and hexamethyldisilazane (HMDS) were ordered from Sigma Aldrich (Korea). A polydimethylsiloxane (PDMS) prepolymer and a curing agent (Sylgard 184 elastomer kit) were purchased from Dow Corning Corporation (USA). Acryl sheet with 30 μm in thickness was ordered from Sejin T. S. Co., Ltd. (Korea). The aptamers were made by Bioneer Corporation (Daejeon, Korea). Alexa Fluor 488 Protein Labeling Kit and Alexa Fluor 546 Protein Labeling Kit were purchased from Invitrogen (USA). Streptavidin coated polystyrene beads (0.5% (w/v), 41 μm diameter) and Vistex 111-50 were ordered from Spherotech (USA) and FSI Coating technologies (USA), respectively. Binding buffer (50mM Tris-HCl, 100mM NaCl, 5mM KCl, pH 7.0) was used for protein-aptamer conjugation. The fluorescence image of the bead array was visualized under a confocal laser microscope (Nikon, ECLIPSE, C1si, Japan). The mass of the aptamers was measured by matrix assisted laser desorption/ionization (MALDI)-TOF MS (Bruker autoflex III, Bruker Daltonics, Germany).

B. Fabrication of 3D hemispherical microstructure

Fig. 1(a) shows the fabrication scheme for a large-scale single bead manipulating microstructure in an acryl sheet. A Si wafer was coated with a 300 nm-thick Si_3N_4 layer and a positive PR, and then a 25 μm -diameter dot array was patterned through the conventional photolithography process. The exposed Si_3N_4 layer was removed by reactive ion etching (RIE) with CF_4 plasma, and the background positive PR was cleaned with acetone. An isotropic wet etching of a Si wafer was performed in a HF , HNO_3 , and CH_3COOH mixture solution (volume of HF , HNO_3 , and CH_3COOH = 20, 35, and 55 mL) to form concave microwells in the Si wafer. The exposed Si_3N_4 layer was removed by reactive ion etching (RIE) with CF_4 plasma, and the background positive PR was cleaned with acetone. An isotropic wet etching of a Si wafer was performed in a HF , HNO_3 , and CH_3COOH mixture solution (volume of HF , HNO_3 , and CH_3COOH = 20, 35, and 55 mL) to form concave microwells in the Si wafer.

FIG. 1. (a) Fabrication process of a large-area hemispherical perforated microwell structure for bead bioassay. (b) SEM images of replicated PDMS microstructures obtained from the wet-etched Si wafer (top panel) and the concave hemispherical microwells in the acryl sheet after hot embossing with a Ni mold. (c) Top, tilted, and enlarged SEM images of the large-scale hemispherical perforated bead microarrays from left to right.

After vigorous washing with distilled water and drying, the remaining Si_3N_4 layer was removed by RIE with CF_4 plasma. The resultant Si wafer which contained concave hemispherical patterns was used as a template for making a hard Ni mold. Through the electroplating process against the Si wafer, the convex micropatterned Ni mold was fabricated. To transfer the hemispherical micropattern onto the polymer matrix, the acryl sheet was hot-pressed by a Ni mold with 25 MPa at 100°C for 15 min. As a result, the concave micropatterned acrylate sheet was generated. To make bottom holes on the hemispherical micropattern, the backside of the acryl sheet was etched by RIE in O_2 plasma. The size of the bottom holes was controlled by tuning the etching time. The resultant acryl sheet retains a large-area hemispherical perforated microwell structure, which has a large hole on the top and a small hole at the bottom. The total number of microwells in the acryl sheet ($6420\ \mu\text{m} \times 2420\ \mu\text{m}$ area) was 1625.

C. An assembled microdevice for microbead manipulation

The bead manipulating microdevice consisted of three layers as shown in Fig. 2(a). The top layer has the PDMS microfluidic channel (width $\times$ length $\times$ height = $600 \times 21\,000 \times 250\ \mu\text{m}$) in which the microbeads were injected and recovered. The middle layer was the 3D hemispherical microstructure patterned acryl layer. The bottom one has the PDMS microchannel (width $\times$ length $\times$ height = $2800 \times 9000 \times 250\ \mu\text{m}$), which was connected with two control lines

FIG. 2. (a) The schematics of an individual layer (left) and the digital image of an assembled microfluidic device (right). (b) The entire process for the high-throughput microbead based aptamer screening in the hemispherical perforated microstructure-integrated device.

to change the flow direction of a bead solution in the top layer. The top and bottom PDMS microchannels were fabricated by a conventional soft lithography procedure and aligned perpendicularly to each other. To bond the acryl sheet with the PDMS layers, each layer was exposed to O₂ plasma for 2 min to generate hydroxyl groups and oxygen radicals on the surface, and then the PDMS and acryl sheet were immediately immersed in a 2% (v/v) GPTMS solution and a 2% (v/v) APTES solution, respectively, at 70 °C for 60 min. This process produced the terminal epoxy-functional groups on the PDMS surface and the amino groups on the acryl surface. After drying with N₂ gas, the functionalized top and bottom PDMS layers were permanently bonded with an acryl sheet at 70 °C for 24 h through the amine-epoxide reaction. Then, the inlet and outlet of the top PDMS layer and the two control lines of the bottom PDMS layer were connected with syringe pumps.

D. Manipulation of microbead capture and retrieval

Prior to bead injection, all the microchannels were filled with a Vistex 111-50 solution and incubated at 70 °C for 1 h to make the microchannel surface hydrophilic. Then, a 1% BSA solution was loaded in the microchannels for 1 h to prevent nonspecific binding of the proteins. After switching the BSA solution to the binding buffer, the microbead solution was introduced. In order to capture the microbeads in the 3D hemispherical microstructure of the acryl layer, the bead solution (5×10^5 in 200 μ L) was introduced and a withdraw mode was set from the control lines of the bottom PDMS layer with a flow rate of 2 mL/h, while the fluid in the top PDMS layer was pulled out to the outlet with a flow rate of 1 mL/h. To release the captured microbeads to the inlet of the top layer, the withdraw mode was changed to the infuse mode with a flow rate of 10 mL/h in the outlet and two control lines.

E. Monitoring of the aptamer and target protein interaction on the large area single bead array

Streptavidin coated polystyrene beads were incubated with a biotinylated aptamer solution for 1 h to link the aptamers on the bead surface. We employed a lysozyme-targeting aptamer (5'-biotin-C₁₈-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3', M.W.: 13 735 g/mol) and a thrombin-targeting aptamer (5'-biotin-C₁₈-GGT TGG TGT GGT TGG-3', M.W.: 5475 g/mol).^{19–23} After vigorous washing with a binding buffer, the aptamer modified polystyrene beads were injected into the microchannel of the top PDMS layer. Then, the microbeads were captured in the hemispherical perforated microwell array through the fluidic control as described above. An Alexa Fluor 488 labelled lysozyme solution or an Alexa Fluor 546 labelled thrombin solution was loaded from the inlet and incubated for 50 min to induce the aptamer-protein interaction. After washing out the excess proteins with a binding buffer, the microbead array was monitored by the confocal microscope to visualize the fluorescence signal on the beads. To confirm the capture aptamer, the beads were recovered by the infuse mode. The biotin-streptavidin binding was cleaved in a 99% formamide solution at 90 °C for 2 min, and then the released biotinylated aptamers were characterized by the MALDI-TOF MS. The matrix solution was prepared by dissolving 35 mg 3-hydroxypicolinic acid and 6 mg ammonium citrate in 0.8 ml of 50% acetonitrile. An aptamer sample was spotted on a stainless steel sample plate with the matrix solution, air dried, and analyzed using a MALDI-TOF MS. All measurements were taken in a linear positive ion mode with a 25 kV accelerating voltage, a 79% grid voltage, and a 160 ns delay time.

III. RESULTS AND DISCUSSION

Fig. 1(a) describes the overall scheme for preparing the hemispherical perforated microwell array. The hemispherical microstructure with an open hole would be adequate not only for localizing the microbeads in the funnel shape and stabilizing the bead position during the bioassay process, but also for manipulating the bead capture and release. To achieve such a unique micropattern, the concave micropatterned Si wafer was fabricated through an isotropic wet

etching as a first step. Isotropic etching on a Si wafer was carried out in a HF, HNO₃, and CH₃COOH mixture with an etching rate of 3–6 $\mu\text{m}/\text{min}$. Fig. 1(b, i–iii) shows the replicated PDMS patterns, which were obtained from the wet-etched Si wafer as a template. As the etching time increased from 10 min to 25 min to 50 min, the diameter and depth are accordingly augmented from 62 and 32 μm (Fig. 1(b, i)) to 94 and 45 μm (Fig. 1(b, ii)) to 254 and 105 μm (Fig. 1(b, iii)).

As a second step, we produced a Ni mold with convex hemispherical micropatterns by a Ni electroplating process against the concave micropatterned Si wafer. The corresponding patterns of a Ni mold could be replicated to the acryl polymer substrate by hot embossing. The acryl sheet was chosen due to the facile bonding with PDMS by surface chemical modification. Other thermopolymers such as polycarbonate, polystyrene, and cyclic olefin copolymer can be selected, too, because hot embossing process is one of the straightforward methods for replicating the nano/microstructure in the thermopolymers. Fig. 1(b, iv–vi) represents the tilted, side, and enlarged view of an acryl sheet. The generated concave hemispherical microwells in the acryl layer were uniform and were well transferred from the Ni mold of Fig. 1(b, iii) without any shape deformation.

The last step was a backside etching to make small holes at the bottom of the hemispheres, which enables easy bead manipulation by controlling hydrodynamic force. The acryl material could be etched out with the RIE in the presence of O₂ plasma. Fig. 1(c, i–iii) shows a top, tilted, and enlarged view of the large-scale hemispherical single bead capture microarrays that retain a large hole on the top and a small one at the bottom.

The size of the bottom holes could be tuned by controlling the RIE exposure time. Fig. 3 displays the change of the bottom hole diameter depending on the RIE time. As the etching time increased, the size of the bottom hole was accordingly enlarged (Fig. 3(a)). Fig. 3(b) shows the relationship between the RIE time and the diameter of the bottom hole. The average hole size was 18, 26, 31, and 41 μm at the etching time of 25, 28, 31, and 36 min, respectively. Thus, we could tune the size of the bottom hole by RIE time as well as the top hole by the mask design depending on the bead type, so the optimal hemispherical microstructure could be fabricated for a variety of the bead based bioassay.

FIG. 3. (a) The diameter change of the bottom holes depending on the RIE time. (b) The relationship between the RIE time and the diameter of the bottom hole.

Fig. 2 describes the entire process for monitoring the high-throughput microbead based aptamer and target protein interaction in the porous hemispherical microstructure-integrated microdevice. Fig. 2(a, left) shows an individual layer (the top PDMS layer, the middle aryl layer, and the bottom PDMS layer) and a digital image of the assembled microfluidic device (Fig. 2(a, right)). The hemispherical microarray patterned aryl layer was bonded with the top and bottom PDMS layers. In the top PDMS layer, the sample solution was loaded in the inlet and recovered in the outlet. In the bottom PDMS layer, two control lines were connected to the microfluidic channel, which functions as a withdraw or infuse mode for bead manipulation. The top PDMS microfluidic channel was positioned orthogonal to the bottom PDMS channel. If the flow of the bottom layer was withdrawn by a syringe pump with a flow rate of 2 ml/h, the hydrodynamic force was exerted from the top layer to the bottom layer through the 3D hemispherical holes, resulting in the microbead directed toward the microhole arrays. Since we controlled the diameter of the top ($60\text{ }\mu\text{m}$) and bottom hole ($25\text{ }\mu\text{m}$), only single polystyrene microbead ($41\text{ }\mu\text{m}$ diameter) would be captured in each microwell and cannot pass through the hole. Thus, single bead array could be uniformly formed by the hydrodynamic fluidic control at the bottom layer with high speed (within 1 min) and high efficiency (almost 100%) [see Fig. 4(a), multimedia view]. If the infuse mode of the syringe pump was applied in the two control lines of the bottom layer as well as the outlet of the top PDMS layer with a flow rate of 10 ml/h, the captured microbeads were released in 10 s to the inlet of the top PDMS layer and could be recovered in the tube for further downstream analysis [see Fig. 4(b) multimedia view)]. This dynamic bead manipulation was possible due to the unique hemispherical microstructure combined with the simple flow control. Compared with the previous reports, which utilized gravity, dewetting, or magnet as a driving force to load the bead in the microwell, our methodology using hydrodynamic force can manipulate the bead with high simplicity, rapidity, and efficiency regardless of the bead type.^{15,17,25}

We applied the proposed bead array microdevice for monitoring the aptamer and target protein interaction. The *in situ* detection of the aptamer-protein complexes and rapid recovery

FIG. 4. (a) The microbeads were located at each microwell by the hydrodynamic fluidic control from the bottom layer with high speed and high efficiency. (b) The captured microbeads were released by applying an infuse mode in the two control lines of the bottom layer. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4903939.1>] [URL: <http://dx.doi.org/10.1063/1.4903939.2>]

of them enables us to collect the target aptamer. First, we confirmed the aptamer-target interaction on the off-chip base by incubating the aptamer labelled polystyrene beads with the target protein (Fig. S1).²⁴ Alexa Fluor 488 labelled lysozymes are bound on the lysozyme-binding aptamer conjugated microbeads, revealing the green fluorescence. In a similar way, Alexa Fluor 546 labelled thrombins are specifically interacted with the thrombin-binding aptamer conjugated microbeads to display the orange fluorescence. These results demonstrated the successful binding of the target proteins to the designated aptamers on the microbeads. Then, we performed the monitoring of the aptamer-target interaction on a chip and the characterization of the aptamer according to the scheme of Fig. 2(b). The lysozyme binding or thrombin binding aptamer conjugated polystyrene microbeads were loaded in the upper PDMS microchannel, and each bead was localized in the hemispherical microarray by applying the withdraw mode in the bottom PDMS channel. Then, fluorescent dye-labelled proteins were continuously injected and the linkage between the aptamer and the target protein was identified by observing the fluorescence signal on the beads. If the microbeads reveal the expected fluorescence, which means that the bound aptamers on the beads correctly captured the target proteins, the protein-aptamer conjugated beads are collected by the back-flow from the bottom PDMS layer. Then, the aptamers are isolated from the proteins and the beads by incubation at 90 °C for 2 min in a 99% formamide solution. Finally, the collected aptamers are characterized by MALDI-TOF MS analysis. Fig. 5(a) shows the bright field microscope image of a lysozyme binding aptamer coated microbead array, a green fluorescence image of the bead array after interaction with Alexa Fluor 488 labelled lysozymes, and the dark fluorescence image of the bead array after collecting the beads from left to right. Similarly, Fig. 5(b) displays the bright field image of a thrombin binding aptamer conjugated microbead array, an orange fluorescence image of the bead array after interaction with Alexa Fluor 546 labelled thrombins, and the dark fluorescence image of the bead array after collecting the beads. These results imply that the aptamers on the microbeads successfully captured the target protein, the binding event could be detected by the fluorescence signal at the single bead level, and the protein-aptamer-microbead complexes could be retrieved for the downstream aptamer characterization.

FIG. 5. (a) The bright field microscope image of a lysozyme aptamer conjugated microbead array, a green fluorescence image of the single bead array after interaction with Alexa Fluor 488 labelled lysozymes, and the dark fluorescence image of the bead array after collecting the beads from left to right. (b) The bright field image of a thrombin aptamer conjugated microbead array, an orange fluorescence image of the single bead array after interaction with Alexa Fluor 546 labelled thrombins, and the dark fluorescence image of the bead array after collecting the beads.

FIG. 6. MALDI-TOF MS data for (a) the lysozyme binding aptamers and (b) the thrombin binding aptamers.

The separated aptamers were analyzed by the MALDI-TOF MS. Since the microbead number could be controllable and the number of the aptamer on the single bead was abundant, the recovered microbeads could provide enough amount of aptamers to be analyzed by the MALDI-TOF MS. Because the molecular weight between the lysozyme-targeting aptamer (M.W.: 13 735 g/mol) and the thrombin-targeting aptamer (M.W.: 5475 g/mol) is quite different, we could distinguish the target aptamer by the mass data. Fig. 6(a) shows the mass peak at 13 798 m/z in which the aptamers were obtained from the green fluorescent microbeads. The mass value was almost matched with the theoretical value of the lysozyme-targeting aptamer. The peak at 5462 m/z in Fig. 6(b) was close to that of the thrombin-targeting aptamer. Therefore, we successfully identified the protein-specific aptamer using the high-throughput single bead microarray device and MALDI-TOF MS. Since the current MALDI-TOF MS technique can distinguish the mass of DNAs at the single Dalton difference with high sensitivity and accuracy, the target aptamer could be easily confirmed among the aptamer library to some extent. If the aptamer libraries are too huge to be screened by the mass spectrometry, the recovered aptamers could be analyzed in the DNA microarray platform as an alternative.

IV. CONCLUSIONS

We fabricated the large-area 3D hemispherical microwell structure, which has a larger hole on the top and a smaller one at the bottom. The diameter of the holes was controlled by the RIE exposure time, so the optimal hemispherical microarray could be prepared with ease

according to the size of the microbeads. The advantages of using such a unique microstructure for a bead-based bioassay are the facile bead manipulation (capture and release regardless of the bead type) by hydrodynamic force, the uniform single bead capture with high speed (within 1 min) and efficiency ($\sim 100\%$), and the accurate and stable localization of each bead. The aptamer screening capability was also executed by monitoring *in situ* aptamer-protein interaction and characterizing the aptamer by the MALDI-TOF MS. We are currently working on the development of an advanced microfluidic device and an optical system to enable the individual bead isolation, so that the aptamer selection among the large pool of aptamer libraries would be possible. In addition to monitoring the aptamer and target protein interaction, the proposed platform can be applied for a variety of bead assays for DNA, protein, and cellular bioanalysis.

ACKNOWLEDGMENTS

This work was supported by the grant from Korea CCS R&D Center (2013M1A8A1040878), and the Engineering Research Center of Excellence Program of Korea Ministry of Science, ICT & Future Planning (MSIP)/National Research Foundation of Korea (NRF) (Grant No. NRF-2014-009799).

- ¹O. Puig, F. Caspary, G. Rigaut, B. Rutz, E. Bouveret, E. Bragado-Nilsson, M. Wilm, and B. Seraphin, *Methods* **24**(3), 218 (2001).
- ²D. M. Rissin, C. W. Kan, L. N. Song, A. J. Rivnak, M. W. Fishburn, Q. C. Shao, T. Piech, E. P. Ferrell, R. E. Meyer, T. G. Campbell, D. R. Fournier, and D. C. Duffy, *Lab Chip* **13**(15), 2902 (2013).
- ³S. Allazetta, T. C. Hausherr, and M. P. Lutolf, *Biomacromolecules* **14**(4), 1122 (2013).
- ⁴M. F. Ali, R. Kirby, A. P. Goodey, M. D. Rodriguez, A. D. Ellington, D. P. Neikirk, and J. T. McDevitt, *Anal. Chem.* **75**(18), 4732 (2003).
- ⁵K. H. Lee, K. Y. Lee, J. Y. Byun, B. G. Kim, and D. M. Kim, *Lab Chip* **12**(9), 1605 (2012).
- ⁶N. Lion, F. Reymond, H. H. Girault, and J. S. Rossier, *Curr. Opin. Biotechnol.* **15**(1), 31 (2004).
- ⁷S. Venkatasubbarao, *Trends Biotechnol.* **22**(12), 630 (2004).
- ⁸X. X. Xu, Z. Y. Li, and A. Nehorai, *Biomicrofluidics* **7**(5) 054108 (2013).
- ⁹Y. Wan, Y. L. Liu, P. B. Allen, W. Asghar, M. A. I. Mahmood, J. F. Tan, H. Duhon, Y. T. Kim, A. D. Ellington, and S. M. Iqbal, *Lab Chip* **12**(22), 4693 (2012).
- ¹⁰W. Yue, H. Zou, Q. Jin, C.-W. Li, T. Xu, H. Fu, L. C. Tzang, H. Sun, J. Zhao, and M. Yang, *Biosens. Bioelectron.* **54**, 297 (2014).
- ¹¹W. H. Tan and S. Takeuchi, *Proc. Natl. Acad. Sci. U.S.A.* **104**(4), 1146 (2007).
- ¹²W. H. Henley, P. J. Dennis, and J. M. Ramsey, *Anal. Chem.* **84**(3), 1776 (2012).
- ¹³V. Sivagnanam, B. Song, C. Vandevyver, and M. A. M. Gijs, *Anal. Chem.* **81**(15), 6509 (2009).
- ¹⁴R. Burger, P. Reith, G. Kijanka, V. Akujobi, P. Abgrall, and J. Ducre, *Lab Chip* **12**(7), 1289 (2012).
- ¹⁵K. D. Barbee, A. P. Hsiao, M. J. Heller, and X. H. Huang, *Lab Chip* **9**(22), 3268 (2009).
- ¹⁶H. C. Tekin, M. Cornaglia, and M. A. M. Gijs, *Lab Chip* **13**(6), 1053 (2013).
- ¹⁷K. D. Barbee and X. H. Huang, *Anal. Chem.* **80**(6), 2149 (2008).
- ¹⁸C. W. Kan, A. J. Rivnak, T. G. Campbell, T. Piech, D. M. Rissin, M. Mosl, A. Peterca, H. P. Niederberger, K. A. Minnehan, P. P. Patel, E. P. Ferrell, R. E. Meyer, L. Chang, D. H. Wilson, D. R. Fournier, and D. C. Duffy, *Lab Chip* **12**(5), 977 (2012).
- ¹⁹J. A. Hansen, J. Wang, A. N. Kawde, Y. Xiang, K. V. Gothelf, and G. Collins, *J. Am. Chem. Soc.* **128**(7), 2228 (2006).
- ²⁰M. C. Rodriguez, A. N. Kawde, and J. Wang, *Chem. Commun.* **2005**(34), 4267.
- ²¹A. K. H. Cheng, B. Ge, and H. Z. Yu, *Anal. Chem.* **79**(14), 5158 (2007).
- ²²N. Hamaguchi, A. Ellington, and M. Stanton, *Anal. Biochem.* **294**(2), 126 (2001).
- ²³H. A. Ho and M. Leclerc, *J. Am. Chem. Soc.* **126**(5), 1384 (2004).
- ²⁴See supplementary material at <http://dx.doi.org/10.1063/1.4903939> for the interaction between the aptamer labelled polystyrene beads and target proteins on the off-chip basis.
- ²⁵Y. Cui, M. T. Bjork, J. A. Liddle, C. Sonnichsen, B. Boussert, and A. P. Alivisatos, *Nano Lett.* **4**(6), 1093 (2004).