A microfluidic detection system based upon a surface immobilized biobarcode assay

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ABSTRACT

The biobarcode assay (BCA) is capable of achieving low detection limits and high specificity for both protein and DNA targets. The realization of a BCA in a microfluidic format presents unique opportunities and challenges. In this work, we describe a modified form of the BCA called the surface immobilized biobarcode assay (SI-BCA). The SI-BCA employs microchannel walls functionalized with antibodies that bind with the intended targets. Compared with the conventional BCA, it reduces the system complexity and results in shortened process time, which is attributed to significantly reduced diffusion times in the micro-scale channels. Raw serum samples, without any pretreatment, were evaluated with this technique. Prostate specific antigen in the samples was detected at concentrations ranging from 40 pM to 40 fM. The detection limit of the assay using buffer samples is 10 fM. The entire assay, from sample injection to final data analysis was completed in 80 min.

1. Introduction

Over the past several years, a number of exciting advances have been made in the field of nanobiodiagnostics, resulting in assays that rival or surpass the selectivity and sensitivity of conventional detection methods (Adler et al., 2008; Alivisatos, 2004; Azzazy et al., 2006; Chan et al., 2002; Ferrari, 2005; Jain, 2005; Kim et al., 2007; Marko et al., 2007; Niemeyer et al., 2005; Rosi and Mirkin, 2005; Zheng et al., 2005). These advances have the potential to dramatically change the way medical diagnosis and treatments are performed in the future. Here, we present a fully integrated chip-based assay for performing detection of prostate specific antigen (PSA) in buffer and serum samples. This marker has been employed to monitor patient progress during treatment of prostate disorders and cancer (Jacobs and Haskell, 1991; Ozata et al., 1997).

Our detection scheme is based upon a modified version of the nanoparticle-based biobarcode assay (BCA). The BCA utilizes gold nanoparticles (Au NPs) functionalized with oligonucleotides (the so-called biobarcodes, which serve as surrogate targets and amplifying agents) and a target-recognition element, which may be an antibody for protein detection or a unique oligonucleotide sequence for nucleic acid detection. The BCA also uses functionalized magnetic microparticles (MMPs), which are adorned with antibodies that bind to the target. In the presence of targets (protein or oligonucleotide molecules) in solution, the MMPs form a sandwich complex with the targets and gold nanoparticles, which can be localized and collected under an applied magnetic field (Goluch et al., 2006a; Shaikh et al., 2005). The barcode oligonucleotide molecules are then chemically released, identified, and quantified.

The BCA is highly sensitive. In the bench-top format, the BCA assay has been shown to achieve low-attomolar sensitivity for protein analytes (Nam et al., 2003), up to five orders of magnitude lower than analogous ELISA technology, the benchmark method for protein detection. The BCA also has exhibited high-zeptomolar sensitivity for nucleic acid targets, rivaling the PCR-amplification method, which requires thermal cycling (Nam et al., 2004). The BCA assay is also highly specific and capable of extensive multiplexing (Stoeva et al., 2006a,b). Therefore, the BCA offers...
several unique diagnostic opportunities including early disease detection, monitoring of disease recurrence, and the possibility of simultaneous multiplexed analysis of a panel of disease markers (Georganopoulou et al., 2005; Stoeva et al., 2006a).

The BCA was originally developed in a bench-top format. Chip-based systems utilizing bio- and nanotechnology are desired as they can provide unparalleled speed, automation, and repeatability (Craighead, 2006; Dittrich et al., 2006; Garstecki et al., 2006; Hatch et al., 2001; Thorsen et al., 2002). The established bench-top BCA protocol cannot be directly transferred to a chip-based format due to unique scaling, materials, and microengineering issues. We have successfully demonstrated a modified BCA that takes advantage of unique microfluid scaling and adjusts for the constraints of lab-on-chip (LOC) systems (Goluch et al., 2006a,b; Shaikh et al., 2005).

Chip-based BCAs developed thus far have employed MMP particles. However, there are a number of issues associated with executing this approach on chip. For example, because of the large volume difference between MMPs and NP probes, it is very easy for NPs to become trapped in the packed bed of MMPs. In practice, this unintentional trapping increases the false-positive rate. Removing non-specifically trapped gold nanoparticles is not practical as it requires extensive wash protocols and substantially increases assay time (by nearly 30 min). Further, the use of MMPs requires the use of switchable electromagnets for the on-chip instrument, increasing the complexity and cost of the system.

A simple alternative is to directly pattern the monoclonal antibodies on the walls of the microfluidic channels. In the presence of targets, the NPs would be bound to the channel wall through the target linker. Instead of forming an MMP-target-NP sandwich in solution and then pulling the sandwich to the reactor sidewall using a magnetic field, the sandwich is formed at the reactor wall. This new surface immobilized biobarcode assay (SI-BCA) protocol eliminates the need to use magnetic microparticles and reduces false-positive readings in microfluidic devices. It also reduces the complexity of the microfluidic system by eliminating the need for creating a magnetic field on demand.

We will later provide analysis to show that the SI-BCA is especially suited for chip-based implementation due to favorable scaling laws. In fact, running the SI-BCA on the bench-top becomes impractical because of the long reaction times required for capturing all of the target molecules from solution.

1.1. Description of the SI-BCA assay

The chip-based assay employs a custom microfluidic chip, which consists of a target capture area and a barcode identification area separated by pneumatically actuated isolation valves (Fig. 1). One wall of the fluid channel in the target capture area is functionalized with monoclonal antibodies specific for a unique epitope on the target antigen. Target antigens present in the sample solution will be trapped in the capture area (Fig. 1B), similar to other microfluidic ELISA approaches (Eteshola and Leckband, 2001; Herrmann et al., 2007; Lai et al., 2004). Second, 30-nm-diameter Au NP probes, co-functionalized with barcode oligonucleotides and an antibody (monoclonal or polyclonal) that recognizes the target protein, are introduced. In the presence of target molecules, which can bind to both the Au NP and the functionalized surface specifically, the Au NPs are anchored to the substrate (Fig. 1D). After washing away non-specifically bound NP probes, we release the barcode strands, acting as surrogates for the target molecule, through a ligand-exchange process induced by dithiothreitol (DTT) (Fig. 1E) (Thaxton et al., 2005). Released barcode strands are transferred to the barcode identification area and identified. The presence, amount, and
sequence of barcode molecules can be detected by a variety of methods. Fig. 1F–H illustrate the high sensitivity scanometric detection method used in these studies (Taton et al., 2000).

The scanometric identification involves three steps: hybridization of the barcode oligonucleotides to the capture strands printed on the chip (Fig. 1F), attachment of 13-nm-diameter Au NP probes to the barcode DNA by hybridization (Fig. 1G), and silver staining of the hybridized probes (Fig. 1H). Note that half of the barcode sequence for each target is the target-reporting oligonucleotide probe, while the other half is identical for all of the barcodes. We use a universal 13-nm-diameter Au NP probe, functionalized with a single oligonucleotide sequence, for the scanometric identification of all barcode DNA strands (Stoeva et al., 2006b). The captured Au NPs promote the reduction of Ag(I) to Ag in the presence of hydroquinone which enhances the signal associated with probe binding (Storhoff et al., 2004). Finally, we determine the presence of the silver-stained probes using a Verigene ID system (Nanosphere Inc.), which records the scattered light from the developed spots after silver amplification.

1.2. Continuous flow reactor design

In LOCs, continuous flow channel reactors are typically used. Since the entire channel volume is relatively small, it is important to use a continuous flow system to accommodate large sample volumes. From a practical point of view, continuous flow eliminates clogging.

It is important to determine whether continuous flow over a single reactive wall will be sufficient in capturing target analytes in our microfluidic device. The relevant parameters in our device are: the channel height $h$ (200 μm), the length of the reactive channel $L$ (2.08 cm), the linear velocity $U$ ($5.56 \times 10^{-1}$ cm s$^{-1}$), the diffusivity of the target protein $D$ ($1 \times 10^{-6}$ cm$^2$ s$^{-1}$), the association and dissociation rate constants for our antibody-target system $k_{on}$ and $k_{off}$ ($6.26 \times 10^4$ M$^{-1}$ s$^{-1}$ and 6.45 $\times 10^{-7}$ s$^{-1}$), the initial concentration of target analyte $C_0$ (10 pM), and the surface density of antibodies $C_{BS}$ (70 fmol mm$^{-2}$). A schematic of our continuous flow reactor is illustrated in Figure 2S in the supplementary material. The following set of equations (Gervais and Jensen, 2006) describes the dynamics of target molecule transport and reaction with surface bound entities:

$$\frac{\partial \Theta_i(\eta, Gr, \tau)}{\partial \tau} = \left( \frac{\partial^2 \Theta_i(\eta, Gr, \tau)}{\partial \eta^2} + \frac{1}{Pe^2} \frac{\partial^2 \Theta_i(\eta, Gr, \tau)}{\partial Gr^2} \right) - \nu(\eta) \frac{\partial \Theta_i(\eta, Gr, \tau)}{\partial Gr},$$

$$\left( 1 \right)$$

$$\frac{\partial \Theta_3(Gr, \tau)}{\partial \tau} = \epsilon Da[\Theta(\eta=0, Gr, \tau)(1-\Theta_3(Gr, \tau)) - K_0 \Theta_3(Gr, \tau)],$$

$$\left( 2 \right)$$

and

$$\frac{\partial \Theta_i(\eta, Gr, \tau)}{\partial \eta} \bigg|_{\eta=0} = \frac{1}{\epsilon} \frac{\partial \Theta_3(Gr, \tau)}{\partial \tau},$$

$$\left( 3 \right)$$

where $\Theta_i$ are normalized concentrations ($\Theta_i = C_i/C_0$), $i = S$ is the concentration of surface bound targets and $i = W$ is the analyte concentration near the reactive wall), $\eta$ is the dimensionless height ($\eta = x/h$), $Gr$ is the Graetz number ($Gr = zh^{-1}Pe^{-1}$), $t$ is the normalized diffusion time ($t = Dt/h^2$), $Pe$ is the Péclet number ($Pe = hU/D$, $\nu(\eta)$ is the normalized velocity profile, $\epsilon$ is the relative adsorption capacity ($\epsilon = C_0h/C_S$), $Da$ is the Damköhler number ($Da = k_{on}C_{BS}h/D$), $K_0$ is the dimensionless equilibrium constant ($K_0 = k_{off}/(k_{on}C_0)$).

The pertinent dimensionless parameters that define the depletion of the target molecule from the bulk solution are the Damköhler number ($Da$) and the Graetz number ($Gr$).

The Damköhler number determines the chance that a target molecule is immobilized once it reaches the surface. When $Da > 1$, the transport to the surface is diffusion limited. Meanwhile, for cases where $Da < 1$, the transport becomes limited by the surface reaction (Glaser, 1993).

The Graetz number is a ratio of the diffusion and convection length scales. At $Gr = 1$, the time required for a particle to diffuse across the channel height equals the time it takes for travel through the channel. For $Gr < 1$, a portion of the analytes in the channel are not able to reach the reactive surface before leaving the reactive region.

In a successful design of channel dimensions and experimental conditions, a majority of target particles must reach the surface ($Gr > 1$) and then must be captured by the surface ($Da > 1$). The reactor design must satisfy these two criteria simultaneously. Channel design and flow rates are also limited by practical issues including fabrication constraints. Our chip design yielded a $Gr$ of 3.32 and $Da$ of nearly 1 (0.657).

We now attempt to quantify the percentage of target molecules that bind with the surface. We solve Eqs. (1)–(3) using the design specifications listed above. Generally, Eqs. (1)–(3) must be solved numerically. However, Gervais and Jensen have shown that by applying the following assumptions to Eqs. (1)–(3), the problem can be simplified and solved analytically.

Such assumptions are summarized here. First, even though the diffusivity in protein-dense biological media can be non-linear (Elowitz et al., 1999), we assume constant diffusivity here because our sample is diluted 3:1 in buffer before being introduced into the device. Second, we assume that the concentration of capture antibodies on the surface of the channel ($C_{BS}$) is much higher than the concentration of the target protein in solution ($C_0$). For our system, even at the highest target concentration of 10 pM, the number of available binding sites on the surface is over 100 times higher than the number of target molecules in the total sample volume. Third, we assume that the association rate ($k_{on}$) is much higher than the dissociation rate ($k_{off}$). Association and dissociation rates ($k_{on}$ and $k_{off}$) for our capture system were obtained using Biacore Surface Plasmon Resonance System (GE Healthcare, Piscataway, NJ). When the above-mentioned conditions are met, the transport in the bulk can then be assumed to be in pseudo-steady state with respect to the surface at all times. Finally, when operating in a microfluidic device, the axial convection is assumed to be much faster than the axial diffusion (Péclet ≫ 1). In our case, Péclet = 500. This means that axial diffusion can be neglected.

When all of the assumptions from the previous paragraph are valid, the transport (Eq. (1)) and reaction (Eq. (2)) equations are no longer coupled by the boundary condition at the reactive wall (Eq. (3)). Eq. (1) simplifies to

$$\frac{\partial^2 \Theta}{\partial \eta^2} = \nu(\eta) \frac{\partial \Theta}{\partial Gr},$$

$$\left( 4 \right)$$

For a single reactive wall, $\nu(\eta)$ is

$$\nu(\eta) = 6\eta(1 - \eta).$$

$$\left( 5 \right)$$

The boundary condition at the reactive wall is

$$\frac{\partial \Theta}{\partial \eta} \bigg|_{\eta=0} = -\frac{1}{\epsilon} \frac{\partial \Theta_3}{\partial \tau} = -Da \Theta_W.$$  

$$\left( 6 \right)$$

The condition at the insulated wall is

$$\frac{\partial \Theta}{\partial \eta} \bigg|_{\eta=1} = 0$$

$$\left( 7 \right)$$

and the initial condition is

$$\Theta(\eta, Gr = 0) = 1.$$  

$$\left( 8 \right)$$
The exact solution to this problem has been recently presented by Gervais and Jensen.

The parameter values for our device were used to calculate the solution to Eq. (4) under conditions (5)–(8). Based on these values, the final concentration of target molecules at the exit of the reaction chamber is determined to be 0.08, meaning that only 8% of the original targets that enter the channel remain in the solution at the exit. In other words, approximately 92% of the target molecules are trapped in the separation area of the device using a continuous flow system. This analysis shows that the continuous flow design of our device successfully removes and immobilizes the majority of protein targets from the sample.

2. Experimental setup

2.1. Experimental protocol

The samples were diluted 3:1 in assay buffer. The samples (20 μL) containing the antigens with different combinations were then passed through the device at a flow rate of 1 μL/min. A wash step (5 μL of assay buffer) was performed next to remove any nonspecifically bound proteins. The flow rate was increased during all wash steps to 2 μL/min.

The solution containing 30-nm-diameter Au NP probe (150 pM) was centrifuged for 8 min at 6,000 rpm to spin down all of the NP probes. The supernatant was removed and the particles were resuspended in assay buffer, back to the concentration of 150 pM. Next, 5 μL of the NP probe solution was flowed through the antigen capture area. The excess Au NP probes were removed from the system by flowing 5 μL of assay buffer.

The control valves were switched to allow the transfer of the released barcodes to the detection area where the barcode DNA hybridized with the immobilized complementary oligonucleotide strands. The barcode DNA strands then were released from the NP probes by flowing 5 μL of 0.5 M DTT (Sigma Ultra, Cat. No. D5545) in scanometric buffer. The excess DTT was removed from the channels by flowing 5 μL of scanometric buffer at a rate of 2 μL/min. Next, 5 μL of 13-nm universal Au NP probe solution (1 nM Au NP in scanometric buffer) was flowed through the channels, followed by 5 μL of 0.5 M aqueous sodium nitrate that removed chloride ions and any unbound NPs. The PDMS piece was removed, the glass slide was rinsed with 0.5 M sodium nitrate and spin dried. The slide was then silver stained for 2 min, rinsed with water, spin dried for 30 s, and visualized with a Verigene ID system (Nanosphere).

2.2. Preparation of glass slides

Localized functionalization of microfluidic channels is a major hurdle (Goluch et al., 2006a,b; Shaikh et al., 2005). For the SI-BCA, it is necessary to immobilize both proteins and DNA with high spatial resolution on a single slide, while still allowing alignment to the PDMS device. To address this problem, we employed a contact microarray printer (LabNext, Chicago, IL) to print both antibodies and DNA on the slide as a series of overlapping spots, akin to dot-matrix printing, as seen in Fig. 2. The overlapping spots form lines on the slide that allow easy alignment to the PDMS chip. A detailed description of the printing procedure is available in the supplementary material.

2.3. PDMS chip fabrication

Microfluidic chips are fabricated by molding two PDMS pieces and bonding them in a layered, registered fashion. One PDMS piece contains fluid channels while a second hosts pneumatic valve control lines (Thorsen et al., 2002). A detailed protocol is described in the supplementary material.

2.4. Instrumentation

The control valve lines in the PDMS chip are dead-end filled with glycerin (Sigma–Aldrich) in order to prevent bubble formation within the fluid channels when the control valves are activated. When the experiment is ready to be run, the preprinted slide is brought to room temperature, rinsed very briefly in DI water and spin dried. The PDMS chip is then reversibly bonded to the preprinted glass slide, maintaining that the printed areas on the slide align with the capture and detection regions of the chip. All reagents and samples are passed through the device in a sequential manner. Vacuum applied in the channels pulls the reagents through the chip, while control valves regulate the pathway that the fluid takes. A custom written Labview program regulates a controller box, which delivers pressurized air and vacuum to the microfluidic device.

2.5. Preparation of 30-nm-diameter gold NP probes

Gold nanoparticles (30 nm, Ted Pella Inc.) were used as received at a concentration of 330 pM. The 30-nm Au NPs were co-functionalized with custom synthesized DNA and the corresponding detection antibodies (polyclonal anti-PSA antibody, AF1344, R&D Systems). The synthesized DNA is attached to the Au
NP S via a triethylthiol bond on the 5′ end (Li et al., 2002). The 3′ end of the DNA contains a tosyl group that reacts with primary amines on the antibodies. The sequence used for the barcode is: 5′-3′-thiol-functionalized oligonucleotides (Sigma–Aldrich). The 13-nm nanoparticles were functionalized with freshly deprotected thiol-functionalized oligonucleotides and attached first, followed by attachment of the barcode DNA. The new approach attaches the barcode DNA before the antibodies are added. The barcode DNA acts as a bifunctional linker with three thiol groups on one side and a tosyl group on the other. The thios form a very strong bond with the gold nanoparticle, while the tosyl groups react with amine groups on the antibodies to form a covalent bond. Both a greater number and much more consistent amounts of barcode DNA are attached per nanoparticle. This new approach also increases the particle stability. Experiments were carried out over the course of 4 weeks with no appreciable loss of sensitivity.

2.6. Preparation of universal gold NP probes

For scanometric detection, gold nanoparticles with a 13-nm average diameter were synthesized by citrate reduction of HAuCl₄ (Sigma–Aldrich). The 13-nm nanoparticles were functionalized with freshly deprotected thiol-functionalized oligonucleotides and salted in a step-wise fashion to 0.3 M NaCl, 10 mM phosphate buffer, 0.01% SDS, pH 7.2 over 24 h. The DNA (IDT Inc.) sequence was 5′-HS-AAA AAA AAA AAA AA-3′. The 13-nm NP probe was then purified from excess DNA with three centrifugations at 14,000 rpm at 20 °C for 25 min. The particles were washed and finally resuspended in 0.5 M NaCl, 10 mM phosphate buffer, 0.01% Tween 20, pH 7.2 at a concentration of ~10 mM, and stored at 4 °C.

2.7. Preparation of assay buffer

First, 32.5 μL of an aqueous 20% Tween 20 solution (Sigma Ultra) and 250 μL of 10% BSA (R&D Systems, Cat. No. DY995) are added to 25 mL of 1× PBS (Invitrogen, Cat. No. 14190-144, Dulbecco buffer). After mixing, 25 mg of poly-acrylic acid (Sigma, Cat. No. 420344) is added to the solution.

2.8. Preparation of scanometric buffer

A 10× PBS solution (Ambion, Cat. No. 9624) is diluted to 2× using milli-Q (Millipore) water. Tween 20 is added to the PBS to a final concentration of 0.01%. The final solution specifications are 0.5 M NaCl, 10 mM phosphate buffer, pH 7.2.

2.9. Samples

Donkey serum (Bioreclamation) was used as received. Samples were prepared by spiking various concentration of prostate specific antigen (Sigma–Aldrich, Cat. No. P3235) into the undiluted donkey serum prior to detection.

3. Results and discussion

We must first prove that our chip design will indeed capture the target protein molecules as predicted. This is accomplished by directly measuring the amount of protein immobilized in the capture region of the channel, which we discuss in Section 3.1.

Once it was established that the target proteins are captured successfully, we then attempted the full experimental protocol where surface immobilization of targets is combined with barcode amplification and scanometric detection to achieve added sensitivity. The results of the full SI-BCA experiments are discussed in Section 3.2.

Tests were conducted with human PSA added to buffer samples (supplementary material) and untreated donkey serum.

3.1. Direct capture and detection of protein targets

It is necessary to demonstrate that the antibodies remain functional after they are patterned on a glass slide. This is achieved by running a simple bench-top experiment. Four sets of anti-PSA antibody spots are patterned on a glass slide and hybridization chambers are placed over each set of spots. The chambers are then filled with solutions containing different concentrations of PSA (blank, 1 pM, 10 pM, and 100 pM) and allowed to incubate for 2 h at 37 °C and 150 rpm. The chambers are disassembled, rinsed, reconstructed and filled with solutions containing 30-nm NP probes. The probes incubate for 1 h at 37 °C and 150 rpm after which time, the chambers are again removed and the slide is rinsed rigorously with sodium nitrate and silver stained. If the antibodies remain functional on the surface, a sandwich will form and be detected since the 30-nm probes have a gold center and therefore catalyze the silver staining reaction the same way as the 13-nm Universal NP probes. Fig. 3 shows the results of one such set of experiments with a blank control, 1 pM PSA, 10 pM PSA, and 100 pM PSA. Concentrations of PSA below 1 pM were undetectable using this approach. The signal response fits an exponential equation $\left[ Y = -0.93803 \exp(-X/(5.53914 \times 10^{12})) + 0.98955 \right]$ with an $R^2$ value of 0.98.
Once the viability was demonstrated on the bench-top, the experiment was tested in a chip-based format. Overlapping spots of antibodies were printed on the slides to form solid lines. Fortunately, the GOPS functionalization can be used for attachment of both DNA and antibodies, as single stranded DNA can be purchased commercially with an amine group terminator and most proteins have amine groups available for binding. A PDMS chip with microchannels was placed over the patterned antibodies and samples containing various concentrations of PSA were flowed through the device. The channels were then flushed with buffer and 5 μL of NP probes were sent through, followed by a wash with sodium nitrate. The PDMS chip was then removed and the slide was silver stained. Fig. 4 shows the results from one such experiment with channels 1 and 2 containing only control serum, channels 3 and 4 containing 400 pM PSA, channels 5 and 6 containing 4 nM HCG as a control, and channels 7 and 8 having 4 nM PSA. These results show that the antibodies patterned on the surface are very selective and that the proteins can be captured using a microfluidic format. Experiments with PSA concentrations below 100 pM did not give a discernable signal. The difference in detection limit versus the bench-top experiment is attributed to the fact that the chip-based format is not allowed to reach equilibrium and as a result each antibody region captures only a few percent of the total targets present in the sample.

3.2. SI-BCA: full assay results

In the previous section, the immobilized target proteins were detected directly with a 1 to 1 ratio of targets to gold nanoparticles. The full potential of the assay is not realized though unless the signal is amplified by the release of barcode DNA. Once all of the parts of the assay were shown to function properly, the complete assay was ready for testing. First, PSA was spiked into assay buffer at 10 pM, 1 pM, and 10 fM concentrations for comparison against a control blank [supplementary material].

PSA detection was then carried out in donkey serum (Fig. 5). Sixteen experiments were run in parallel on one microfluidic chip. The serum is diluted 3:1 in buffer before being loaded onto the chip. Four independent replicate experiments were carried out using a control serum sample and final PSA concentrations in serum of 10 pM, 100 fM, and 10 fM. The concentrations in the graph are for diluted serum that was loaded into the device, which matches the detection limit obtained by PSA spiked directly into buffer. Acrylic acid in the buffer prevents non-specific adsorption of protein in the channel. The serum sample was not pretreated. It was used as obtained from the company. The experiment was carried out using a serum sample and completed in 80 min counting from the first sample being loaded to visualization of the results after silver staining. The data was fit to an exponential equation $Y = -0.61383 \exp(-X/(2.00103 \times 10^{-13})) + 0.99869$ with an $R^2$ value of 0.79.

4. Conclusions

We have developed and validated a novel chip-based approach for carrying out biomarker detection using functionalized gold nanoparticles. Compared with the bench-top BCA, the chip-based system decreases the reagent volumes, shortens the assay time, and achieves considerably high sensitivity and selectivity. The shortened assay time is attributed to small diffusion times that result in micro-scale channels versus bench-top approaches. Our system also eliminates the need for several pieces of equipment used in the bench-top assay including incubators, vortexes, and centrifuges.

The channel design is tailored for the SI-BCA. A low aspect ratio channel (200 μm wide × 15 μm tall) creates target capture and detection regions that promote target interactions with the functionalized walls. Our results establish that molecular diffusion coupled with the increased local concentration within the microchannels provides sufficient antibody/antigen and DNA/DNA
interactions to eliminate the need for heated incubation and vortexing that is typically employed in commercial techniques.

The complexity of the new chip-based SI-BCA is much lower compared to previous chip-based BCAs as well (Goluch et al., 2006a; Shaikh et al., 2005). Magnetic particles and instrumentation for manipulating them are not required. There is no incubation time in the assay, and wash steps are shortened. Further, the chance of trapping nanoparticle probes is greatly reduced by the elimination of MMPs from the protocol.

The chip layout was changed significantly for this version of the BCA. The design now allows for 16 independent experiments to be run simultaneously. Our chip design also allows the identification of many unique barcode sequences by simply printing additional texting that is typically employed in commercial techniques.

The detection limit of the assay is 10 fM with buffer samples. Serum samples can be used in the assay without any prior pretreatment. For prostate specific antigen detection in serum samples, the detection limit of the assay is 10 fM with buffer samples.

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Appendix A. Supplementary data


References