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Ultrasensitive DNA Microarray Biosensing via *in Situ* RNA Transcription-Based Amplification and Nanoparticle-Enhanced SPR Imaging

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Supporting Information

ABSTRACT: DNA microarrays are invaluable tools for the detection and identification of nucleic acids in biosensing applications. The sensitivity and selectivity of multiplexed single-stranded DNA (ssDNA) surface bioaffinity sensing can be greatly enhanced when coupled to a surface enzymatic reaction. Herein we describe a novel method where the specific sequence-dependent adsorption of a target ssDNA template molecule onto an ssDNA-modified gold microarray is followed with the generation of multiple copies of ssRNA via *in situ* surface transcription by RNA polymerase. The RNA created on this "generator" element is then detected by specific adsorption onto a second adjacent "detector" element of ssDNA that is complementary to one end of the ssRNA transcript. SPR imaging is then used to detect the subsequent hybridization of cDNA-coated gold nanoparticles with the surface-bound RNA. This RNA transcription-based, dual element amplification method is used to detect ssDNA down to a concentration of 1 fM in a volume of 25 μL (25 zeptomoles).

The simultaneous detection of multiple DNA or RNA sequences at femtomolar concentrations in a microarray format is a challenging yet necessary stepping stone for the implementation of a variety of new diagnostic biosensing and biomarker discovery applications.[1−3] PCR amplification methods with fluorescence detection have sufficient sensitivity to detect single sequences of DNA at these concentrations but are difficult to implement for large numbers of sequences and do not always correctly reproduce the relative concentrations of multiple target oligonucleotides. Other surface enzymatic reaction schemes have been used in conjunction with both fluorescence imaging and surface plasmon resonance imaging (SPRI) with some success.[4,5] For example, in a previous paper we have detected DNA reaction products via a DNA polymerase (Klenow fragment) extension reaction on microarrays in conjunction with nanoparticle-enhanced SPRI.[6] The most advanced eventual application of DNA microarrays would be to create proteomic microarrays via the canonical information flow of biological systems (i.e., the surface enzymatic conversion of DNA to RNA to proteins), but this requires much more complex surface enzymatic processing. Thus, to date the direct microarray detection of RNA and proteins encoded by an extremely small number of DNA templates has not been widely implemented.[7−9] As a first step in this direction, in this paper we demonstrate a highly sensitive isothermal surface enzymatic method for detecting ssDNA analytes at femtomolar concentrations via the microarray quantitation of *in situ* transcribed RNA at picomolar concentrations. A target template ssDNA molecule is first specifically adsorbed onto a complementary surface-bound promoter sequence in a microarray, and then an *in situ* surface RNA polymerase reaction is used to transcribe multiple ssRNA copies from this "generator" microarray element (see Figure 1). The transcribed ssRNA is then hybridized to a DNA oligonucleotide on a second nearby "detector" element and is detected with nanoparticle-enhanced SPRI.[10,11]

Our demonstration of this transcription-based detection methodology employed a 16 element DNA microarray that was created using 1 mm diameter gold thin (45 nm) film spots on a SF10 glass substrate. Amine-modified ssDNA was immobilized onto the microarray elements using a polyGlu/EDC/NHSS attachment chemistry described previously.[12] Sixteen ssDNA elements were created (see Figure 1): four generator elements (G) containing an ssDNA promoter sequence complementary to the ssDNA template, eight detector elements (D) containing ssDNA partially complementary to the ssRNA transcripts, two positive control elements (+C) with ssDNA complementary to the ssDNA-modified gold nanoparticles, and two negative control elements (−C) with a noncomplementary ssDNA 30-mer to detect any nonspecific adsorption. The specific DNA sequences are given in the Supporting Information.

Template ssDNA was specifically adsorbed via base pairing to form duplexes ("base pairing adsorption") on the generator elements and then used for the *in situ* surface transcription of ssRNA by RNA polymerase. Base pairing adsorption was accomplished by exposing the array to a 25 μL volume of template ssDNA solution with concentrations ranging from 10 pM down to 1 fM in a phosphate buffered saline solution (Na-PBS, 10 mM sodium phosphate, 0.3 M NaCl, 5 mM MgCl, and 2.7 mM KCl), pH 7.4. The total time required for this experiment to provide optimum results is governed by the hybridization time necessary for the template DNA to absorb onto the surface bound DNA assay.[13] Thus, the minimum exposure time varied from 45 min for 10 pM to 4 h for 100 fM and to 36 h for a 1 fM concentration. To absolutely ensure complete template binding, the adsorption time was kept constant at 36 h for all samples. The RNA transcription reaction time and SPR imaging were kept constant at 2 h and 12 min, respectively. The slides were rinsed with PBS

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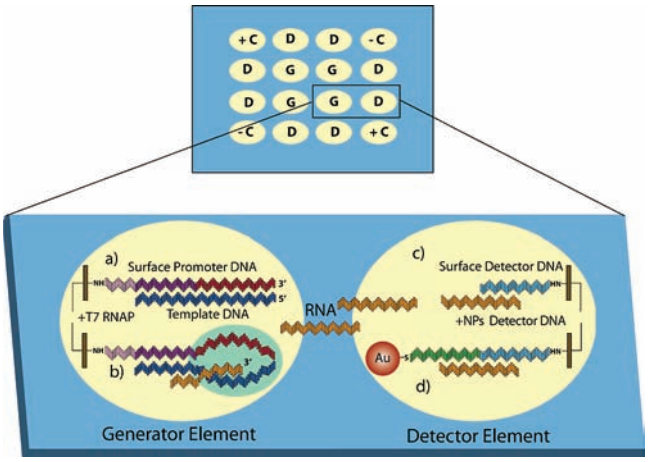

Figure 1. Schematic diagram showing the surface chemistry of generator and detector elements. (a) On the generator elements a surface promoter DNA is covalently attached to the gold surface via an amide bond using polyGlu/EDC/NHSS chemistry and then is base-paired with template DNA from solution. (b) The surface is exposed to a solution of T7 RNA polymerase and rNTPs to produce multiple copies of RNA. (c) The RNA reaches the detector elements through diffusion in the solution and hybridizes with the surface detector DNA. (d) The amount of template DNA is quantitated by measuring the amount of transcribed RNA on the detector element via RNA hybridization with DNA-modified AuNPs in an SPR imaging instrument.

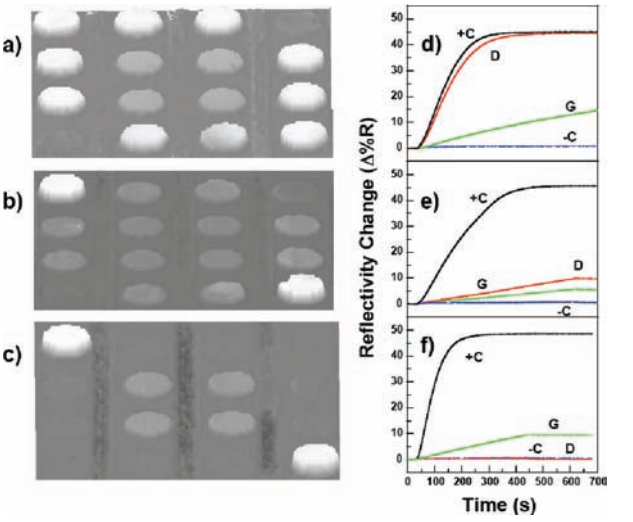

Figure 2. SPRI difference images showing four different types of active areas: generator (G), detector (D), positive control (+C), and negative control (−C) spots that were reacted with (a) 10 pM, (b) 100 fM, and (c) 0 fM template DNA (Control) and the corresponding kinetics of each of the four different elements (d−f).

and water and then placed in a 25 μL Frame-Seal incubation chamber (BioRad) at 37 °C for 2 h. In this chamber, ssRNA was produced from the surface-bound templates by an *in vitro* RNA transcription (IVTx) reaction. As the ssRNA was generated, it diffused to the adjacent detector elements and hybridized onto immobilized complementary ssDNA (see Figure 1). The diffusion time from a G element to an adjacent D element is at most a couple of minutes as the interelement spacing is approximately 1 mm.

After the RNA transcription and hybridization adsorption, the slides were rinsed with PBS to remove any enzyme and unincorporated ribonucleotides and then placed directly in an SPRI cell for the nanoparticle-enhanced detection of the transcribed RNA. SPRI images and kinetic curves were obtained as the array was exposed for 700 s to a 4 nM solution of ssDNA-modified 13 nm gold nanoparticles (AuNPs) that were partially complementary to the ssRNA as depicted in Figure 1d. A control experiment slide was prepared under similar conditions with the modification that no target DNA was used in the hybridization reaction. SPRI difference images and kinetic curves for three DNA template concentrations (10 pM, 100 fM, and 0 fM) are shown in Figure 2, while the data for the lowest concentration observed, 1 fM, are included in the Supporting Information.

The use of AuNPs for enhanced-SPRI have been described and optimized previously.[5b,10,12a,14] A diameter of 13 nm for the AuNPs was demonstrated as optimal for (i) DNA surface attachment chemistry, (ii) signal enhancement for near-infrared SPRI measurements, and (iii) low nonspecific adsorption of the nanoparticles. The largest enhancements have been recently observed with gold nanorods.[15]

Four kinetic curves are shown for each of the template DNA concentrations in Figure 2d−f. The positive control elements (+C, black curves) have a differential reflectivity change (Δ%R) of 45 ± 5%, corresponding to the formation of a full gold nanoparticle monolayer. The negative control (−C, blue curves) has a Δ%R of approximately 0.3%, showing that there is minimum nonspecific adsorption of gold nanoparticles onto the ssDNA microarray. The generator elements (G, green curves) also show some nanoparticle adsorption with a Δ%R of 10−20%. The adsorption of nanoparticles onto the generator elements (G) is attributed to the base pairing of ssDNA-modified gold nanoparticles with the residual ssDNA surface promoter molecules that did not form a duplex with a complementary target DNA template, and varied from sample to sample. However, nonspecific adsorption onto the G elements does not affect the measurements; AuNPs adsorption onto the negative control array elements (−C) was always minimum. This verifies the two-element methodology that separates the surface generation chemistry from the surface detection chemistry. The partitioning of elements is essential to achieve the lowest nonspecific binding on the detector elements when enzymatic reactions are employed on surfaces.

Most importantly in Figure 2, the detector elements (D, red curves) clearly show Δ%R values that vary systematically between 0% and the positive control value (ca. 45%) depending on the ssDNA template concentration. A more complete plot of the DNA template concentration dependence of Δ%R for the detector elements is shown in Figure 3.

Also included in Figure 3 is the Δ%R for an experiment that had no template DNA (red curve). These data clearly demonstrate that the detector element Δ%R can be used to reliably track the ssDNA template concentration, with the lowest detectable concentration of 1 fM, and a maximum concentration of ca. 10 pM (corresponding to the formation a full gold nanoparticle monolayer). Since a 25 μL solution was used in these measurements, the 1 fM detection limit corresponds to approximately 15 000 molecules.

From these SPRI measurements, we can conclude that (i) RNA is made at the generator elements via an *in vitro* surface transcription reaction, (ii) RNA molecules diffuse to the detector elements and hybridize to the detector elements, and (iii) nanoparticle-enhanced SPRI can be used to detect the ssRNA created from DNA template solutions from 1 fM to 10 pM.

How many copies of ssRNA are made from each template molecule? This number can be estimated by performing reverse transcription and quantitative PCR (RT-qPCR) of the ssRNA created in

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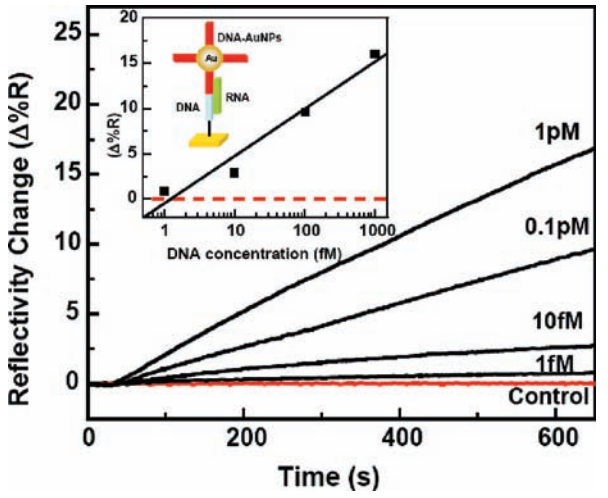

Figure 3. Kinetic curves corresponding to AuNPs binding by DNA–RNA hybridization to the detector elements for a series of template DNA concentrations: 1 pM, 0.1 pM, 10 fM, 1 fM, and a negative control. The inset figure plots the differential reflectivity change (Δ%R) at 700 s versus the template DNA concentration on a logarithmic scale.

the surface transcription solution. The results for several samples are shown in the Supporting Information (Figure S3). RT-qPCR analysis indicates that an amplification factor of 2000 was obtained from the transcription process. For example, the specific adsorption of template DNA onto the generator spots from a 100 fM target solution yielded an approximately 200 pM ssRNA solution in the 25 μL Frame Seal volume, which was then detected with the nanoparticle-enhanced SPRI measurements. The 1 fM limit of detection for template DNA is reasonable given that the limit of detection for ssRNA with nanoparticle-enhanced SPRI is approximately 1–5 pM.[10,11]

In summary, we have demonstrated a highly sensitive DNA detection assay that uses surface-based RNA transcription to detect DNA templates in a dual element microarray format at concentrations from 10 pM down to 1 fM. While only one DNA template was used in these demonstration experiments, the method could easily be extended to multiple DNA templates (and their detection via RNA adsorption to unique sequences) by adding more elements to the microarray. Additionally, the surface-based RNA transcription reaction presented here is more than just another method of detecting DNA; it can also be used for the preparation of RNA aptamer microarrays and the eventual translation of the RNA into proteins for the fabrication of proteomic microarrays.

■ ASSOCIATED CONTENT

Supporting Information. Experimental Section, Figures S1–S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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