Sequential Strand Displacement Beacon for Detection of DNA Coverage on Functionalized Gold Nanoparticies

ARTICLE *in* ANALYTICAL CHEMISTRY · MAY 2014

Impact Factor: 5.64 · DOI: 10.1021/ac501341t · Source: PubMed

5 AUTHORS, INCLUDING:

Rebecca Paliwoda
University of Alberta
3 PUBLICATIONS **6** CITATIONS
SEE PROFILE

Feng Li
Brock University
25 PUBLICATIONS **1,150** CITATIONS
SEE PROFILE

Michael Scott Reid
University of Alberta
3 PUBLICATIONS **13** CITATIONS
SEE PROFILE

X. Chris Le
University of Alberta
248 PUBLICATIONS **7,757** CITATIONS
SEE PROFILE

analytical chemistry

Article

pubs.acs.org/ac

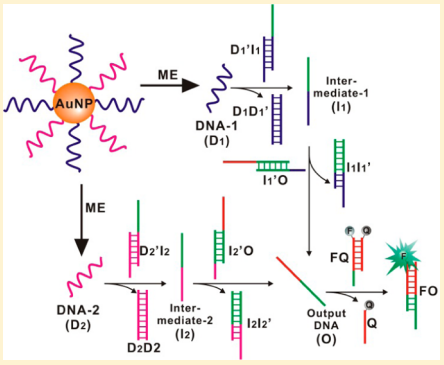
Sequential Strand Displacement Beacon for Detection of DNA Coverage on Functionalized Gold Nanoparticles

Rebecca E. Paliwoda,[†,§] Feng Li,[‡,§] Michael S. Reid,[†] Yanwen Lin,[†] and X. Chris Le*[,†,‡]

[†]Department of Chemistry, and [‡]Department of Laboratory Medicine and Pathology, Division of Analytical and Environmental Toxicology, University of Alberta, Edmonton, Alberta T6G2G3, Canada

Supporting Information

ABSTRACT: Functionalizing nanomaterials for diverse analytical, biomedical, and therapeutic applications requires determination of surface coverage (or density) of DNA on nanomaterials. We describe a sequential strand displacement beacon assay that is able to quantify specific DNA sequences conjugated or coconjugated onto gold nanoparticles (AuNPs). Unlike the conventional fluorescence assay that requires the target DNA to be fluorescently labeled, the sequential strand displacement beacon method is able to quantify multiple unlabeled DNA oligonucleotides using a single (universal) strand displacement beacon. This unique feature is achieved by introducing two short unlabeled DNA probes for each specific DNA sequence and by performing sequential DNA strand displacement reactions. Varying the relative amounts of the specific DNA sequences and spacing DNA sequences during their coconjugation onto AuNPs results in different densities of the specific DNA on AuNP, ranging from 90 to 230 DNA molecules per AuNP. Results obtained from our sequential strand displacement beacon assay are consistent with those obtained from the conventional fluorescence assays. However, labeling of DNA with some fluorescent dyes, e.g., tetramethylrhodamine, alters DNA density on AuNP. The strand displacement strategy overcomes this problem by obviating direct labeling of the target DNA. This method has broad potential to facilitate more efficient design and characterization of novel multifunctional materials for diverse applications.



Multifunctionality that integrates targeting ligands, drugs, imaging moieties, and many other functional groups into a single unit is a key component to the design of next-generation DNA nanomaterials and bioassays.[1−4] Recent efforts have emphasized the development of methods that are able to control and tune the number of each functional group, enabling well-defined materials in applications including diagnostic, imaging, and drug delivery.[5,6] Among DNA-based materials, DNA-functionalized gold nanoparticles (DNA−AuNPs) are promising candidates for constructing precise, multifunctional materials due to the well-established conjugation strategies, superior optical and electrical properties, and the unique photothermal properties.[7−10] For example, by coconjugating multiple DNA oligonucleotides on the same AuNP, Prigodich et al.[11] have developed multiplexed nanoflares that are able to detect and regulate multiple mRNA in living cells. By using a similar strategy, Diaz et al. have developed a series of multifunctional DNA−AuNPs that have well-defined spatial organization.[5] Our group has developed binding-induced molecular translators that rely on coconjugating signaling DNA and affinity DNA (e.g., aptamer) onto the same AuNP for homogeneous protein sensing.[12] An increasing number of methods focus on constructing multiplexed DNA−AuNPs for diverse nanomaterial assembly,[13] sensing,[12,14−17] and drug delivery.[4,11] However, there is no simple method capable of determining the density or surface coverage of each DNA oligonucleotide functionalized on multiplexed DNA−AuNPs, i.e., the averaged DNA coverage of each oligonucleotide that is measured from a population of AuNPs.

Characterizing the surface coverage of DNA oligonucleotides conjugated onto a AuNP surface is critical both for understanding the interactions between the DNA and nanoparticles (e.g., DNA conformation, hybridization efficiency) and for optimizing the sensitivity, stability, and efficacy of nanoparticle-based sensors and therapeutic carriers.[18−21] Currently, the conventionally used fluorescence turn-on method measures the density of a fluorescent dye-labeled DNA on AuNPs, but it cannot measure unlabeled DNA.[22,23] To detect multiple DNA on the AuNP, researchers must label each DNA sequence with a unique fluorescent dye; this increases the cost. When multiple dyes are used in the same assay, factors such as overlaps in emission spectra and strong interactions between the dyes and AuNPs must be considered, which limits the choice of potential dyes.[24] Other methods, including the use of DNA intercalating dyes, e.g., OliGreen (ssDNA dye)[25] and real-time PCR,[26] lack the specificity to distinguish each DNA oligonucleotide coconjugated on AuNPs. These methods may also require complicated instrumentation and operations. The objective of this study is to develop a simple, label-free, and low-cost

Received: April 13, 2014
Accepted: May 21, 2014
Published: May 21, 2014

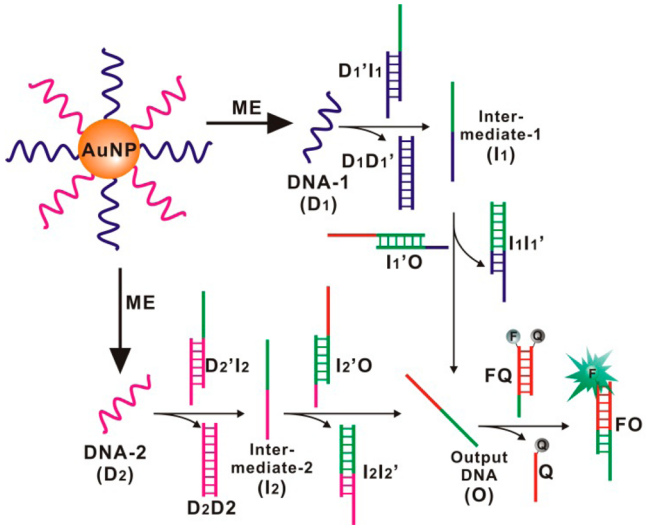
   dx.doi.org/10.1021/ac501341t | *Anal. Chem.* 2014, 86, 6138−6143

method that is able to specifically measure multiple DNA oligonucleotides on AuNPs. The main feature of our technique is a unified, predesigned DNA beacon that acts by toehold-mediated DNA strand displacement.

DNA strand displacement reactions, which are initiated through a short sticky end of the target DNA (known as DNA toehold), play a central role in dynamic DNA nanotechnology.[27] Due to their superior kinetics and modularity, DNA strand displacement reactions have been widely used to design diverse nanostructures/devices for a variety of applications, ranging from DNA computing, to biosensing, and to programming biological functions of living systems.[27] Taking advantage of the highly efficient DNA toehold-mediated strand displacement networks, Picuri et al.[28] and Li et al.[29] have developed DNA-based molecular translators that are able to convert any DNA or protein target into a unique output DNA. Inspired by this, we describe here a toehold-mediated strand displacement strategy for characterizing DNA–AuNPs. Our central hypothesis is that different DNA molecules conjugated to AuNPs can be converted into a single predesigned output DNA through sequential DNA strand displacement reactions. The quantitative information on each DNA sequence can then be readily obtained by measuring the amount of output DNA produced from the specific strand displacement reactions.

Scheme 1 shows the principle of the sequential strand displacement beacon strategy to quantify DNA from functionalized AuNPs. All DNA oligonucleotides on AuNPs are first released by using competing thiols, e.g., mercaptoethanol (ME). To quantify one specific DNA sequence (e.g., DNA-1), we designed a pair of unmodified DNA probes ($\mathbf{D_1'I_1}$ and $\mathbf{I_1'O}$) so that strand displacement reactions occur only in the presence of the target DNA-1 ($\mathbf{D_1}$). An intermediate strand ($\mathbf{I_1}$) can quickly be released from $\mathbf{D_1'I_1}$ through a toehold-mediated DNA strand displacement reaction. $\mathbf{I_1}$ then triggers the subsequent release of the output DNA **O** through another toehold-mediated DNA strand displacement between $\mathbf{I_1}$ and $\mathbf{I_1'O}$. The resulting output DNA **O** can turn on the fluorescence of a beacon (**FQ**) by displacing the quencher-labeled DNA strand **Q** from the **FQ** probe through toehold-mediated DNA strand displacement. Similarly, to detect another DNA sequence (e.g., DNA-2), we designed a pair of unlabeled DNA probes, $\mathbf{D_2'I_2}$ and $\mathbf{I_2'O}$. The first strand displacement reaction results in the release of the intermediate strand $\mathbf{I_2}$, which then triggers the second strand displacement reaction, releasing the output DNA **O**. This output DNA **O** triggers the third strand displacement reaction with the beacon **FQ**, giving rise to detectable fluorescence. Using our sequential strand displacement strategy and a single beacon, we can easily quantify any DNA from AuNPs, by simply designing a pair of unlabeled DNA probes. The design of these DNA probes is simple, because their sequences are predefined by the target DNA and the output DNA, i.e., one fraction of the intermediate DNA **I** is part of the target DNA sequence and the other fraction is part of the output DNA sequence. The application of our strategy is flexible. In principle, the sequential strand displacement strategy can be applied to any DNA sequence that is long enough (e.g., >18 nt) to trigger a toehold-mediated DNA strand displacement reaction between target DNA and the intermediate DNA. For a longer DNA strand, a fraction of its sequence can be used instead of the entire target DNA.

Scheme 1. Schematic Illustrating the Use of Sequential DNA Strand Displacement Reactions and Displacement Beacon to Quantify Multiple DNA Oligonucleotides on the Same AuNP



RESULTS AND DISCUSSION

To examine the feasibility of the sequential strand displacement beacon strategy, we first designed a specific target DNA $\mathbf{D_1}$, output DNA **O**, and strand displacement beacon **FQ** that can only be specifically turned on by **O**. On the basis of the DNA sequences of $\mathbf{D_1}$ and **O** (Supporting Information Table S1), we then designed a pair of DNA probes ($\mathbf{D_1'I_1}$ and $\mathbf{I_1'O}$). $\mathbf{I_1}$ consists of two predesigned sequences: a 17-nt region (blue color in Supporting Information Table S1) that is identical to a portion of the target DNA $\mathbf{D_1}$ and a 26-nt sequence (green) that is identical to a portion of the output DNA **O** (Figure 1A and Supporting Information Table S1). Figure 1B shows the overall kinetic profile of the sequential DNA strand displacement reactions from $\mathbf{D_1}$ (target) to **FO** (signal read-out). A continuous increase in fluorescence signal (blue curve in Figure 1B) is observed over the course of 120 min after the addition of 50 nM of DNA-1 ($\mathbf{D_1}$) to an incubation solution containing $\mathbf{D_1'I_1}$, $\mathbf{I_1'O}$, and **FQ**. Only background fluorescence is observed from the negative control that contains all reagents ($\mathbf{D_1'I_1}$, $\mathbf{I_1'O}$, and **FQ**) but no target DNA (red curve in Figure 1B). These results suggest that our strand displacement beacon can quickly respond to the target DNA. There is no apparent increase in background fluorescence generated over 2 h after premixing all of the probes and reagents. Calibrations between the fluorescence intensities and concentrations of $\mathbf{D_1}$ (Figure 1C) are linear within the concentration range from 0 to 30 nM, at both 30 min ($r^2 = 0.9967$) and 60 min ($r^2 = 0.9966$). To test the specificity of the approach, we used the probes that are specifically designed for the target DNA-1 ($\mathbf{D_1}$) and conducted experiments to detect the target DNA and a nonspecific DNA. Figure 1D shows that the assay gives a concentration-dependent fluorescence response from the target DNA (blue circles) and that there is no fluorescence increase from the analysis of the nonspecific DNA (red squares). The results suggest that the sequential strand displacement beacon method is able to quantify specific DNA sequences without interference from other DNA present in the same sample.

To demonstrate an application of this technique, we quantified the surface density of a DNA sequence conjugated to AuNPs. We first conjugated $\mathbf{D_1}$ to AuNPs through sulfur–

dx.doi.org/10.1021/ac501341t | Anal. Chem. 2014, 86, 6138–6143

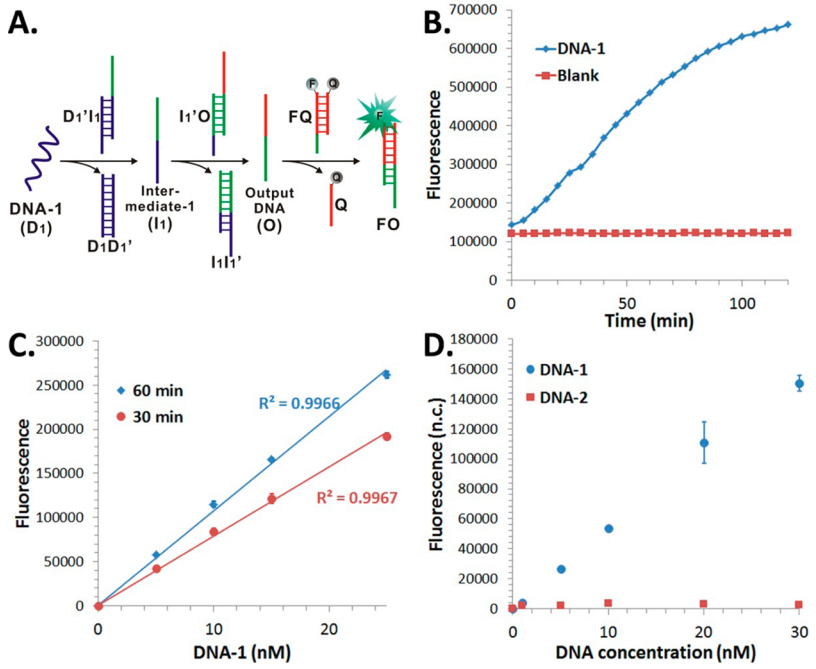


Figure 1. (A) Schematic illustrating the sequential DNA strand displacement reactions and strand displacement beacon (**FQ**) to quantify DNA-1 ($\mathbf{D_1}$) in PBS buffer. (B) Kinetics of sequential DNA strand displacement reactions observed from monitoring the specific DNA-1 and reagent blank. (C) Fluorescence intensity from the displacement beacon analysis as a function of concentrations of DNA-1 ($\mathbf{D_1}$) in solution. (D) Specificity test of the sequential strand displacement beacon assay, showing concentration dependence for the specific DNA-1 and negligible background from a nonspecific DNA (DNA-2).

gold bonds according to a previously reported method.[12] The concentration of $\mathbf{D_1}$ and AuNPs used in the reaction was in a ratio of 1000 to 1. We then released $\mathbf{D_1}$ from AuNPs by adding 20 mM ME. The DNA concentration in this solution was subsequently determined using the sequential strand displacement beacon assay. A mixture of beacon probes ($\mathbf{D_1'I_1}$, $\mathbf{I_1'O}$, and **FQ**) was added to the solution and incubated for 60 min in the dark. In parallel, unconjugated $\mathbf{D_1}$ served as an external standard for calibration (Figure 2A). We are able to quantify the amount of specific DNA ($\mathbf{D_1}$) released from the gold nanoparticle ($\mathbf{D_1}$–AuNPs). Under the given conditions of DNA conjugation to the AuNPs, we measured the density of the DNA on AuNPs to be 201 ± 11 oligonucleotides per AuNP (Figure 2A). This measured value is consistent with previously reported DNA coverage on 20 nm AuNPs under similar conditions.[23,30] To further validate our result, we used a previously reported OliGreen assay[25] (Supporting Information Figure S1) to measure the density of DNA on AuNPs, and we obtained a value of 207 ± 22 oligonucleotides per AuNP. The good agreement between the values obtained with the strand displacement beacon assay (201 ± 11) and the OliGreen assay (207 ± 22) supports the accuracy of the assay.

We have further applied the strand displacement beacon technique to the detection of different DNA densities on AuNPs. We prepared a series of DNA–AuNPs with different densities by coconjugating DNA-1 and a short polyT DNA oligonucleotide (spacer oligo). The overall number of DNA oligonucleotides on each AuNP was kept constant. By tuning the ratio between DNA-1 and the spacer oligo from (1000:0, 750:250, 500:500, 250:750, and 0:1000) at the conjugation step, we were able to achieve different densities of DNA-1 on the AuNP. As shown in Figure 2B, the measured densities of DNA-1 on AuNP decrease as we reduce the amount of DNA-1 at the conjugation step. The results demonstrate that our assay

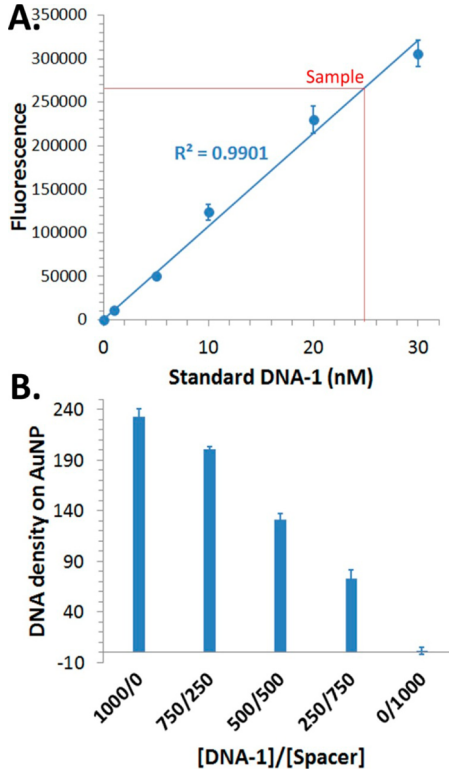


Figure 2. (A) Quantifying the concentrations of DNA oligonucleotides on DNA–AuNPs using the sequential strand displacement beacon strategy. (B) Quantifying DNA densities from five different DNA–AuNPs that were prepared under the conditions of varying DNA-1 to DNA spacer ratios. The total amount of DNA during the conjugation step was kept constant.

dx.doi.org/10.1021/ac501341t | *Anal. Chem.* 2014, 86, 6138–6143

is able to quantify a range of DNA densities on AuNPs. The measurement background is negligible when no DNA-1 is added during the conjugation step. This is consistent with the excellent specificity of the assay.

Unlike other existing methods that require labeling multiple DNA oligonucleotides with different fluorescent dyes, our technique has the ability to quantify multiple unlabeled DNA oligonucleotides coconjugated on the same AuNP. As an example, we show the detection of two DNA sequences ($\mathbf{D}_1$ and $\mathbf{D}_2$) coconjugated to the same AuNPs (Table 1). We measured the density of $\mathbf{D}_1$ and $\mathbf{D}_2$ in parallel by applying specific DNA probes: $\mathbf{D}_1'\mathbf{I}_1$ and $\mathbf{I}_1'\mathbf{O}$ for detection of $\mathbf{D}_1$ and $\mathbf{D}_2'\mathbf{I}_2$ and $\mathbf{I}_2'\mathbf{O}$ for detection of $\mathbf{D}_2$. The measured values are 93 ± 6 DNA sequence $\mathbf{D}_1$ per AuNP and 117 ± 4 DNA sequence $\mathbf{D}_2$ per AuNP (Table 1). We then separately conjugated $\mathbf{D}_1$ and $\mathbf{D}_2$ to different AuNPs and determined the densities of each sequence on AuNPs. We kept the total amount of DNA constant for the different conjugation experiments. The measured values, 222 ± 8 and 233 ± 12, are consistent with the sum of $\mathbf{D}_1$ and $\mathbf{D}_2$ coconjugated on the same AuNP (210 ± 10) (Table 1). The results further confirm that our strand displacement beacon method has the ability to quantify multiple DNA oligonucleotides from AuNPs.

Table 1. DNA Densities on Multifunctionalized $\mathbf{D}_1$−$\mathbf{D}_2$−AuNP and Monofunctionalized $\mathbf{D}_1$−AuNP and $\mathbf{D}_2$−AuNP[a]

density (no. per AuNP)	$\mathbf{D}_1$−$\mathbf{D}_2$−AuNP	$\mathbf{D}_1$−AuNP	$\mathbf{D}_2$−AuNP
$\mathbf{D}_1$ (DNA-1)	93 ± 6	222 ± 8	0
$\mathbf{D}_2$ (DNA-2)	117 ± 4	0	233 ± 12
total	210 ± 10	222 ± 8	233 ± 12

[a]The total amount of DNA was kept constant between different DNA sequences when they were conjugated to AuNPs. Results were obtained with the strand displacement beacon assay.

Having established the method to determine unlabeled DNA oligonucleotides on AuNPs, we examined how the fluorescent labeling used in the conventional method could affect the DNA density on AuNPs. We tested fluorescein (FAM) and tetramethylrhodamine (TAMRA) as they are the two most widely used fluorescence labels for measuring DNA oligonucleotides by fluorescence turn-on assays. The results are summarized in Table 2. When the DNA is labeled with FAM, the density measured (226 ± 10) using a fluorescence turn-on assay is comparable to the density of the unlabeled DNA (222 ± 8) measured using the strand displacement beacon method. However, when the DNA is labeled with TAMRA, the density of this DNA conjugated on AuNPs is 153 ± 5, a decrease of more than 30% from the unlabeled DNA (222 ± 8). The values obtained from the strand displacement beacon method (147 ± 2) and from the fluorescence turn-on assay (153 ± 5) are consistent. These results confirm that the TAMRA labeling of DNA decreases the number of the DNA molecules being conjugated on the surface of AuNPs. This observation is consistent with previous reports that TAMRA has strong interaction with the surface of AuNPs.[31] Thus, TAMRA labeling may alter the interaction between DNA and AuNPs, leading to a decrease in surface coverage of DNA.

Table 2. DNA Densities on Various DNA−AuNP Conjugates: $\mathbf{D}_1$−AuNP, FAM−$\mathbf{D}_1$−AuNP, and TAMRA−$\mathbf{D}_1$−AuNP[a]

density (no. per AuNP)	strand displacement method	fluorescence turn-on assay
$\mathbf{D}_1$ (DNA-1)	222 ± 8	N.A.[b]
FAM−$\mathbf{D}_1$	N.A.[b]	226 ± 10
TAMRA−$\mathbf{D}_1$	147 ± 2	153 ± 5

[a]Density of $\mathbf{D}_1$−AuNP was measured by the sequential strand displacement assay. Density of FAM−$\mathbf{D}_1$−AuNP was measured by the fluorescence turn-on assay. Density of TAMRA−$\mathbf{D}_1$−AuNP was measured by both the fluorescence turn-on assay and the sequential strand displacement method. [b]N.A.: not applicable.

In summary, we have successfully developed a simple, low-cost strand displacement beacon method that is able to directly measure the average number of DNA molecules on each AuNP. Our sequential strand displacement beacon method does not require fluorescent labeling of the target DNA, thereby eliminating the effect of fluorescent dyes on the surface coverage of DNA. Particularly, multiple DNA oligonucleotides that are coconjugated on the same AuNP can be quantified specifically through unique DNA strand displacement reactions and a unified displacement beacon. Furthermore, our method has a subnanomolar detection limit. This is comparable to those obtained by most fluorescence-based assays, e.g., fluorescence turn-on assay (OliGreen assay). Another benefit is that the strand displacement probes do not need to be purified with HPLC; a simple desalting procedure is sufficient (Supporting Information Figure S2). Eliminating the HPLC purification step reduces the reagent cost. Our universal strand displacement beacon strategy is not limited to DNA−AuNPs but can be expanded to other DNA hybrid materials, allowing for better characterizations and applications.

MATERIALS AND METHODS

Materials and Reagents. Solution of gold nanoparticles (AuNPs) (20 nm in diameter, 7.0×10^{11}) was purchased from Ted Pella (Redding, CA). Tris−HCl buffer (1 M, pH 7.4), NaCl, 100× Tris EDTA buffer (1.0 M Tris−HCl, 0.1 M EDTA, pH 8), and 2-mercaptoethanol (ME) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Tween 20 and $MgCl_2$ were purchased from Fisher Scientific (Nepean, ON, Canada). OliGreen ssDNA binding dye was purchased from Life Technologies (Burlington, ON, Canada). All DNA (Supporting Information Table S1) was synthesized and purified by Integrated DNA Technologies Inc. (Coralville, IA). Nanopure H_2O (>18.0 MΩ), purified using an Ultrapure Milli-Q water system, was used for all experiments.

Preparation of DNA-Functionalized Gold Nanoparticle. Thiolated DNA oligonucleotides ($\mathbf{D}_1$, $\mathbf{D}_2$, $\mathbf{D}_1$−FAM, $\mathbf{D}_1$−TAMRA, Supporting Information Table S1) were used in the preparation of DNA−AuNPs. These DNA oligonucleotides were diluted to 5 μM in 200 μL with Tris−HCl buffer. This solution was then mixed with 1 mL of AuNPs (1 nM) and left to incubate in the dark overnight. To this mixture, 50 μL of 3 M NaCl was slowly added followed by 15 s of sonication. This process was repeated three times with 30 min intervals to final salt concentration of 300 mM. After incubation overnight in the dark, the solution of DNA−AuNPs was then centrifuged at 13 000 rpm for 30 min to separate DNA−AuNPs from the unreacted reagents. DNA−AuNPs were then washed twice with 10 mM Tris−HCl (pH 7.4) and finally redispersed in 200 μL of 20 mM Tris−HCl. Functionalized AuNPs were stored at 4 °C in the dark until used.

Preparation of DNA Probes for Strand Displacement Beacon FQ and Sequential Strand Displacement

dx.doi.org/10.1021/ac501341t | Anal. Chem. 2014, 86, 6138−6143

Reactions. DNA probe ($\mathbf{D_1}'\mathbf{I_1}$ as an example) was prepared for sequential strand displacement reaction by mixing 100 μL of 5 μM $\mathbf{D_1}'$ and 100 μL of 5 μM intermediate strands $\mathbf{I_1}$ in TE-Mg buffer. The mixture was heated to 90 °C for 5 min, and then the solution was allowed to cool down slowly to 25 °C in a period of 2 h. Similarly, displacement beacon **FQ** was prepared by mixing 100 μL of 5 μM DNA probe **F** and 100 μL of 10 μM probe **Q**. The mixture was heated to 90 °C for 5 min, and then the solution was allowed to cool down slowly to 25 °C in a period of 2 h.

Measuring DNA Density on AuNPs Using Sequential DNA Strand Displacement Reactions. DNA oligonucleotides were first released from the AuNPs by adding 80 μL of freshly prepared 50 mM ME into 20 μL of the DNA–AuNP solution. This mixture was then incubated in the dark at room temperature for 3 h. Solution containing DNA strand displacement probes and displacement beacon was added to the mixture to a final concentration of 50 nM each. An aliquot of this mixture (90 μL) was added to a 96-well microplate, and fluorescence was measured using a multimode microplate reader with an excitation/emission wavelength of 485 nm/535 nm for FAM-labeled DNA and excitation/emission wavelength of 535 nm/595 nm for TAMRA-labeled DNA. All DNA–AuNPs were prepared in duplicate under identical conditions, and each DNA–AuNP solution was analyzed in triplicate. Therefore, each DNA density value was obtained from an average of six measurements. For calibration, external DNA standards (identical DNA oligonucleotides but not conjugated to AuNP) with varying concentrations were treated and measured under the same conditions as for DNA–AuNP samples.

Measuring DNA Density on AuNPs Using Fluorescence Turn-On Assay. Similar to the strand displacement beacon assay, FAM-labeled or TAMRA-labeled DNA oligonucleotides were first released from DNA–AuNPs by adding 80 μL of freshly prepared 50 mM ME into 20 μL of the DNA–AuNP solutions. After incubating in the dark for 3 h, fluorescence measurements were performed using a multimode microplate reader as described above. For calibration, FAM-labeled ($\mathbf{D_1}$–FAM, Supporting Information Table S1) or TAMRA-labeled DNA ($\mathbf{D_1}$–TAMRA, Supporting Information Table S1) oligonucleotides with varying concentrations were used as external standards and were measured in the same way as the DNA–AuNPs. Once the released DNA has been quantified by the external standards, we further calculate the DNA density on AuNP by the following equation: density = $(C_{DNA}VN_A)/(VD_{AuNP})$, where C_{DNA} is the measured DNA concentration, V is the volume of DNA–AuNP solution, N_A is the Avogadro's constant, and D_{AuNP} is the density of AuNPs. The density of AuNPs (7.0×10^{11} particles/mL) was provided by the vendor (Ted Pella).

Measuring DNA Density on AuNPs Using OliGreen Assay. Similar to the sequential strand displacement beacon assay, unlabeled DNA oligonucleotides ($\mathbf{D_1}$ or $\mathbf{D_2}$) were first released from 20 μL of DNA–AuNPs with 80 μL of freshly prepared 50 mM ME. Standard DNA oligonucleotides with varying concentrations were treated the same way as the DNA–AuNPs. OliGreen dye was prepared as specified by the manufacturer and added to DNA solutions. After 5 min, 100 μL of each mixture was transferred into a 96-well microplate. Fluorescence was measured using the multimode microplate reader with an excitation/emission wavelength of 485 nm/535 nm for OliGreen dyes. Intercalation of the OliGreen dye into the targeted DNA oligonucleotides gave rise to fluorescence.

■ ASSOCIATED CONTENT

Supporting Information

DNA sequences (Table S1), results from an alternative analysis (Figure S1), and a comparison of sequential strand displacement beacon performed using different DNA probe reagents (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 1-780-492-6416. Fax: 1-780-492-7800. E-mail: xc.le@ualberta.ca.

Author Contributions

§R.E.P. and F.L. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Natural Sciences and Engineering Research Council of Canada, the Canadian Institutes of Health Research, the Canada Research Chairs Program, Alberta Health, and Alberta Innovates.

■ REFERENCES

(1) Ferrari, M. *Nat. Rev. Cancer* **2005**, *5*, 161–171.
(2) Tan, S. J.; Kiatwuthinon, P.; Roh, Y. H.; Kahn, J. S.; Luo, D. *Small* **2011**, *7*, 841–856.
(3) Duncan, R. *Nat. Rev. Cancer* **2006**, *6*, 688–701.
(4) Zhang, L.; Gu, F. X.; Chan, J. M.; Wang, A. Z.; Langer, R. S.; Farokhzad, O. C. *Clin. Pharmacol. Ther.* **2008**, *83*, 761–769.
(5) Diaz, J. A.; Grewer, D. M.; Gibbs-Davis, J. M. *Small* **2012**, *8*, 873–883.
(6) Li, X.; Guo, J.; Asong, J.; Wolfert, M. A.; Boons, G.-J. *J. Am. Chem. Soc.* **2011**, *133*, 11147–11153.
(7) Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2010**, *49*, 3280–3294.
(8) Khlebtsov, N.; Bogatyrev, V.; Dykman, L.; Khelebtsov, B.; Staroverov, S.; Shirokov, A.; Matora, L.; Khanadeev, V.; Pylaev, T.; Tsyganova, N.; Terentyuk, G. *Theranostics* **2013**, *3*, 167–180.
(9) Zhang, H.; Li, F.; Dever, B.; Li, X.-F.; Le, X. C. *Chem. Rev.* **2013**, *113*, 2812–2841.
(10) Song, S.; Qin, Y.; He, Y.; Huang, Q.; Fan, C.; Chen, H.-Y. *Chem. Soc. Rev.* **2010**, *39*, 4234–4243.
(11) Prigodich, A. E.; Randeria, P. S.; Briley, W. E.; Kim, N. J.; Daniel, W. L.; Gijohann, D. A.; Mirkin, C. A. *Anal. Chem.* **2012**, *84*, 2062–2066.
(12) Li, F.; Zhang, H.; Lai, C.; Li, X.-F.; Le, X. C. *Angew. Chem., Int. Ed.* **2012**, *51*, 9317–9320.
(13) Song, T.; Liang, H. *J. Am. Chem. Soc.* **2012**, *134*, 10803–10806.
(14) Huang, Y.; Zhao, S.; Liang, H.; Chen, Z.-F.; Liu, Y.-M. *Chem.—Eur. J.* **2011**, *17*, 7313–7319.
(15) Song, S.; Liang, Z.; Zhang, J.; Wang, L.; Li, G.; Fan, C. *Angew. Chem., Int. Ed.* **2009**, *48*, 8670–8674.
(16) Hazarika, P.; Ceyhan, B.; Niemeyer, C. M. *Small* **2005**, *1*, 844–848.
(17) Liu, J.; Lu, Y. *Adv. Mater.* **2006**, *18*, 1667–1671.
(18) Hill, H. D.; Millstone, J. E.; Banholzer, M. J.; Mirkin, C. A. *ACS Nano* **2009**, *3*, 418–424.
(19) Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K. R.; Han, M. S.; Mirkin, C. A. *Science* **2006**, *312*, 1027–1030.
(20) Diaz, J. A.; Gibbs-Davis, J. M. *Small* **2013**, *9*, 2862–2871.
(21) Seferos, D. S.; Prigodich, A. E.; Giljohann, D. A.; Patel, P. C.; Mirkin, C. A. *Nano Lett.* **2009**, *9*, 308–311.

(22) Demers, L. M.; Mirkin, C. A.; Mucic, R. C.; Reynolds, R. A., III; Letsinger, R. L.; Elghanian, R.; Viswanadham, G. *Anal. Chem.* **2000**, *72*, 5535−5541.
(23) Hurst, S. J.; Lytton-Jean, A. K. R.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313−8318.
(24) Stokes, R. J.; Macaskill, A.; Lundahl, P. J.; Smith, W. E.; Faulds, K.; Graham, D. *Small* **2007**, *3*, 1593−1601.
(25) Liu, C.-W.; Huang, C.-C.; Chang, H.-T. *Langmuir* **2008**, *24*, 8346−8350.
(26) Kim, E.-Y.; Stanton, J.; Vega, R. A.; Kunstman, K. J.; Mirkin, C. A.; Wolinsky, S. M. *Nucleic Acids Res.* **2006**, *34*, e54.
(27) Zhang, D. Y.; Seelig, G. *Nat. Chem.* **2011**, *3*, 103−113.
(28) Picuri, J. M.; Frezza, B. M.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2009**, *131*, 9368−9377.
(29) Li, F.; Zhang, H.; Wang, Z.; Li, X.; Li, X.-F.; Le, X. C. *J. Am. Chem. Soc.* **2013**, *135*, 2443−2446.
(30) Li, F.; Zhang, H.; Dever, B.; Li, X.-F.; Le, X. C. *Bioconjugate Chem.* **2013**, *24*, 1790−1797.
(31) Maxwell, D. J.; Taylor, J. R.; Nie, S. *J. Am. Chem. Soc.* **2002**, *124*, 9606−9612.