

# COLORIMETRIC SCREENING OF OLIGONUCLEOTIDE BASED ON THE HYBRIDIZATION-MEDIATED GROWTH SIZE OF GOLD NANOPARTICLE PROBES

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## ABSTRACT

In this work, we propose a novel method for the colorimetric screening of oligonucleotide based on the hybridization-mediated growth size of gold nanoparticle probes. DNA targets which are complementary, three mismatched and uncomplimentary to the probes can be apparently differentiated by the proposed method that enables a short duration of the colorimetric detection in 1 min with satisfactory detection limitation of the targets 100 nM. We believe that this method is able to be applied to the point-of-care screening and the single-nucleotide polymorphism (SNP) diagnosis of oligonucleotide if the whole assay process is optimally modified.

## KEYWORDS

Colorimetric screening, gold nanoparticle, oligonucleotide, hybridization

## INTRODUCTION

Gold nanoparticles (AuNPs) are increasingly applied in biochemistry and engineering because of their size-dependent optical properties, great surface modifiability and biocompatibility [1]. A colorimetric method to identify DNA was proposed involving cross-linking DNA hybridization, utilizing nanoparticles capped with probe DNA to detect target DNA [2]. Another colorimetric method was demonstrated to detect a terminus-single-base mismatched DNA target by non-cross-linking DNA hybridization [3]. For a rapid and facile DNA screening, we report a novel approach to identify DNA samples colorimetrically based on the growth size of the AuNP probe dominated by the hybridization characteristics between the probe and samples. This approach has advantages relative to previous methods [2,3]: no requirement of temperature control, satisfactory detection limitation of DNA (~ 100 nM), and brief colorimetric duration (< 1 min).

## EXPERIMENT

Figure 1 illustrates our scheme to screen DNA colorimetrically. Initially, AuNP modified with probe DNA (PDNA), termed AuNP probe, hybridizes with DNA samples in the split-and-recombination microreactor (SAR-reactor) [4]. The mobile hybridization [5] of the probe and samples occurring in the SAR-reactor achieves complete hybridization in less than ten seconds, a duration much less than for traditional static hybridization, indicating that the microreactor improves the rapidity of this scheme. The hybridized AuNP probes are collected in a centrifuge tube, followed by addition of  $\text{NH}_2\text{OH}$  and  $\text{HAuCl}_4$  in sequence so as to induce the reduction of gold metal on the probes [6]. The hybridization characteristics between probes and samples are strongly related to their complementarity, and affect the growth of the hybridized probes and the resulting colorimetric properties.

Figure 2 shows the colorimetric results from various DNA samples, specifically target DNA (TDNA), single-mismatched DNA (SMDNA), three-mismatched DNA (TMDNA), and fully uncomplimentary DNA (UNDNA) (Table 1). The hues for TDNA and SMDNA are both blue whereas those for TMDNA and for UNDNA are purple and pink; the probes without additional DNA grow to exhibit pink. TDNA, TMDNA and UCDNA are differentiable with this scheme by naked eyes. Figure 3 shows the surface plasmon resonance (SPR) maximum is at 562 nm for UCDNA-hybridized AuNP probes, near that for no DNA-hybridized AuNP probe, implying these hybridized probes have similar growth sizes (~ 80 nm). The UCDNA is least likely to affect the growth of the probes because the hybridization (binding strength) between the UCDNA and the probes is weak. Compared to no DNA-hybridized AuNP probe, the SPR maximum of TDNA-hybridized AuNP probes reveals a significant red shift (from 562 to 605 nm) on the addition of the TDNA, whereas a moderate red shift of the SPR maximum occurs from 562 to 588 nm on the addition of the TMDNA. The growth sizes of the probes are hence increased with the increasing binding strength and with the decreasing extent of mismatch between the probes and samples, confirming the concept of our approach. We intend to improve the differentiation between TDNA-hybridized AuNP probes and SMDNA-hybridized AuNP probes by modulating the buffer solution.

This approach involving a microreactor technique to decrease the duration of DNA hybridization and a novel AuNP-based colorimetric assay is promising to realize facile and reliable DNA screening.

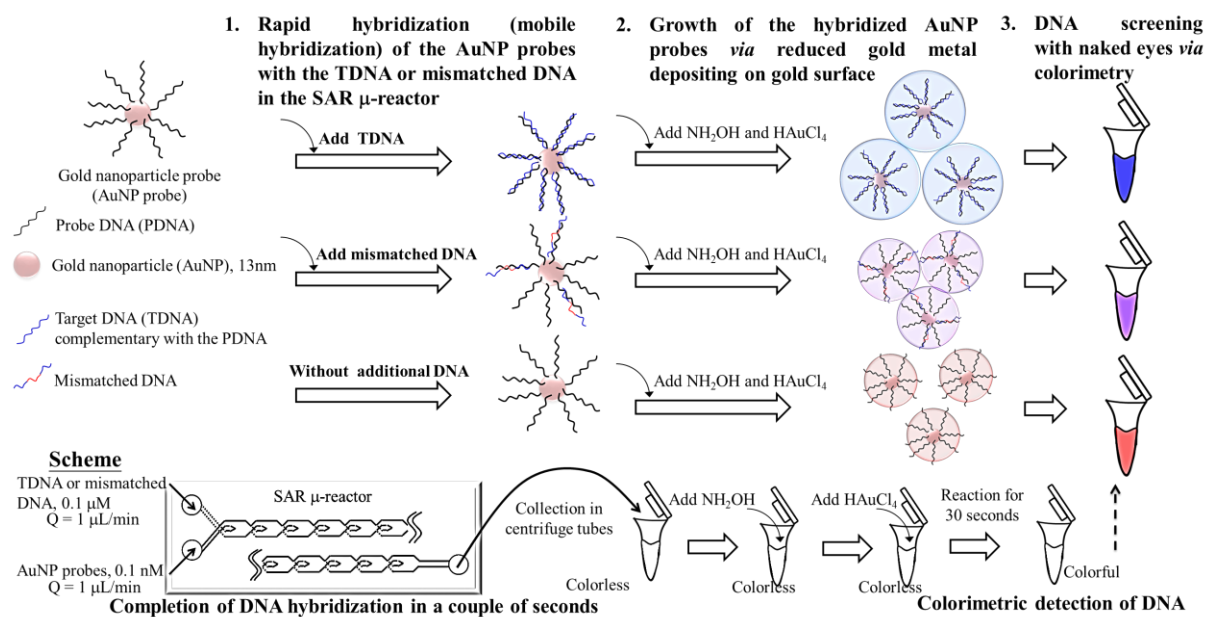


Figure 1. Schematic illustration of the hybridization-mediated growth of gold nanoparticle probes for rapid DNA screening. DNA samples and AuNP probes are separately dissolved in buffer solutions (0.2 M NaCl), and are respectively injected into the SAR  $\mu$ -reactor (channel length 105 mm) to hybridize; the hybridized AuNP probes solution is collected.  $\text{NH}_2\text{OH}$  (15 mM, 40  $\mu$ L) and  $\text{HAuCl}_4$  (1mM, 10  $\mu$ L) are added to the solution in sequence to perform growth (synthetic reaction) of the hybridized AuNP probes for 30 s.

Table 1. Sequence of probe and DNA samples used in this work.

DNA (oligonucleotide)	Sequence (5' to 3')
Probe DNA (PDNA)	GAGCTGGTGGCGTAGGCAAG
Target DNA (TDNA)	CTTGCCTACGCCACCAGCTC
Single mismatched DNA (SMDNA)	CTTGCCTACTCCACCAGCTC
Three mismatched DNA (TMDNA)	CTTGCCTACTTTACCAGCTC
Uncomplementary DNA (UCDNA)	TTTTTTTTTTTTTTTTTTTTTTT



Figure 2. Experimental photographs of various regions (a to g) of the device (Case B). Scale bar = 100  $\mu$ m.

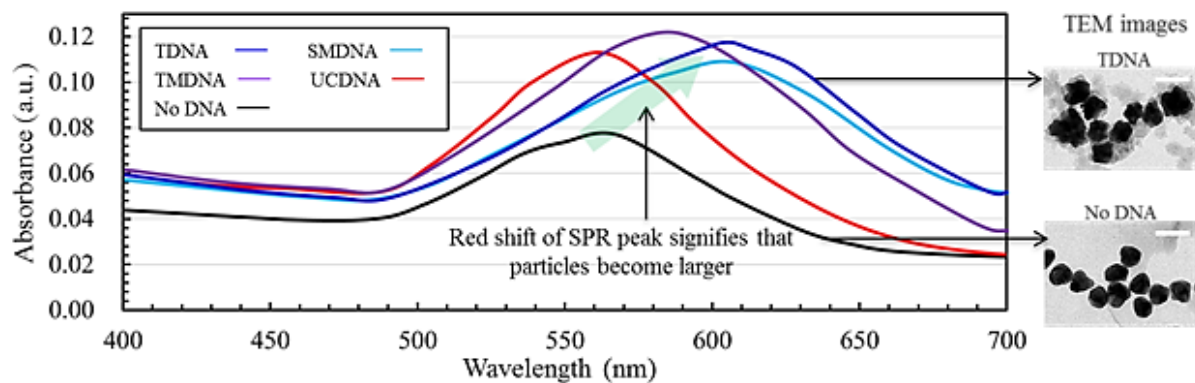


Figure 3. UV-visible spectra of various hybridized probes after growth (reduction of gold metal on the surface of the probes). The SPR maxima for TDNA-hybridized, SMDNA-hybridized, TMDNA-hybridized, UCDNA-hybridized and no DNA-hybridized AuNP probes are at 605, 604, 588, 562 and 563 nm respectively. Scale bar in TEM images is 100 nm.

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