



Short communication

Optimizing Au-nanoprobes for specific sequence discrimination

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ABSTRACT

Gold nanoparticles functionalized with thiol-oligonucleotides are ideal platforms for detection of specific DNA sequences. Here we evaluate the effect of single base mismatches in hybridization efficiency according to the position of the mismatch, base pairing combination and thiol-oligonucleotide density in terms of specificity and efficiency of target recognition. Hybridization efficiency and single-nucleotide polymorphism discrimination at room temperature is maximized at a density of 83 ± 4 thiol-oligonucleotides per 13.5 nm gold nanoparticle (24 pmol/cm^2), and when the mismatch is localized at the 3'-end of the Au-nanoprobe, i.e. away from the gold nanoparticle surface.

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1. Introduction

A new era of molecular diagnostics has emerged through the development of detection methods based on Au-nanoprobes, i.e. gold nanoparticles (AuNP) derivatized with thiol-modified oligonucleotides, for the selective and specific detection of nucleic acid sequences (DNA and RNA) [1,2]. These approaches rely on the specific molecular recognition of the target sequence and the associated effects of hybridization on the behavior of the AuNP. The hybridization efficiency between Au-nanoprobes and fully complementary nucleic acid targets has already been extensively studied [3–6], showing that the length of the thiol-oligonucleotide influences hybridization efficiency—longer sequences or spacer segments facilitate hybridization. Also, it has been shown that the density of thiol-oligonucleotides covering the AuNP surface influences the number of complementary targets able to hybridize to the Au-nanoprobe, i.e. a linear increase in complementary target hybridization is observed as more functionalized oligonucleotides become available for hybridization [7]. It has also been shown that a stronger interaction between the thiol-oligonucleotides' sequence and the AuNPs' surface seriously hamper hybridization [8].

Characterization of the effects on Au-nanoprobe specificity with single base resolution (single-nucleotide polymorphisms, SNP) is an important factor in molecular diagnostics. Thus far, the effect in hybridization efficiency to an Au-nanoprobe of a target har-

boring a single base mismatch has not been characterized. In the present report, we show the effects of a single base mismatch in hybridization efficiency according to the position of the mismatch, base pairing combination and Au-nanoprobe density. These data could help Au-nanoprobe design for improved target hybridization with concomitant increase in specificity for SNP discrimination at room temperature, resulting in the optimization of several existing Au-nanoprobe based approaches [3,9–13].

2. Materials and methods

All unmodified and thiol-modified ssDNA oligonucleotides were synthesized by STAB Vida, Lda. (Portugal)—see [Supplementary Material](#) for sequence details. All chemicals were from Sigma Aldrich and of the highest purity available. The AuNPs were prepared by the citrate reduction method described by Lee and Meisel [14] and characterized by Transmission Electron Microscopy (TEM). Au-nanoprobes were prepared by derivatizing the surface of 13.5 nm AuNPs with 20-mer thiol-modified ssDNA oligonucleotides using a well-established salt aging procedure [15]. To obtain different AuNPs coverage, a combination of different AuNP/thiolated-oligonucleotide ratios (up to 1:600) and electrolyte concentration (up to 0.7 M NaCl) were used during Au-nanoprobe synthesis—see [Supplementary Material](#) for specific conditions. The number of oligonucleotides for each Au-nanoprobe was determined using an oligonucleotide intercalating dye—Oligreen (Invitrogen, USA), after displacement of the immobilized thiol-oligonucleotides with 100 mM dithiothreitol. The hybridization efficiency was assessed by a fluorescence-based technique adapted

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Table 1
Effect of mismatch position in hybridization efficiency.

Hybridized targets per Au-nanoprobe (average \pm S.D.)				Fully complementary	Non-complementary
Single base mismatch (G-G) position ^a					
nt1	nt4	nt16	nt20		
8.42 \pm 0.83	9.69 \pm 0.87	15.61 \pm 0.21	20.10 \pm 0.55	19.15 \pm 0.39	2.98 \pm 0.70

^a Considering nucleotide 1 (nt1) at the 3'-end base of the Au-nanoprobe oligonucleotides.

from Demers et al. [7]. Forty-mer ssDNA oligonucleotides were used as targets and a fluorescein-modified 20-mer oligonucleotide (6FAM-3'), complementary to the 5'-end of the target oligonucleotide, was used to quantify hybridization to the Au-nanoprobe. Hybridizations were carried out in 10 mM phosphate buffer (pH 8) containing 20 mM NaCl, by mixing target and fluorescent probe, both 1 μ M with 2.5 nM Au-nanoprobe. Following denaturation at 95 °C for 10 min, the samples were allowed to hybridize at room temperature for 30 min, washed twice and re-dispersed in 10 mM phosphate buffer (pH 8). Samples were then denatured using sodium hydroxide (final concentration of 50 mM) and let to rest for 2 h at room temperature. After centrifuging the samples at 21,460 \times g for 20 min, the supernatant was collected to capture fluorescence spectra in a PerkinElmer LS45 Fluorescence Spectrometer (USA) using an Ultra-Micro quartz cell (Höllma, Germany). Calibration curves were generated by preparing a sequential dilution of the fluorescein-modified oligonucleotide (concentration range: 0.0016–0.1 μ M) under the same conditions as the samples. Absorption spectra of the samples pellet were determined in a UNICAM, model UV2, UV-vis spectrophotometer with Ultra-micro quartz cells (Höllma, Germany), as to correct for loss of Au-nanoprobe during the washing process. All assays were performed in triplicate.

3. Results and discussion

The Au-nanoprobes' hybridization efficiency was evaluated as function of different features of the Au-nanoprobe/target conjugates, such as the position of the mismatch, base pairing combination and thiol-oligonucleotide density.

The effect of the single base mismatch position on hybridization efficiency was assessed by engendering a guanidine-guanidine (G-G) mismatch at several base positions along the sequence of the Au-nanoprobe. Because G-G mismatches yield the strongest interaction among the non-Watson-Crick base pairing [16], they should enhance discrimination of the occurring phenomena at the molecular level. The best mismatch discrimination was found to be at the 3'-end of the Au-nanoprobe (i.e. nt1)—see Table 1. As the mismatch position approaches the 5'-end of the Au-nanoprobes' sequence, the discrimination between fully complementary and mismatched targets decreases, and when the mismatch is located at the 5'-end (i.e. nt20), no discrimination is observed. This decline in sequence specificity may be due to the decreased availability of the nucleotide bases close to the AuNPs' surface for base pairing interaction relative to the outer bases [5]. This way, the destabilizing effect of the mismatch in stable duplex formation and target hybridization efficiency is higher when the mismatch is located at the 3'-end of the Au-nanoprobe. This effect has been previously observed in Au-nanoprobe colorimetric single-nucleotide polymorphism (SNP) detection [11].

We further evaluated the effect of all possible base pair combinations at the 3'-end of the Au-nanoprobe. Our results show that all complementary targets hybridized in higher numbers than targets harboring a mismatch, and non-complementary targets showed negligible hybridization (Fig. 1). Hybridization of mismatched base pair variants indicates that the type of the mis-

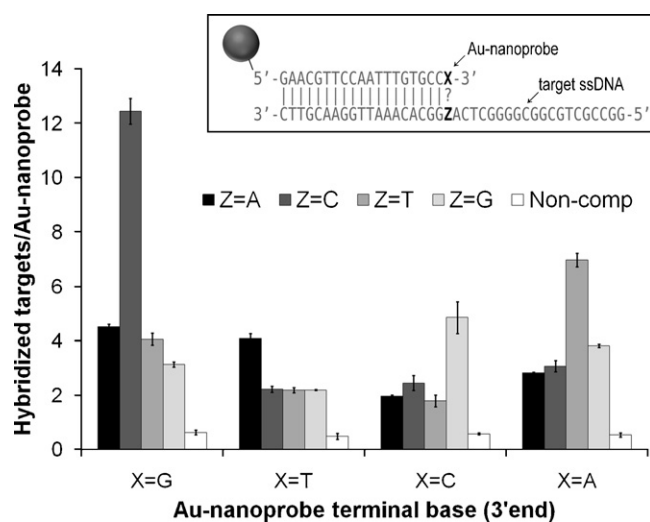


Fig. 1. Effect of base pairing at the 3'-end of the Au-nanoprobe on hybridization efficiency. The variable X translates for the Au-nanoprobe 3'-end base and the variable Y translates for the target base interacting with X, as shown in the inset scheme of the Au-nanoprobe and ssDNA target. Error bars represent standard deviation.

matched base pair has little or no effect in hybridization efficiency. The observed variation in mismatch discrimination between different Au-nanoprobes is likely to be due to different Au-nanoprobe densities. To confirm this hypothesis, we investigated the effect of Au-nanoprobe density in single base mismatch discrimination (G-G). The maximum efficiency of fully complementary target hybridization was observed for 83 ± 4 oligos/AuNP, corresponding to a density of 24 pmol/cm² (Fig. 2). This value is in good

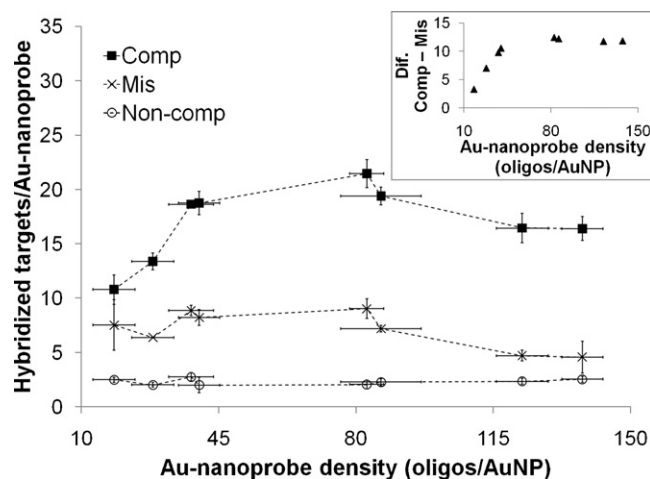


Fig. 2. Effect of Au-nanoprobe density in hybridization efficiency with: a fully complementary target – Comp; a target harboring a single base mismatch G-G at the 3'-end of Au-nanoprobe – Mis; a non-complementary target – Non-comp. Error bars represent standard deviation and dashed lines are guides for the eye. Inset: Difference between the number of complementary and mismatched targets hybridized to the Au-nanoprobe in relation to its oligo density.

accordance with the results observed by Demers and co-workers, where a linear increase in hybridization efficiency between the Au-nanoprobes and fully complementary targets was observed up to a density of 20 pmol/cm² [7]. Below this value, hybridization efficiency has not yet reached the maximum, as fewer Au-nanoprobe oligonucleotides are present to hybridize. For densities above this maximum, more Au-nanoprobe bound oligonucleotides are available for hybridization but the increasing electrostatic repulsion between the targets and the probe together with increasing steric hindrance possibly hampers target hybridization. A similar effect is observed for discrimination between complementary and mismatched targets, i.e. the difference between the number of complementary targets and mismatched targets hybridized to the Au-nanoprobe increases gradually until reaching a plateau beyond which mismatch discrimination remains unaltered (Fig. 2, inset). This maximum discrimination level corresponds to an Au-nanoprobe density associated to the maximum hybridization efficiency. For lower densities, the destabilizing effect of the mismatch in the duplex stability should become less pronounced as the Au-nanoprobes' nucleotide bases become more available to interact with their complementary target bases.

4. Conclusions

We demonstrate that thiol-oligonucleotide density at the AuNPs' surface and mismatch localization within the Au-nanoprobe sequence influence target hybridization and single base mismatch discrimination at room temperature. Hybridization efficiency and SNP discrimination at room temperature is maximized for an Au-nanoprobe density of 24 pmol/cm² and when the mismatch is localized at the 3'-end of the Au-nanoprobe. These aspects should be taken into consideration when optimizing Au-nanoprobes for molecular diagnostic methods, in particular when maximum specificity for single base resolution is required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.colsurfb.2010.01.007.

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