Two-Color Labeling of Oligonucleotide Arrays via Size-Selective Scattering of Nanoparticle Probes

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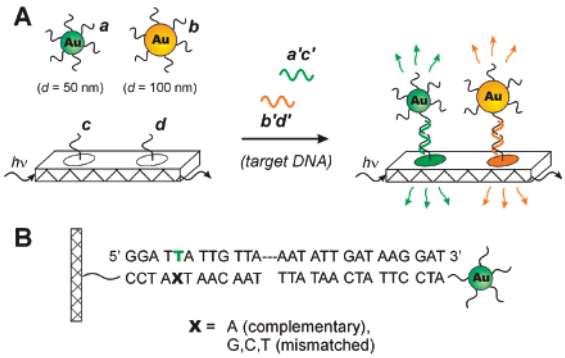
Combinatorial oligonucleotide array (or "gene chip") technology depends on the quantitative detection of target DNA hybridized to complementary array elements. We recently reported a "scanometric" method for detecting DNA targets hybridized to gene arrays in which oligonucleotide-functionalized, 13 nm diameter gold nanoparticles serve as the indicators of target hybridization to the chip.[1] Because of the unusually sharp temperature-induced dissociation (or "melting") profiles of the nanoparticles from the surface of the array, the selectivity of the scanometric DNA detection system was intrinsically higher (by a factor of 4) than that of a conventional array system based upon fluorophore probes. In addition, enlarging the array-bound nanoparticles by gold-promoted reduction of silver(I) permitted the arrays to be imaged, in black-and-white by a flatbed optical scanner, with 100 times the sensitivity than that typically observed by confocal fluorescence imaging of fluorophore-labeled gene chips. The scanometric method was successfully used for DNA base mismatch identification and shows promise for use in single nucleotide polymorphism analysis[2] or identification of genetic disease mutations.[3] However, researchers interested in using gene chips for studying gene expression often require the additional capability of multicolor labeling and imaging of DNA microarrays to perform difference analysis of expressed mRNA in test and reference samples.[4]

Metal nanoparticles that differ in size and composition can be designed to scatter light of different wavelengths according to their distinct surface plasmon resonances.[5] Scattered light from different sized nanoparticles has been used in histochemical imaging,[6,7] but nanoparticle labels have not been used for multicolor labeling in array-based detection schemes. Particles of varying size and composition, which have been modified with a dense layer of oligonucleotides, can be used to yield DNA detection systems with multicolor capabilities and, in principle, the selectivity advantages observed in the scanometric detection system. Herein we report that imaging the light scattered by oligonucleotide-functionalized, 50 and 100 nm diameter Au nanoparticle probes hybridized to targets captured by a DNA array can be used to identify two target sequences in one solution. In addition, we show that, as with the scanometric array method, the unique melting properties of nanoparticle probes lead to enhanced sequence selectivity when DNA arrays are imaged by scattered light from bound nanoparticles.

The DNA sequences of the array capture strands, the oligonucleotide-functionalized nanoparticle labels, and the targets to be detected were designed to cohybridize in a three-component sandwich assay (Scheme 1).[1] Test oligonucleotide targets were synthesized by automated solid-phase synthesis, and oligonucleotide arrays were fabricated on glass microscope slides using

Scheme 1



literature procedures.[1,8] Gold nanoparticles (50 and 100 nm in diameter) were functionalized with dithiane-terminated oligonucleotides and isolated by literature methods.[9] In a typical experiment, oligonucleotide−nanoparticle conjugates and oligonucleotide targets were cohybridized to the DNA arrays in 0.3 M PBS hybridization buffer[10] at room temperature for 2 h.[11] The arrays were then washed with clean buffer to remove unhybridized target and nanoparticle probes. The array slides were mounted on a microscope stage and illuminated in the plane of the slide by a fiber optic illuminator;[12] in this configuration, the slide served as a planar waveguide, preventing any light from reaching the microscope objective by total internal reflectance. Wherever nanoparticle probes were attached to the surface of the waveguide, however, evanescently coupled light[13] was scattered out of the guide plane and was imaged as bright, colored spots on a dark background. In these experiments, green light (λ_{max} = 542 nm) was observed wherever 50 nm Au particles were attached to the waveguide, and orange light (λ_{max} = 583 nm) was similarly observed for attached 100 nm particles. Although scattering of light from large (≥200 nm) selenium particles attached to planar waveguides has been used to image DNA arrays,[14] two features of the system we describe herein distinguish it from previous work: (i) the remarkable sequence selectivity caused by the sharp melting profiles inherent to densely functionalized, oligonucleotide−gold nanoparticle hybrids and (ii) the observation that different particle sizes, and in principle, different particle compositions, provide multicolor analysis of oligonucleotide arrays.

To test the potential of this system to do multiple-color analysis of two different DNA targets in one solution, a mixture of 10 nM 50 nm particles functionalized with DNA sequence ***a***, 3.5 nM 100 nm Au particles functionalized with DNA sequence ***b***, and 200 nM synthetic oligonucleotide targets ***a′c′*** and ***b′d′*** was exposed to a glass slide spotted with sequences ***c*** and ***d*** (Scheme 1A).[11] In the presence of both targets ***a′c′*** and ***b′d′***, the two different nanoparticle probes were successfully and independently hybridized to the corresponding complementary slide spots, as demonstrated by the scattered green light imaged at the test spot for sequence ***a′c′*** and the scattered orange light at the test spot

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(10) 0.3 M NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.
(11) Further details of array hybridization, imaging, and sensitivity are given in the Supporting Information.
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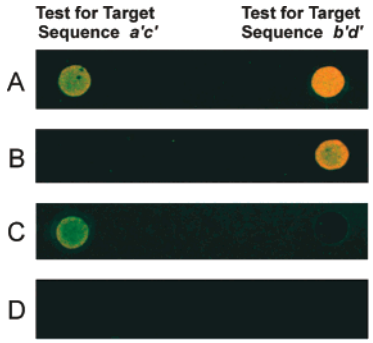

Figure 1. Microscope images of model DNA arrays functionalized with oligonucleotide sequences ***c*** (left) and ***d*** (right) and incubated with a solution of 50 nm diameter Au nanoparticles functionalized with sequence ***a*** (10 nM), 100 nm diameter Au nanoparticles functionalized with sequence ***b*** (3.5 nM), and oligonucleotide target(s) ***a′c′*** and/or ***b′d′*** (200 nM).[11] (A) Array and nanoparticles hybridized with both targets ***a′c′*** and ***b′d′***. (B) Array and nanoparticles hybridized with only target ***b′d′***. (C) Array and nanoparticles hybridized with only target ***a′c′***. (D) Array and nanoparticles incubated in the absence of target. Sequences of DNA used: ***a***, 3′ **TTA TAA CTA TTC CTA A_{20}** 5′-steroid disulfide; ***b***, 3′ **CTC CCT AAT AAC AAT A_{20}** 5′-steroid disulfide; ***c***, 3′ **A_{20} TAT TCT TCC TAT AAT** 5′; ***d***, 3′ **A_{20} TCC CAA TAT AAC ATC** 5′; ***a′c′***, 3′ **TAG GAA TAG TTA TAA ATT ATA GGA AGA ATA** 5′; and ***b′d′***, 3′ **ATT GTT ATT AGG GAG GAT GTT ATA TTG GGA** 5′.

for sequence ***c′d′*** (Figure 1A). The low scattered light intensity measured away from the spots is a reflection of low nonspecific binding of the nanoparticles to the slide surface and the exceptional stability of these nanoparticle probes. In the presence of only target ***a′c′*** or ***b′d′***, only the green light or the orange light is observed, respectively (Figure 1B,C). Little background signal is observed in either case at the noncomplementary spot. Scattered light due to hybridized nanoparticle probes could be distinguished from background signal in the presence of as little as 1 pM of target,[11] which is comparable to the sensitivity of conventional fluorophore-based array detection. Note that no scattered light was observed when the DNA functionalized microscope slide was treated with the two different nanoparticle probes in the absence of targets (Figure 1D).

To test the selectivity of this new scattering detection system based on nanoparticle probes, we synthesized model DNA arrays containing four elements, with each element bearing capture strands containing one of the four possible nucleotides at a particular sequence position. These arrays were incubated at room temperature with a solution that was 200 nM in a target complementary to one of the four elements (X = A, Scheme 1B) and 10 nM in 50 nm diameter nanoparticle probes.[11] The arrays were then washed to remove unhybridized target and probes and resubmerged in clean hybridization buffer. The dissociation of the hybridized nanoparticles from the array surface was then induced and continuously observed by gradually raising the temperature of the buffer. As long as this temperature did not exceed the melting temperature (T_m) of either the complementary or the mismatched DNA duplexes, scattered light was observed at all four array elements (Figure 2A), indicating that no dissociation had taken place. However, as the temperature of the buffer was further increased, the nanoparticle probes dissociated from the slide in the order of the thermal stability of the varied base pair (T:T ≈ C:T < G:T < A:T, Figure 2A). Because the dissociation of nanoparticles from the slide was observed in real time, the optimal stringency temperature, selectivity, and target sequence could all readily be determined visually as the experiment progressed. In addition, quantitative melting profiles for all array elements were generated and compared simultaneously by measuring the signal intensity at each element in real time (Figure 2B). At the temperature of optimal stringency (55 °C, shown by the vertical line in Figure 2B), the signal at the complementary (X = A) element is five times higher than that of the element

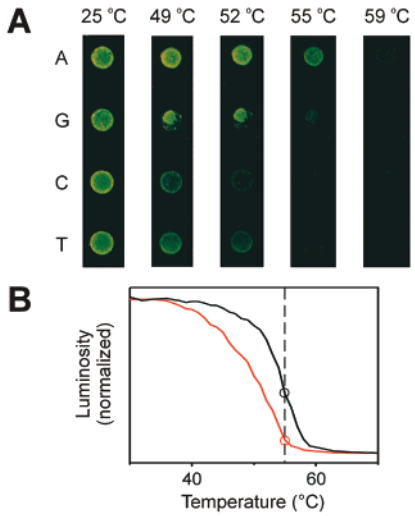

Figure 2. (A) Microscope images of single nucleotide polymorphism (SNP) arrays labeled with 50 nm diameter Au nanoparticle probes. Arrays functionalized with sequences shown in Scheme 1B were incubated with oligonucleotide-functionalized particles (10 nM) and a synthetic oligonucleotide target (200 nM) complementary to only one of the four array sequences (X = A); after washing and resuspending the array in clean hybridization buffer, images were taken at the temperatures shown as the buffer was gradually heated and stirred.[11] (B) Melting profiles of 50 nm Au nanoparticle probes from the DNA array surface at elements X = A and G, determined by quantitative analysis of array images (such as those in part A) obtained during the melting experiment.[17] At T = 55 °C (shown by the vertical line), taken from the corresponding image in part A, the fraction of remaining hybridized particle label is 0.38 for X = A and 0.08 for X = G.

containing a G:T "wobble" mismatch. This represents significantly higher sequence selectivity than previously observed for molecular fluorophore probes and arrays with identical sequences.[1] As was observed for smaller (13 nm diameter) nanoparticles via the black and white scanometric approach,[1] the higher selectivity of the 50 nm particle probes compared to fluorophore-labeled DNA is a direct consequence of the narrow temperature range over which the nanoparticles dissociate from the array surface; the full width at half-maximum for the first derivative of the curves shown in Figure 2B is 5 °C, compared to 18 °C for fluorophore-labeled DNA with an identical sequence.[1] Results presented here demonstrate that this selectivity also can be used in conjunction with multicolor labeling of multiple DNA targets on the same chip.

The DNA array imaging technique described here, based on scattered light from oligonucleotide-functionalized nanoparticles, is a sensitive, ultraselective, multicolor labeling method for DNA arrays. This approach should be extendable to additional colors using nanoparticles of different compositions and sizes.[15] In addition, the use of particles with higher scattering coefficients may permit this system to rival the high sensitivity of the scanometric nanoparticle system[1] and waveguide-based fluorescence arrays.[16]

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Supporting Information Available: Details of oligonucleotide synthesis, nanoparticle functionalization, DNA array fabrication, array imaging, spectral analysis of surface-bound particles, and melting experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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