DNA Detection

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One-Step Homogeneous Detection of DNA Hybridization with Gold Nanoparticle Probes by Using a Linear Light-Scattering Technique**

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As the human genome project has uncovered the full sequence of human genomes and post-genome technologies have been rapidly developed, detection of specific DNA sequences has become increasingly important in the diagnosis of hereditary diseases, detection of infectious agents, discovery of gene-targeted drugs, and a variety of biomedical studies. Many detection systems make use of solid-phase sandwich DNA hybridization methods that contain oligonucleotide probes covalently linked to reporter groups.[1] These assays have several merits, such as high specificity, low background, and high throughput. However, they also have a fundamental weakness in that they usually need a long dwell time owing to several cycles of consecutive hybridization and washing steps. In addition, the requirement of washing steps to remove the unhybridized probes precludes the use of real-time hybridization studies and the local detection of specific nucleic acids in living cells. As a way to circumvent the limitations, several schemes have been put forward for detecting specific nucleic acids in homogeneous solution,[2] such as fluorescence polarization,[3,4] fluorescence resonance energy transfer,[5–8] TaqMan,[9,10] molecular beacons,[11,12] and light-up probes.[13,14] Among these assays, molecular beacons have been widely used for detection of DNA hybridization in homogeneous solutions[2] and gene expression in living cells[15,16] owing to their high specificity and high sensitivity (0.3-nM detection limit).[17] However, they suffer from a difficult synthesis, are susceptible to photobleaching, and are expensive to employ.

In recent years, great attention has been focused on the use of metal nanoparticle labels on oligonucleotide probes to overcome the problems associated with fluorescent labels. Mirkin and co-workers first developed an entirely new colorimetric detection scheme for DNA hybridization in a homogeneous solution based on aggregation of oligonucleotide-functionalized gold nanoparticles that are directed by the target DNA and the distance-dependent optical properties of gold nanoparticles.[18,19] However, the main limitation of these approaches is their low sensitivity, which is still not as good as the best results from fluorophore-based assays.[20] To improve the sensitivity, many detection techniques, such as the scanometric method,[21] surface plasmon resonance (SPR),[22] laser diffraction,[23] colorimetric scatter on glass waveguide,[24] surface-enhanced Raman spectroscopy,[25] and array-based electrical detection,[26] have been developed. These approaches can push the detection limits of target DNA to picomolar, and even femtomolar values. However, all these assays need to detect gold nanoparticles on a solid phase and inevitably need a sophisticated process and expensive instruments.

Herein, we report a highly sensitive light-scattering assay for DNA hybridization in a homogeneous solution by using gold nanoparticles as the labels of oligonucleotide probes. The assay relies on the observation of greatly enhanced light scattering that originated from the aggregation of oligonucleotide-functionalized gold nanoparticles directed by the target DNA. Notably, a gold-nanoparticle-based hyper-Rayleigh scattering (nonlinear light scattering) has recently been explored for the detection of DNA hybridization in which 10 nM target DNA can be detectable.[27] However, here, the light scattering can be easily measured by using a common spectrofluorimeter equipped with a 150-W high pressure Xenon lamp.[28] Hence it should be considered as linear light scattering because the nonlinear light scattering can only be observed with laser excitation. With the proposed light-scattering assay, target DNA with a concentration as low as 0.1 pM can be detected and no washing steps are needed. The sensitivity increases by at least four orders of magnitude greater over that of the gold-nanoparticle-based colorimetric method[18] and the hyper-Rayleigh scattering assay.[27] Moreover, the light-scattering assay exhibits a high degree of discrimination between perfectly matched target DNA and targets with single base-pair mismatches.

As shown in Figure 1, the target DNA is a 30-base fragment of human p53 gene (exon 8). Probe 1 and probe 2, made of 15 bases, are complementary to part of the target DNA. By functionalizing the gold nanoparticles with either 3′ or 5′ termini of the thiol-capped oligouncleotide probes, the gold nanoparticles would align in a tail-to-tail (a), tail-to-head (b), or head-to-head (c) fashion onto the target DNA. Owing to many oligonucleotide probes immobilized on the surface of one nanoparticle, the hybridization in each alignment fashion can result in the formation of extended polymeric network aggregates.[18]

Figure 2 shows that the light-scattering intensity of the gold nanoparticles modified with probe 1 and probe 2 is very weak in the absence of target DNA. The light scattering would be only Rayleigh scattering because the diameter of the gold nanoparticles ($\approx$13 nm) is smaller than 1/20 of the incident wavelengths in the range of 260–700 nm.[29] A weak resonance Rayleigh scattering (RSS) peak can be observed at 545 nm, which is near the maximum absorbance wavelength (520 nm) of the gold nanoparticles. The light-scattering intensities of the gold nanoparticles can be greatly enhanced by the addition of complementary target DNA in the

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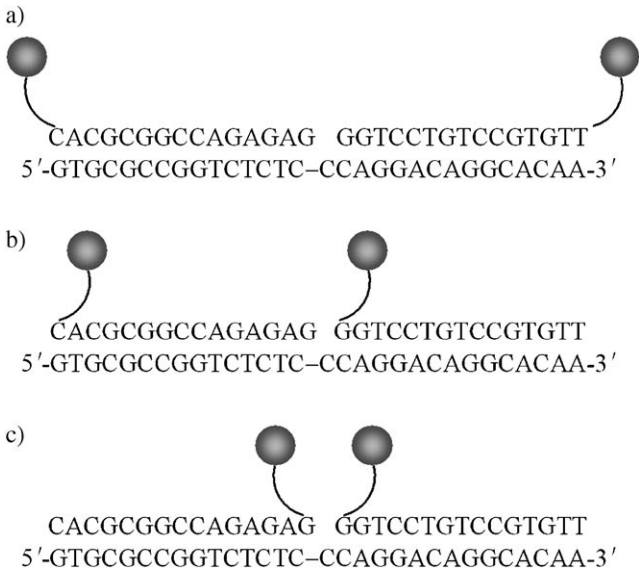

Figure 1. The three fashions of gold nanoparticles modified respectively with probe 1 and probe 2 at the 3′ or 5′ terminus aligning onto a target DNA. a) tail to tail, b) tail to head, c) head to head.

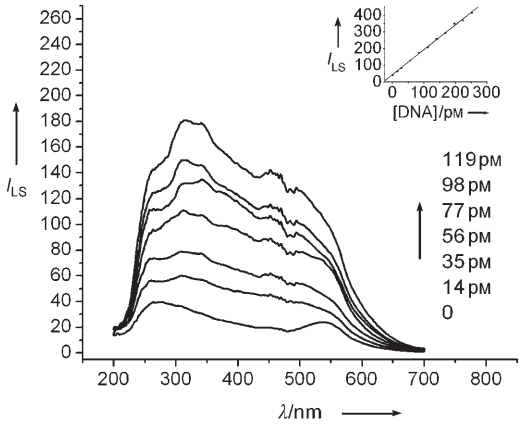

Figure 2. The light-scattering spectra of gold nanoparticles modified respectively with probe 1 and probe 2 in the absence and in the presence of the complementary DNA target at different concentrations. The inset shows the calibration curve of the target-DNA concentration versus the intensity of the light scattering (I_{LS}). The gold nanoparticles are align in the tail-to-tail fashion onto the target DNA. The DNA hybridization is performed in phosphate buffer solution (10 mM; pH 7.0) containing NaCl (0.7 M), and the hybridization time is 5 min at room temperature.

wavelength range of 250–650 nm. Interestingly, however, the RRS peak disappears, which is likely because the size of the aggregates induced by the DNA hybridization exceeds the range to produce RRS.[30] It can also be seen from Figure 2 that the enhanced light-scattering intensities linearly increase and the light-scattering spectra maintain almost the same pattern as the concentration of the target DNA increases. This means that the light scattering of gold nanoparticle aggregates formed in solution has no obvious wavelength selectivity. However, on the glass surface, different-sized gold nanoparticles can scatter light at different wavelengths.[31] Furthermore, the color of scattered light of the gold nanoparticles modified with oligonucleotide probes is different from that of the aggregates of the gold nanoparticles that are directed by the hybridization between the probes and the complementary target DNA.[24] Therefore, the great enhancement of light scattering induced by DNA hybridization in solution cannot be simply explained by the increased size of the formed aggregates of gold nanoparticles because the aggregates have a polymeric network structure and are still water soluble in the solution.

In the polymeric network structure of the gold nanoparticle aggregates, the gold nanoparticles are interlocked by multiple short duplex segments[18] in which the gold nanoparticle concentration would be much greater than that of the surrounding solution. Namely, the aggregation of the gold nanoparticles directed by DNA hybridization can lead to the great concentration fluctuation of the gold nanoparticles in the solution, which can consequently induce the great enhancement of light scattering according to Einstein's fluctuation theory for light scattering. Therefore, light scattering is a very sensitive and powerful technique for the detection of aggregation and self-assembly of nanoparticles in a solution.

The quantitative assessment of the proposed light-scattering assay for DNA hybridization is based on monitoring the dependence of the light-scattering intensity at 315 nm on the target-DNA concentration. As shown in the inset of Figure 2, the corresponding calibration plot of light-scattering intensity versus the target-DNA concentration is linear over the range of 0.7–119 pM. The correlation equation is $I_{LS} = 44.6 + 1.49c$ (where I_{LS} = the intensity of light scattering and c = the target-DNA concentration in pM) with a correlation coefficient of $r = 0.9980$. A series of 11 repetitive measurements of target DNA (0.7 pM) were used to evaluate the precision, and the relative standard deviation (RDS) was determined to be 2.6%. The corresponding detection limit (3σ, $n = 11$) was estimated to be 0.1 pM.

A variety of factors can affect the aggregation of the gold nanoparticles in the DNA hybridization system.[32] For a given system, salt concentration and the gold nanoparticle position on the oligonucleolide probes (either 3′ or 5′ terminus), that is, the alignment fashions of the gold nanoparticles on the DNA hybrids, are critical. Our experimental results show that the light-scattering intensity that resulted from the DNA hybridization almost linearly increases with an increase in the salt concentration, from 0.25 M to 0.7 M, and then levels off. This result is consistent with the conclusion that electrostatic interactions (repulsion between gold nanoparticles) are the dominant factors that affect stability of the DNA hybrids.[32] Owing to a screening effect of the salt, high salt concentration can minimize the electrostatic repulsion resulting in to more hybridization events taking place. On the other hand, the tail-to-tail arrangement is more favorable to the aggregates directed by the DNA hybridization among the three alignment fashions because the tail-to-tail fashion results in the longest interparticle distance, which then exhibits the smallest particle-to-particle repulsion. Therefore, the salt concentration of 0.7 M and the tail-to-tail fashion are employed for the proposed light-scattering assay for DNA hybridization.

The specificity of the proposed light-scattering assay is evaluated by detecting the light-scattering intensities arising from a complementary target, one-base-mismatched DNA strand, in which the point mutation of C to T corresponds to the single-nucleotide polymorphism (SNP) of Arg283His in the human p53 gene (exon 8) and a three-base-mismatched DNA strand. All of DNA molecules tested in the experiment are at a concentration of 66 pM.

Figure 3 shows that a well-defined light-scattering response is observed for the complementary target DNA, whereas the three-base-mismatched DNA strand yields a

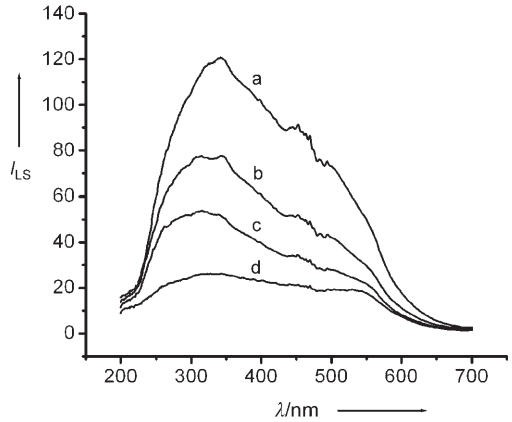

Figure 3. The light-scattering spectra of the gold nanoparticle probes in the presence of perfectly complementary DNA (a), one-base-mismatched DNA strands (b), three-base-mismatched DNA strands (c) at a concentration of 66 pM, and in the absence of the DNA target (d). The pH of the solutions are adjusted to be 7.0 by phosphate buffer solution (10 mM) containing NaCl (0.7 M). The hybridization time is 5 min at room temperature.

weaker light-scattering signal. It was also found that the light-scattering intensity of the one-base-mismatched DNA strand is much smaller (only 55%) than that of the perfectly complementary target. Therefore, with the proposed light-scattering assay, the single-base-mismatched DNA strands can be directly discriminated from the perfectly complementary targets without stringently controlling the temperature, indicating the high specificity of the assay. Controlling the temperature would be expected to differentiate the single-base mutation from the perfectly complementary targets and to apply to SNP analysis.

In summary, we have demonstrated a new gold-nanoparticle-based light-scattering strategy for detecting sequence-specific DNA in homogeneous solution. The sensitivity of the light-scattering assay compares favorably with the previously reported homogeneous detection methods for DNA hybridization, and the assay can be accomplished with one-step operation by using a common spectrofluorimeter. The light-scattering assay also exhibits such high specificity that a one-base difference in DNA targets can be directly discriminated without controlling the temperature. More importantly, it has been demonstrated that the light-scattering technique is a simple and sensitive tool for detecting the aggregation of nanoparticles in homogeneous solution. The nanoparticles functionalized by appropriate biomolecules can provide a variety of specific recognition and bioaffinity interactions which can result in the nanoparticle aggregation.[33] Therefore, the proposed light-scattering method would be expected to be extended for immunoassays, drug discovery based on the interactions between drugs and DNA (or protein) targets, etc. On the other hand, the homogeneous assay should be suited for monitoring the synthesis of specific nucleic acids in real time. If the gold nanoparticles functionalized with oligonucleotide probes are introduced into living cells, the light-scattering assay can probably open new avenues for the analysis of gene expression in living cells by using fluorescence microscopy with appropriate designs.

Experimental Section

Tetrachloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Sinopharm Group Chemical Reagent Co. (Shanghai, China). All of the oligonucleotides used in this paper were purchased from TaKaRa Biotechnology (Dalian, P.R. China) Co., Ltd. The sequences are as follows:

probe 1:	5′-(SH-C_6)-(T)10-TTG TGC CTG TCC TGG-3′
	5′-TTG TGC CTG TCC TGG-$(T)_{10}$-(C_3-SH)-3′
probe 2:	5′-GAG AGA CCG GCG CAC-$(T)_{10}$-(C_3-SH)-3′
	5′-(SH-C_6)-(T)10 -GAG AGA CCG GCG CAC-3′
complementary target strand:	5′-GTG CGC CGG TCT CTC CCA GGA CAG GCA CAA-3′
one-base-mismatched strand:	5′-GTG CGT CGG TCT CTC CCA GGA CAG GCA CAA-3′
three-base-mismatched strand:	5′-GTG CGT CGG TTT CTC TCA GGA CAG GCA CAA-3′

Twice-distilled deionized water was used throughout. NaH_2PO_4, NaOH, and NaCl (Third Chemical Reagent Factory of Tianjin, Tianjin, China) were of analytical reagent grade and used to prepare the buffer solution.

A F-4500 spectrofluorimeter (Hitachi, Japan) was used to measure the light-scattering spectra and light-scattering intensities. A WH-861 vortex mixer (Huangjin Instrumental Co., Jiangsu, China) was used to blend the solutions. The TEM images of the colloidal gold were acquired on a JEM-1200EX II transmission electron microscope (JEOL, Japan).

Gold nanoparticles were prepared by the reduction of $HAuCl_4$ by trisodium citrate (Tianjin Beichen Chemical Reagent Co., Tianjin, China) according to the literature,[34] and the TEM image demonstrated a average diameter of 13 nm. Colloidal gold-labeled oligonucleotides were synthesized by derivatizing the gold-nanoparticle solution with 3′- and 5′-alkanethiol-capped oligonucleotides according to the previous report, but with some modifications (see the Supporting Information).[35]

In a typical experiment for DNA detection, a homogeneous three-component sandwich assay is used in the hybridization reaction. Briefly, under ambient temperatures, a solution of the gold nanoparticles functionalized with probe 1 (1 mL) and a solution of the gold nanoparticles functionalized with probe 2 (1 mL) in phosphate buffer solution (10 mM; pH 7.0) containing NaCl (0.3 M) were added to a dry 5-mL test tube. The NaCl concentration in the solution was then adjusted to 0.7 M. Afterward, a series of dilutions of target DNA dissolved in phosphate buffer solution (10 mM; pH 7.0) were pipetted into the test tubes by using microsyringes. The mixtures were blended by the WH-861 vortex mixer and heated at 70 °C for 5 min. After the mixtures were cooled to room temperature, another 5 min was needed to complete the hybridization. The light-scattering spectra were then recorded by synchronously scanning the excitation and

emission monochromators from 200 nm to 700 nm (namely, $\Delta\lambda = 0$) by using the F-4500 spectrofluorimeter.

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