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Functional magnetic nanoparticle-based trapping and sensing approaches for label-free fluorescence detection of DNA

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ABSTRACT

In this study, a label-free fluorescence detection method for DNA was designed using functional magnetic nanoparticles (MNPs) as affinity probes. With the advantage of magnetic feature, MNP-based affinity probes can be easily manipulated for trapping and sensing target species. Two types of MNP-based nanoprobes for trapping and detecting target DNAs were fabricated. The basic strategy for this approach is the use of trapping probes to concentrate target DNAs selectively from complex samples. The detection probes are then used as fluorescence reporters to explore the level of the target species. Trapping probes were constructed by covalently immobilizing probe DNA molecules complementary to the target DNA. Detection nanoprobes were made by linking a fluorescent dye, riboflavin-5′-monophosphate (RFMP), onto the surface of the core/shell Fe_3O_4@Al_2O_3 MNPs via Al-phosphate chelation. The fluorescence derived from RFMP molecules became invisible when molecules were attached onto the MNP surface. However, after phosphorylated species (e.g., DNA molecules) replaced RFMP from the surface of the RFMP-Fe_3O_4@Al_2O_3 MNPs under microwave heating for 15 s, the RFMP molecules released from the MNPs enhanced the fluorescence intensity in the solution. Based on the measurement of the fluorescence intensity, the level of target DNA in the samples was determined. The remaining DNA molecules on the RFMP-Fe_3O_4@Al_2O_3 MNPs were characterized by using matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). The detection limit for DNA was as low as 40 pM using this approach.

1. Introduction

DNA carries informative genetic codes. Thus, it has been used as the main target in biodiagnostics and biochemical analyses [1–3]. Electrochemistry and optical spectroscopy have been used as analytical tools for DNA detection [1–6]. For high throughput DNA analysis, DNA chips are the typical choice [7–11]. However, fluorescence-labeled nucleic acid probes are usually required [9,10]. Furthermore, amplifying DNA prior to chip analysis may be necessary because of its insufficient sensitivity. Although real-time polymerase chain reaction (PCR) can provide the required sensitivity for DNA detection [12], it is generally unsuitable for high throughput analysis because of its time-consuming experimental steps and the high cost of reagents. Alternatively, analytical methods based on label-free fluorescence detection [8,13–15], and molecular beacons [9,16,17] have recently attracted much attention. For example, intercalating dyes like ethidium bromide [15] have been demonstrated as highly sensitive toward double strand DNA. Combining the ability to concentrate traces of target DNA from sample solutions and eliminating unwanted interference are desirable. Developing new methods to improve analysis performance can be beneficial for DNA analysis.

The rapid growth of nanotechnology has opened new avenues for progress in DNA-sensing methods. Functionalized gold nanoparticles (NPs) immobilized with DNA probes have been used extensively in the detection of complementary target DNA based on observations of color changes in NPs [1,6]. Additionally, functional magnetic nanoparticle (MNP)-based approaches have recently received much attention [18–21]. We herein propose a new approach, whose basic strategy is the employment of two types of functional MNPs, trapping probes and detection probes, with microwave heating in order to develop a rapid, simple, and sensitive method for DNA analysis. Specifically, two types of MNP-based nanoprobes, one for DNA trapping and the other for DNA detection, were fabricated. With advantages on magnetic property, the trapping nanoprobes were easily used in the selective concentration and isolation of target DNAs from the complex samples. Detection probes were used as activated fluorescence reporters for the target DNA species that were selectively concentrated by the magnetic trapping probes. Iron oxide MNP-based nanoprobes for targeting DNA (Fe_3O_4@DNA MNPs) were constructed onto the surface of the MNPs by covalently

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immobilizing DNA molecules complementary to the target DNA. Aluminum oxide-coated iron oxide (Fe_3O_4@Al_2O_3) MNPs could interact with phosphorylated species [22–27]. The detection nanoprobes were constructed by simply linking an inexpensive fluorescent dye, i.e., riboflavin-5′-monophosphate (RFMP), onto the surface of the core/shell of Fe_3O_4@Al_2O_3 MNPs (RFMP-Fe_3O_4@Al_2O_3 MNPs) via Al-phosphate chelation [27]. RFMP could emit fluorescence at the wavelength of 530 nm with the excitation wavelength at 450 nm. The fluorescence derived from the RFMP molecules was invisible when the molecules attached onto the surface of the MNPs (i.e., quenching effect). After the phosphorylated species (i.e., DNA molecules) replaced the RFMP from the surface of the Fe_3O_4@Al_2O_3 MNPs, the light derived from the released RFMP in the solution was readily turned on under excitation light. The level of DNA in the samples was determined quantitatively based on the measurements of fluorescence intensity in the solution. To confirm further the aforementioned results, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was employed to characterize the DNA molecules attached to the detection probes. Qualitative and quantitative information for target DNA could be readily provided using this sensing method.

2. Material and methods

2.1. Reagents

Sodium chloride was purchased from Merck (Seelze, Germany). Iron(III) chloride hexahydrate, tri-sodium citrate-2-hydrate, di-ammonium hydrogen citrate, trifluoroacetic acid (TFA), and sodium sulfite anhydrous were obtained from Riedel-de Haën (Seelze, Germany). Tetraethyl orthosilicate (TEOS) and glutaraldehyde were obtained from Fluka (Buchs, Switzerland). Ferrous chloride, hydrochloric acid (36.5–38%), acetonitrile, ammonium bicarbonate, and sodium silicate (35%) were purchased from J.T. Baker (Phillipsburg, NJ). Aluminum isopropoxide and 2′,4′,6′-trihydroxyacetophenone monohydrate (THAP) were purchased from Aldrich (Milwaukee, WI). Aqueous ammonium hydroxide (33%), sodium hydroxide, tetramethyl ammonium hydroxide pentahydrate, (3-aminopropyl)triethoxysilane (3-APTES), 2-(*N*-morpholino)ethanesulfonic acid (MES), sodium phosphate monobasic, sodium phosphate dibasic heptahydrate, and RFMP were obtained from Sigma (St. Louis, MO). Ethanol (99.5%), acetone (99.9%), and methanol (99.9%) were obtained from ECHO (Miaoli, Taiwan). All the oligonucleotides were purchased from MD Bio Inc. (Taiwan).

2.2. Preparation of trapping probes

Fe_3O_4 MNPs immobilized with DNA molecules that were complementary to target DNA were generated. Initially, the Fe_3O_4 MNPs were prepared using the co-precipitation method. $FeCl_3$ (5.4 g) and $FeCl_2$ (2 g) were dissolved in a hydrochloride solution (2 M, 25 mL). The gas in the reaction flask was pumped out and the flask was filled with nitrogen. An ammonia solution (25%, 40 mL) was slowly added to the solution with vigorous stirring under a nitrogen atmosphere. The mixture was stirred at room temperature for 1 h. The generated MNPs were rinsed with deionized water (40 mL × 3), followed by re-suspension in deionized water (40 mL) prior to use.

To prepare silane-coated Fe_3O_4 MNPs (Fe_3O_4@TEOS MNPs), the as-generated Fe_3O_4 MNPs (1.0 g) were isolated from the MNP suspension solution obtained above and the MNPs were re-dispersed in ethanol/deionized water (32 mL/8 mL) in a flask. The flask was filled with nitrogen by covering with a balloon with nitrogen gas. The nanoparticle solution was then sonicated for 10 min in an ultrasonicator to thoroughly disperse the MNPs in the solution. TEOS was quickly injected into this suspension using a syringe, followed by ultrasonication for 10 min. Aqueous ammonia (10%, 2.0 mL) was quickly injected into the mixture using a syringe. The resulting solution was continually ultrasonicated for another 10 min. The mixture was then stirred at 40 °C for 12 h. The as-prepared Fe_3O_4@TEOS MNPs were magnetically isolated from the solution, rinsed with methanol (40 mL × 3) and deionized water (40 mL × 2), and re-suspended in methanol (40 mL).

The as-prepared Fe_3O_4@TEOS MNPs were modified further with APTES to generate amino-terminal functional groups on their surfaces. That is, the Fe_3O_4@TEOS MNPs (32 mg) were rinsed with acetone (10 mL × 2) and then covered with nitrogen. A solution containing APTES (0.25 mL) and acetone (12.5 mL) was added to the above MNP suspension, after which the solution was stirred at room temperature. After 12 h, the generated Fe_3O_4@APTES MNPs were rinsed with ethanol (10 mL × 3) to remove excess reagents. The MNPs were then re-suspended with a saline sodium citrate (SSC) buffer (5 mL). The SSC buffer was prepared by diluting a mixture of NaCl (2 M) and sodium citrate (0.2 M, pH 7.4) by a factor of 13. The Fe_3O_4@APTES MNP suspension obtained above was stirred with glutaraldehyde (5 mL) prepared in the SSC buffer (20 mL) under nitrogen for 12 h to generate MNPs with terminal aldehyde groups. The generated MNPs were rinsed with SSC buffer (10 mL × 3). Fig. S1a and b presents the TEM image and size distribution of the resulting MNPs. The size of the MNPs was estimated at 12.4 ± 2.5 nm. The MNPs have superferromagnetic property according to the hysteresis curve (Fig. S1c), and the saturation magnetism (M_H) was estimated to be ~24.5 emu/g.

The glutaraldehyde-bound MNPs (50 μg) obtained above were then cross-linked with NH_2-derivatized DNA (10^{-6} M, 0.25 mL) prepared in an MES buffer (50 mM, pH 6.3) via imine binding. The mixture was reacted under stirring for 8 h. Alternatively, DNA-bound MNPs could be readily generated with similar DNA binding capacity by reacting the MNPs with the DNA under microwave heating (450 W) for 30 s. The resulting DNA-immobilized MNPs (Fe_3O_4@DNA) were magnetically isolated, followed by rinsing with the MES buffer (250 μL × 2). The resulting Fe_3O_4@DNA MNPs were used as the DNA-targeting probes in the study. The binding amount of the DNA on the MNPs was estimated by measuring the difference at 260 nm of the optical density ($OD_{260\,nm}$) of the DNA solution obtained before and after reaction. The DNA binding amount on the MNPs was estimated at 2.40 ± 0.04 nmol/mg.

2.3. Preparation of RFMP-Fe_3O_4@Al_2O_3 MNPs

Iron(III) chloride ($FeCl_3{\cdot}6H_2O$, 6.48 g) was dissolved in hydrochloric acid (2 M, 12 mL) and then diluted with 100 mL of water. The mixture was degassed using a vacuum pump. Sodium sulfite (50 mL, 0.08 M) was slowly added to the above solution under nitrogen with stirring at room temperature. Subsequently, ammonia (5%, 45 mL) was added slowly to the reaction solution with vigorous stirring at room temperature. The mixture was left to react for 30 min in a water bath at 70 °C. The magnetic nanoparticles were rinsed three times with deionized water, followed by re-suspending in tetramethylammonium hydroxide/deionized water (1 g/40 mL) and stirring for 1 h. The Fe_3O_4 MNPs (0.2 g) dissolved in deionized water (40 mL) were first prepared in a flask. The gas in the flask was removed by a pump, and then the flask was filled with nitrogen. The suspension was well-dispersed under ultrasonication for 1 h. Sodium silicate (0.6%, 40 mL) was quickly injected into the flask. The mixture was stirred in an oil bath at 35 °C for 24 h. The generated Fe_3O_4@Silicate MNPs were rinsed with deionized water (40 mL × 3), followed by re-suspension in deionized water (40 mL) and ultrasonication for 30 min. Alumina-coated Fe_3O_4@Silicate MNPs (Fe_3O_4@Al_2O_3) were then generated by ultrasonicating the MNP suspension with aluminum isopropoxide

Table 1
List of DNA used in this study.

DNA	Sequences	MW
Probe	5′($NH_2C_6H_{12}$)-AAAAA AATCG TGCGT GTAAT CTTGA-3′(OH)	7892.26
Target	5′($HOPO_3$)-TCAAG ATTAC ACGCA CGA-3′(OH)	5555.63
M1	5′($HOPO_3$)-TCAAG ATTAC ACTCA CGA-3′(OH)	5530.62
M2	5′($HOPO_3$)-TCAAT ATTAC ACTCA CGA-3′(OH)	5505.06
M3	5′($HOPO_3$)-TCAAT ATTAC ACTCA CTA-3′(OH)	5480.59
R1	5′($HOPO_3$)-AAAAA AACAG GTGCC GAGAT AGATG-3′(OH)	7861.14
R2	5′($HOPO_3$)-AAAAA AACAG GTGCC GAGAT AGATG GTAGA TAGAG CCGTG-3′(OH)	12,580.19
R3	5′($HOPO_3$)-GTCCA ATGAT TAGAT GTACA T-3′(OH)	6523.27

(15 mg) for 30 min. The mixture was stirred in an oil bath at 80 °C for 1 h, while placing it in an ultrasonicator to sonicate for 1 min at 15-min intervals to thoroughly disperse the MNPs in the solution. After 1 h, the temperature of the reaction was elevated to 90 °C. The cap was opened for 30 min to remove the generated isopropanol during reaction. The mixture was refluxed at 90 °C for 2 h. After cooling to room temperature, the as-prepared Fe_3O_4@Al_2O_3 MNPs were rinsed with deionized water (40 mL × 3) and re-suspended in deionized water (40 mL). Fig. S2a and b presents the TEM image and size distribution of the resulting MNPs. The size of the MNPs was estimated to be 22.6 ± 3.8 nm. The MNPs have superferromagnetic property according to the magnetism result, and the saturation magnetism (M_H) was estimated to be ~63.9 emu/g (Fig. S2c). On the basis of the energy dispersive X-ray analysis (EDAX) result (Fig. S2d), the MNPs contain Fe, Si, O, and Al, which provided the evidence that alumina had been immobilized on the MNPs.

We then bound fluorescent dye, that is, RFMP, onto the surface of the Fe_3O_4@Al_2O_3 MNPs (RFMP-Fe_3O_4@Al_2O_3 MNPs) via phosphate-Al chelation [27] by incubating the Fe_3O_4@Al_2O_3 MNPs (50 μg) and RFMP (1.5×10^{-5} M, 1 mL) prepared in a solution of 0.075% TFA/50 mM ammonium bicarbonate (9/1, v/v) (buffer A, 1 mL) for 2 h. We have previously demonstrated the effectiveness of microwave heating in accelerating the trapping of target species [27]. Alternatively, RFMP can bind onto the surface of the Fe_3O_4@Al_2O_3 MNPs under microwave heating for 60 s (power: 450 W) with similar binding results, while the cap of the sample vial was kept open during incubation. The binding amount of RFMP onto the MNPs was estimated by measuring the fluorescence intensity ($\lambda_{excitation}$ = 450 nm; $\lambda_{emission}$ = 530 nm) of the RFMP solution obtained before and after interaction with the MNPs. To reduce the possibility of over-estimating the binding amount, the isolated MNP-RFMP conjugates were repeatedly rinsed with buffer A (1 mL × 2). The fluorescence intensity ($\lambda_{excitation}$ = 450 nm) of the rinse solution at 530 nm was recorded and taken into consideration as a factor for non-specific binding. The binding amount was estimated based on an RFMP calibration curve. The maximum binding amount of RFMP on the Fe_3O_4@Al_2O_3 MNPs was estimated to be ~73 nmol/mg.

2.4. Using Fe_3O_4@DNA to trap target DNA

The sample containing the target DNA was prepared in a phosphate buffered saline (PBS, pH 8) consisting of Na_2HPO_4 (0.1 M), NaH_2PO_4 (0.1 M), and NaCl (0.5 M). When conducting the trapping experiment, the DNA sample (0.25 mL) was heated to 95 °C for 10 min. Subsequently, the sample was mixed with the Fe_3O_4@DNA MNPs (50 μg) and incubated in a refrigerator (4 °C) for 30 min. Table 1 details the DNA sequences used in this study. The Fe_3O_4@DNA-target species conjugates were separated from the solution via magnetic isolation. The supernatant was collected to estimate the trapping amount of the target DNA on the Fe_3O_4@DNA MNPs based on the measurement of $OD_{260\,nm}$ using ultraviolet/visible (UV/Vis) absorption spectroscopy.

2.5. Using RFMP-Fe_3O_4@Al_2O_3 as detection probes for DNA

The MNP-target species conjugates were re-suspended in deionized water (40 μL). The temperature of the solution was raised to 47 °C first and maintained at the same temperature for 10 min to release mismatched DNA for fluorescence measurement, while the remained MNP-target species conjugates were re-suspended in deionized water (40 μL). Subsequently, the temperature of the remained MNP-target species were raised to 60 °C and maintained at the same temperature for 10 min to dissociate the target DNA from the conjugates. To conduct the detection experiment for target DNA, the supernatant was acidified by mixing with a solution (10 μL) of 0.45% TFA/0.1 M ammonium bicarbonate (3/1, v/v). The acidified supernatant (50 μL) was mixed with the RFMP-Fe_3O_4@Al_2O_3 (10 μg) and incubated in a microwave oven (power: 900 W) for 15 s. We found that the results obtained from incubation of the acidified supernatant with the RFMP-Fe_3O_4@Al_2O_3 (10 μg) under vortex mixing for 2 h were similar to those obtained from incubation of the same mixture under microwave heating for 15 s. Thus, all the results presented in this work were obtained by conducting label-free fluorescence detection of DNA under microwave heating. After incubation, the supernatant was observed by either the naked eye under UV light irradiation or fluorescence spectroscopy for the analysis of target DNA. The isolated MNPs from the solution were rinsed with buffer A followed by re-suspension in PBS buffer (pH 8, 50 μL). Phosphate in the PBS buffer was used to replace the target DNA from the surface of the MNP-target DNA conjugates. The supernatant was isolated by magnetic isolation. We took 1 μL of the supernatant to mix with equal volume of MALDI matrix (1 μL) before MALDI MS analysis. The MALDI matrix was prepared by dissolving THAP (30 mg/mL) in acetonitrile/diammoniumhydrogen citrate (0.2 M) (2/1, v/v). The mixture (1 μL) was deposited on a sample target. After solvent evaporation, the sample was ready for MALDI MS analysis. All the DNA mass spectra were obtained at negative ion mode.

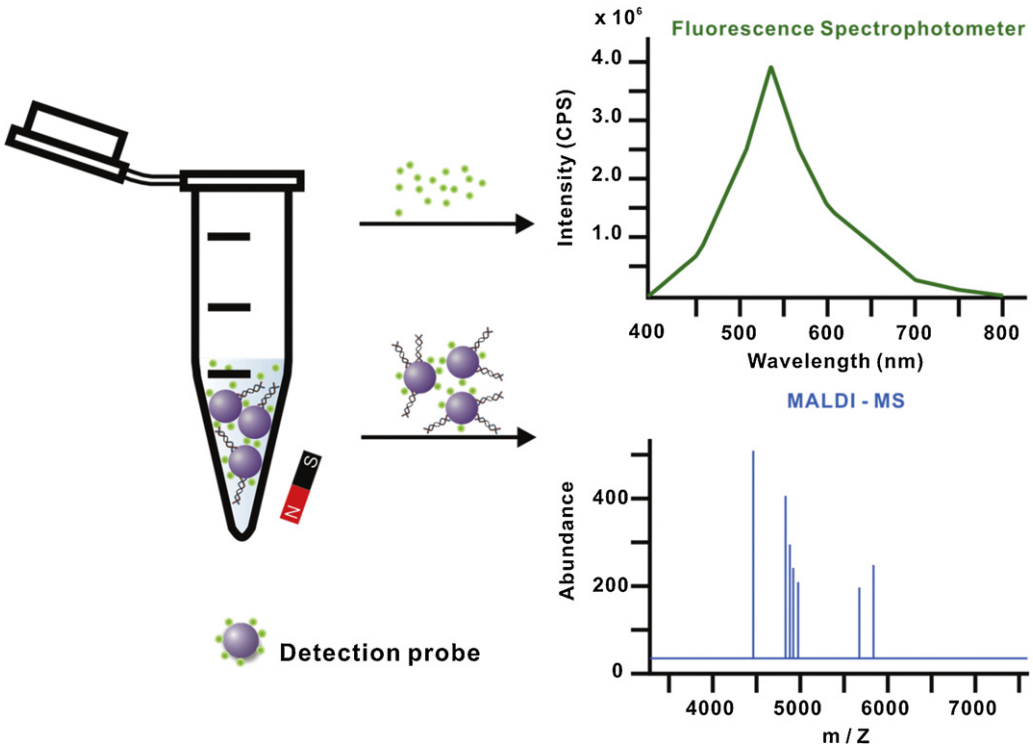
2.6. Instrumentation

Mass spectra were obtained using a Bruker Daltonics Biflex III time-of-flight mass spectrometer (Bremen, Germany) equipped with a 337-nm nitrogen laser. Liner and negative ion mode were selected when analyzing DNA samples. The MS parameters were set as follows: IS1, 19 kV; IS2, 18.18 kV; lens, 9.8 kV; pulsed ion extraction (PIE), medium; laser repetition rate, 10 Hz. TEM images were obtained from a JEOL 2000FX instrument (Tokyo, Japan). EDAX data was obtained from a JEOL JSM-7401F instrument (Tokyo, Japan). Absorption spectra were performed using a Varian Cary 50 absorption spectrofluorophotometer (Melbourne, Australia). Fluorescence spectra were obtained using a Horiba Jobin Yvon FluoroMax-3 spectrophotometer (NJ, USA). The magnetic measurements were obtained from a Quantum Design Magnetic Property Measurement System (MPMS XL-7) (CA, USA).

3. Results and discussion

3.1. Binding capacity of the trapping probes for target DNA

The trapping capacity of the DNA bound iron oxide magnetic nanoprobes with their complementary DNA (i.e., target DNA) mainly depends on the amount of the probe DNA molecules immobilized on the nanoparticles. Thus, we initially estimated the binding amount of the DNA immobilized on the surface of the Fe_3O_4@DNA MNPs, which was estimated at 2.40 ± 0.04 nmol/mg.



Scheme 1. Using RFMP-Fe_3O_4@Al_2O_3 MNPs as the detection probes for target DNA.

When employing the MNPs for interaction with their target DNA, the binding amount of the target DNA on the MNPs were estimated at 2.39 ± 0.15 nmol/mg. This is based on the measurement of $OD_{260\,nm}$ of the target DNA solution before and after binding with the Fe_3O_4@DNA MNPs. Results indicate that the binding affinity of the targeting MNPs to their complementary DNA is >95%. Thus, we then used the as-prepared MNPs as probes for the following experiments.

3.2. Using RFMP-Fe_3O_4@Al_2O_3 MNPs as the detection probes for target DNA

In this study, we tried to demonstrate functionalized magnetic nanoparticles can be used as the trapping and detection probes for DNA followed by quantitative and qualitative analysis by fluorescence spectroscopy and MALDI MS, respectively (Scheme 1). Initially, we demonstrated our RFMP-Fe_3O_4@Al_2O_3 MNPs could be used as the detection probes for quantizing target DNA from samples. Fig. 1a presents the plot of the fluorescence intensity of the supernatant as a function of the concentration of the target DNA in the sample solution when RFMP-Fe_3O_4@Al_2O_3 MNPs are used as detection probes. The results indicate that the fluorescence intensity derived from the RFMP is proportional to the concentration of the target DNA from 0.04 to1000 nM ($Y = 0.0019 \times X + 0.1054$, $R^2 = 0.9896$), indicating the potential of this approach for quantitative analysis of target DNA. As the concentration of the target DNA in the sample reached >1 μM, the plot in Fig. 1a became flat when it approached saturation point. When the DNA concentration was increased to 3 μM, the fluorescence intensity decreased because of the self-absorption effect at this level. Above this concentration,

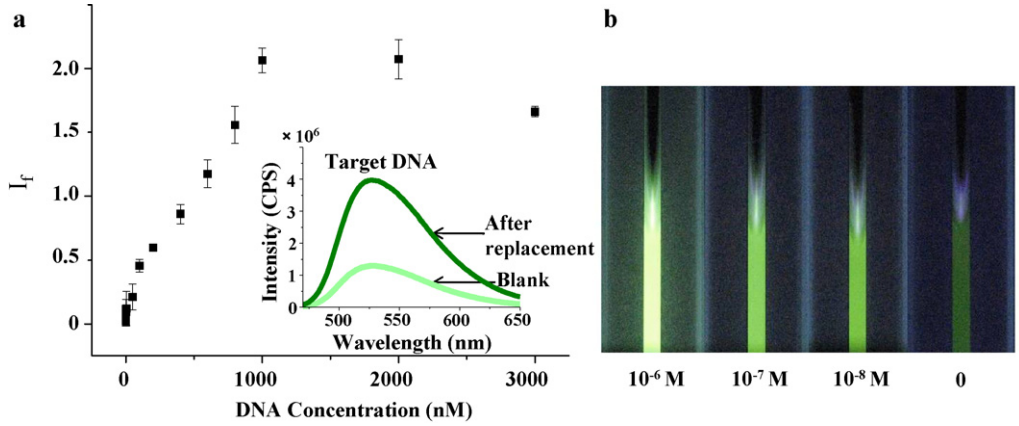


Fig. 1. (a) Plot obtained by plotting the fluorescence intensity (I_f) of the supernatants as a function of the concentration of the target DNA in the sample solution when using the RFMP-Fe_3O_4@Al_2O_3 MNPs as the detection probes. I_f stands for $(I_s - I_0)/I_0$ (I_s: the fluorescence intensity of the supernatant; I_0: the fluorescence of blank background). The inset is the representative fluorescence spectrum of the supernatant obtained after using the detection probes to interact with the sample containing the target DNA (10^{-6} M). (b) Photographs of the supernatant obtained after using the detection probes to interact with the samples containing target DNA at the concentrations of 10^{-6} M, 10^{-7} M, 10^{-8} M, and 0 (from left to right), under illumination of a UV lamp ($\lambda_{max} = 365$ nm). Three duplicates were conducted for each experiment.

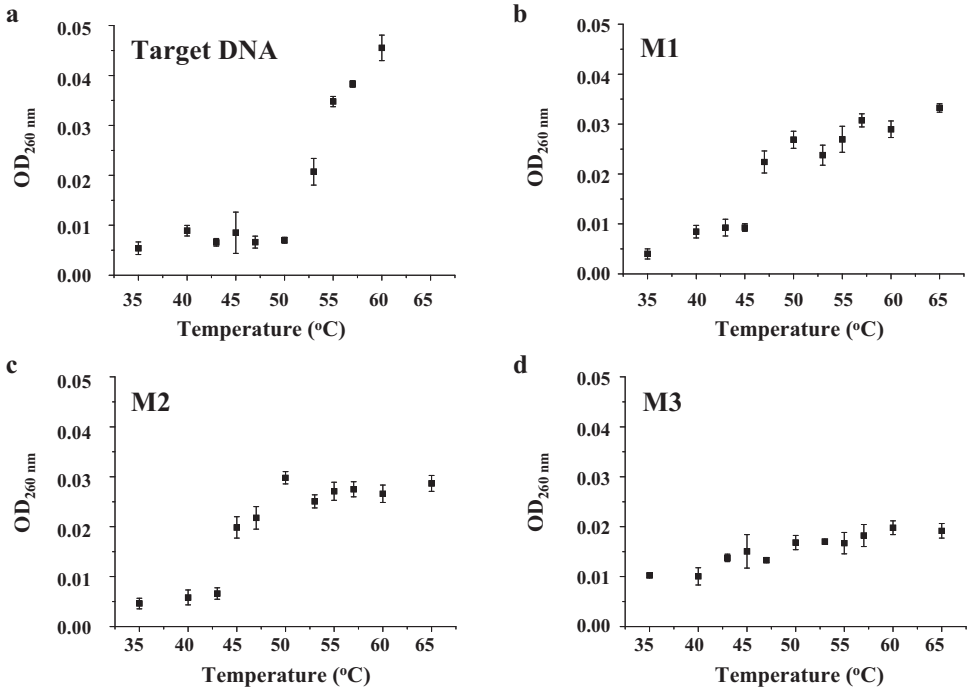


Fig. 2. Examination of the melting temperature for (a) target DNA, (b) M1, (c) M2, and (d) M3 by plotting the intensity of the $OD_{260\,nm}$ representing the amount of the dissociated DNA species from the MNPs in the solution at different temperatures. Three duplicates were conducted for each experiment.

the absorption and fluorescence bands of RFMP overlap noticeable, leading the self-absorption effect apparent. The detection limit was as low as 40 pM (250 μL). The inset in Fig. 1a shows the resulting fluorescent spectrum obtained after the detection probes were combined with the sample containing the target DNA at 1 μM concentration. Fig. 1b presents the photograph of the obtained supernatant after incubating the detection probes with the target DNA under UV lamp illumination (λ_{max} = 365 nm). The tube on the right-hand side is the blank control. Green fluorescence derived from RFMP was observed in the control tube because the RFMP molecules were attached to the MNPs via Al-phosphate chelation. The distribution of RFMP on the MNPs and the solution would achieve equilibrium after incubation for a certain period. Therefore, a portion of RFMP molecules in the solution and visible fluorescence in the control tube were observed. The dissociation constant (k_d) was $\sim 6.82 \times 10^{-7}$ M [27] based on the Langumir adsorption equation. The tubes from the left-hand to the right-hand side correspond to the detected samples containing 10^{-6}, 10^{-7}, 10^{-8}, and 0 M of the target DNA. Apparently, the fluorescence intensity of the sample containing 10^{-8} M differed from that of the blank control; that is, when the concentration of the target DNA was $\geq 10^{-8}$ M, the DNA presence in the sample can be observed by the naked eye.

3.3. Using non-target DNA as the sample

The possibility of using the proposed approach in distinguishing DNA with mismatches from the target DNA was also examined. M1, M2, and M3 with one, two, and three base mismatches, respectively, to the target DNA and the R1, R2, and R3 with random sequences were used as samples (Table 1). Fig. S3a–f shows the representative absorption spectra of the supernatants obtained after using Fe_3O_4@DNA MNPs to probe the target species from the samples (0.25 mL) containing M1, M2, M3, R1, R2, and R3 before and after trapping. The intensity changed remarkably at $OD_{260\,nm}$ before and after probing by Fe_3O_4@DNA MNPs for the samples containing M1, M2, and M3, but changes were hardly observed in the samples containing R1, R2, and R3. The binding amounts of M1, M2, and M3 on the Fe_3O_4@DNA MNPs were estimated at 1.77 ± 0.04, 1.35 ± 0.17, and 1.23 ± 0.08 nmol/mg, respectively. These values were much lower compared with those obtained from interactions with the complementary target DNA. The affinity capacity of the Fe_3O_4@DNA MNPs for the DNA species was closely related to the number of mismatched bases in the sequences. Almost no binding interactions took place between Fe_3O_4@DNA MNPs and R1, R2, and R3 (Fig. S3).

3.4. Examination of melting temperature of DNA samples

The melting temperatures of the complementary target DNA and the mismatched DNA molecules differed. Thus, the melting temperatures of these DNA species were examined using Fe_3O_4@DNA MNPs to trap the DNA samples individually. Next, the

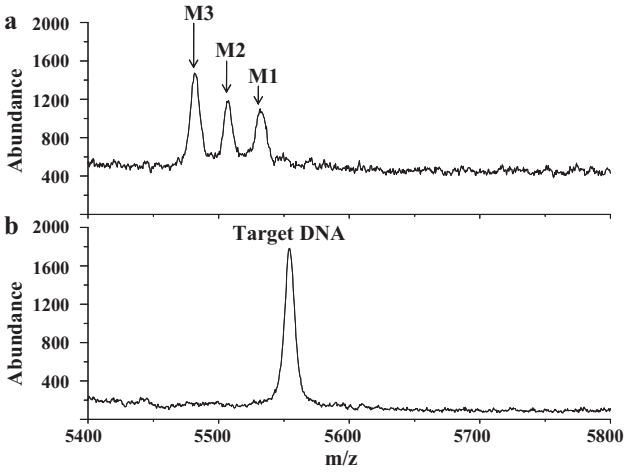


Fig. 3. MALDI mass spectra of the supernatants obtained by elevating the temperature of the MNP-target species conjugate sample to (a) 47 °C followed by elevating to (b) 60 °C. Three duplicates were conducted for each experiment.

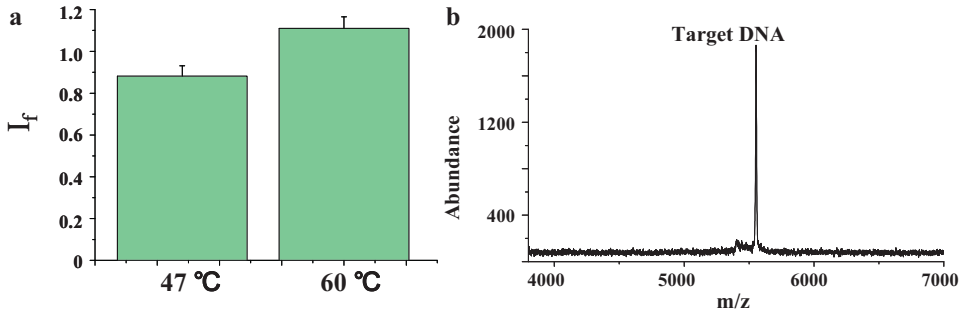


Fig. 4. (a) Bar graph of the fluorescence intensity (I_f) of the supernatants obtained by using the RFMP-Fe_3O_4@Al_2O_3 MNPs as the detection probes to interact with the released DNA from the samples for obtaining Fig. 3a and b. (b) MALDI mass spectrum obtained by releasing the target species from the RFMP-Fe_3O_4@Al_2O_3 MNP-target species conjugates. I_f stands for $(I_s - I_0)/I_0$ (I_s: the fluorescence intensity of the supernatant; I_0: the fluorescence of blank background). Three duplicates were conducted for each experiment.

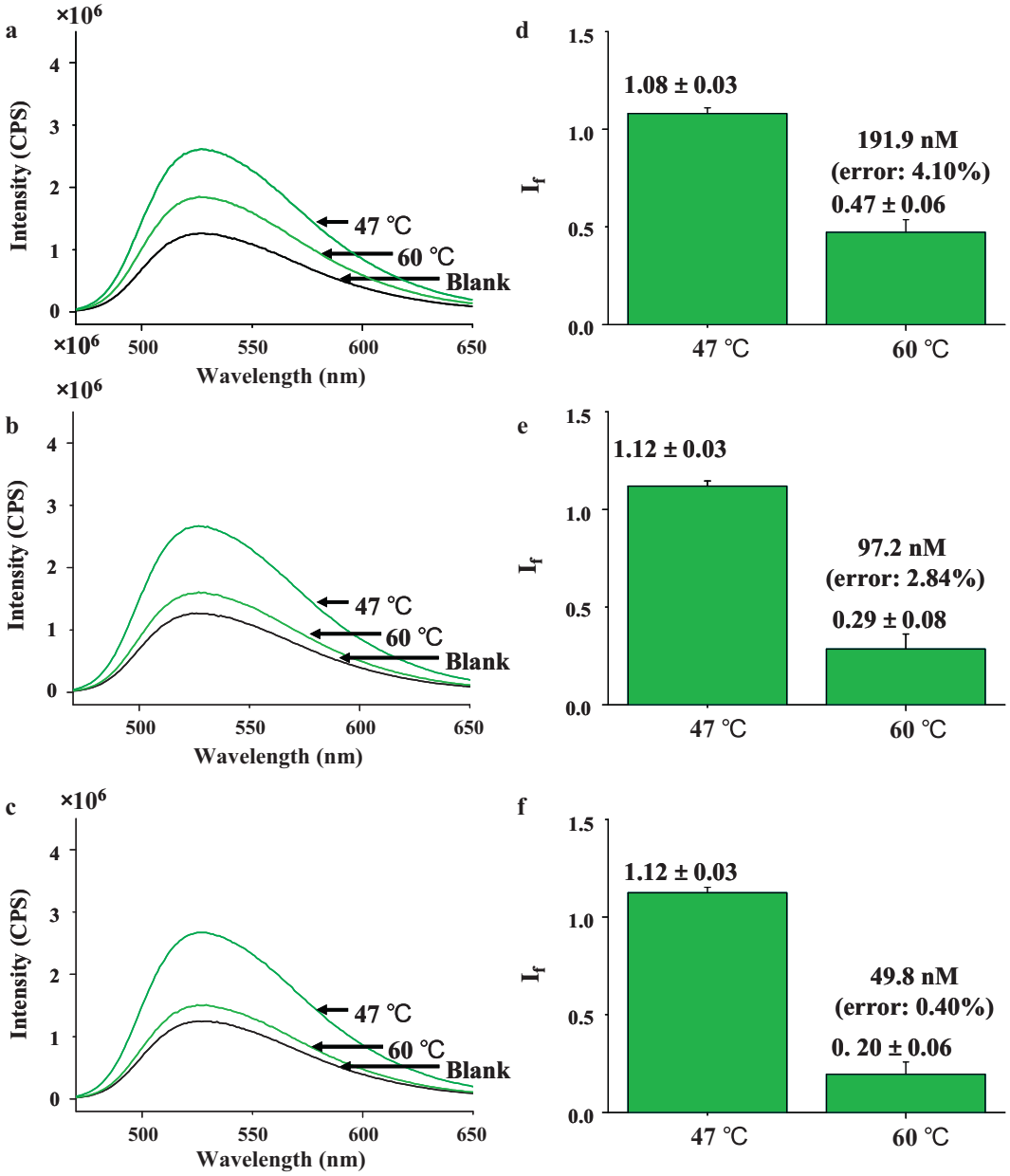


Fig. 5. (a–c) Fluorescence spectra of the supernatants obtained by using the RFMP-Fe_3O_4@Al_2O_3 MNPs as the detection probes to interact with the released DNA from the samples A, B, and C. Panels (d–f) are their corresponding bar graphs. Three duplicates were conducted for each experiment.

trapped DNA species were released from the MNPs by elevating the temperature. Fig. 2 shows the resulting curves obtained by plotting the intensity of the $OD_{260\,nm}$, representing the amount of the DNA species dissociated from the MNPs in the solution at different temperatures. The melting temperature of the target DNA was ~53 °C, while that for M1 and M2 were 46 °C and 44 °C, respectively (Fig. 2b and c). M3 exhibited an even lower melting temperature (~35 °C) because of the three mismatched bases found in its sequence. When the temperature of the solution containing the Fe_3O_4@DNA-target species conjugates was elevated to >46 °C, the conjugates started to release M1, M2, and M3. After discharging these mismatched DNA molecules from the conjugates, only the target DNA molecules remained on the Fe_3O_4@DNA MNPs. The target DNA would then be released from the MNPs by elevating the solution temperature to >53 °C.

3.5. Using MALDI MS to confirm the results

MALDI-MS was used to confirm the released DNA molecules from the MNP-target species conjugates obtained at different temperatures. Fig. 3a presents the MALDI mass spectrum of the supernatant obtained by elevating the temperature of the MNP-target species conjugate sample from the incubated Fe_3O_4@DNA with a sample containing the target DNA, M1, M2, and M3. Then, magnetic isolation was employed at 47 °C. Three ion peaks representing M1, M2, and M3 appear at *m*/*z* 5530, 5505, and 5480, respectively. When temperature was increased to 60 °C, only the peak at *m*/*z* 5555 representing the complementary target DNA is observed in the mass spectrum (Fig. 3b). The results imply that employing the proposed approach in distinguishing single-base mismatched DNA from complementary target DNA might be possible by managing dissociation temperatures.

3.6. Using the RFMP-Fe_3O_4@Al_2O_3 MNPs as the qualitative reporters for DNA

The levels of the released DNA molecules above were further determined by employing RFMP-Fe_3O_4@Al_2O_3 as detection probes. Fig. 4a presents that bar graph of the resultant supernatant when using the RFMP-Fe_3O_4@Al_2O_3 MNPs as detection probes to interact with the released DNAs from the samples for obtaining Fig. 3. The bar on the left-hand side correspond to the supernatant obtained at 47 °C (Fig. 3a), while the band on the right-hand side correspond to the supernatant that was further elevated to 60 °C (Fig. 3b). The fluorescence intensity obtained from the supernatant containing M1, M2, and M3 were lower than that of the supernatant containing the target DNA. The results imply that the binding affinity of the Fe_3O_4@DNA is better for the target DNA that for the DNA species containing mismatched bases, although the original sample contained the same concentrations (500.0 nM). The concentration of the target DNA (528.7 nM) based on the fluorescence result (I_f = 1.11) might have deviated from the true concentration (500.0 nM) by only 5.74%. The DNA species trapped by the RFMP-Fe_3O_4@Al_2O_3 MNPs were confirmed by MALDI-MS. Fig. 4b presents the corresponding MALDI mass spectrum obtained from the supernatant by releasing target DNA using PBS (pH 8) from the isolated RFMP-Fe_3O_4@Al_2O_3-target species conjugates. Only a peak at *m*/*z* 5555 derived from the complementary target DNA was observed in the mass spectrum. The result confirms that the appearance of RFMP in the solution results from the replacement of RFMP on the RFMP-Fe_3O_4@Al_2O_3 MNPs by the target DNA. The result further indicates that the proposed approach can specifically detect target DNA by providing quantitative and qualitative information using the designed MNPs as the affinity and reporter probes.

In addition, we further used the samples containing the same concentration of the DNA M1, M2, and M3 (500.0 nM each) with different concentrations of target DNA (200.0 nM, 100.0 nM, and 50.0 nM, which were named as the samples A, B, and C, respectively), to examine the capability of this approach in quantitative analysis of target DNA in mixtures. Fig. 5 displays the resulting fluorescence spectra and the plots of these samples obtained at different discharging temperatures by employing RFMP-Fe_3O_4@Al_2O_3 as detection probes. The level of the target DNA in the samples A, B, and C was estimated to be 191.9, 97.2, and 49.8 nM, respectively, based on the results in Fig. 5 and the calibration curve provided in Fig. 1. The experimental errors deviated from the true concentrations are less than 5%. The results indicate again that our approach is suitable for estimation of the level of target DNA in complex samples.

4. Conclusions

An approach for the rapid detection of DNA from sample solutions is demonstrated. Microwave heating is employed to assist in the fabrication of detection probes and to accelerate the detection processes for the target DNA. The full period for analysis is only about 1 h. Furthermore, placing multiple samples into the microwave oven during the sensing process is possible. The features suggest the possibility of employing the proposed approach for high throughput analysis. The capability of the Fe_3O_4@DNA trapping probes to concentrate traces of target DNAs from sample solutions is one of the merits of this approach. The detection limit is as low as 40 pM. Additionally, fluorescent dye-labeled nucleotides are not required; only an inexpensive RFMP dye is used in this label-free approach. Furthermore, examining the sensing results through the naked eye is possible when the DNA concentration in the sample is ≥10 nM. As the proposed approach mainly relies on the use of extremely small quantities of functional nanoparticles, sample consumption is therefore possible for subsequent minimization. This approach is potentially suitable for the rapid sensing of target DNAs from sample solutions in terms of qualitative and semi-quantitative high throughput analysis.

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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.talanta.2011.08.061.

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