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ORIGINAL PAPER

A Novel Label-Free microRNA-155 Detection on the Basis of Fluorescent Silver Nanoclusters

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Abstract In this paper, a new approach for microRNA-155 (miRNA-155) detection was described based on the fluorescence quenching of oligonucleotide-templated silver nanoclusters (DNA-AgNCs). The specific DNA scaffold with two different nucleotides fragments were used: one was enriched with a cytosine sequence fragment (C12) that could result in DNA-AgNCs with a high quantum yield via a chemical reduction method, and the other was the probe fragment (5- CUGUUAAUGCUAAUCGUG-3) which could selectively bind to the miRNA-155. Thus, the as-prepared AgNCs could exhibit quenched fluorescence when binding to the target miRNA-155. The fluorescence ratio of the DNA-AgNCs was quenched in a linearly proportional manner to the concentration of the target in the range of 0.2 nM to 30 nM with a detection limit of 0.1 nM.

Keywords MicroRNA-155 · Silver nanoclusters · Fluorescence · Quenching

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Introduction

MicroRNAs (miRNAs) are the important small RNA which regulates diverse gene expression at the DNA post-transcriptional level. MiRNAs are considered as important biomarkers since abnormal expression of specific miRNAs is associated with many diseases such as cancer and diabetes. Therefore, it is important to develop biosensors to quantitatively detect miRNA expression levels [1].

MiRNA-155 is one of the most important miRNAs processed from an exon of a non-coding RNA transcribed from the *B-cell Integration Cluster* (BIC) located on chromosome 21 [2]. miRNA-155 is overexpressed or mutated in various malignant tumor cells, such as hepatocellular carcinoma (HCC) [3, 4], non-small cell lung cancer (NSCLC) [5], breast cancer [6], colon cancer [7], chronic lymphocytic leukemia (CLL) [8], etc. Also, it has been demonstrated that miRNA-155 plays a key role in the mammalian immune system [9]. Due to its important roles, detection of miRNA-155 as a biomarker in certain types of cancer could be reasonable and effective. Attempts for developing a rapid, accurate, sensitive and quantitative method for miRNA-155 detection would be imperative and meaningful work.

Researchers have focused their attention on the development of methodologies for detecting miRNAs. All of those methods, however, have embraced several remaining challenges. Microarray hybridization-based methods are high throughput methods but with relatively low sensitivities and time-consuming [10]. Although both Northern [11] and qRT-PCR-based methods [12] show highly sensitive, but they are also time-consuming which is somewhat inappropriate to integrate for point-of-care detection platforms. Moreover, these traditional methods for miRNA detection usually employ labels including fluorescent dyes [10], radioactive probes [11], or other probes [12], [13]. Those labels impose limitations on

reliability in that fluorescent dyes emit light only for a short time due to photobleaching and radioactive probes suffer from difficulties in handling and disposal [14]. To address these limitations, researchers have recently focused on emerging nanotechnology based methods as alternatives to the traditional methods.

Very recently, there has been an explosion of interest in fluorescent silver nanoclusters (AgNCs) synthesis and their application in the area of bioassays [15]. AgNCs with a few atoms, exhibiting size-dependent fluorescence emission, have been developed as a new class of fluorophores. The synthesis of fluorescent AgNCs using DNA as scaffolds in aqueous solution has attracted extensive attention [16]. The DNA scaffolded AgNCs (DNA-AgNCs) exhibit outstanding spectral and photophysical properties, and the photoluminescence (PL) emission band can be fine-tuned just by changing the sequence of DNA [17]. They display excellent photostability, subnanometer size, nontoxicity, biocompatibility, and thus well-suited as a fluorescent probe for biochemical applications. Recent years have witnessed the rapid development of AgNC-based fluorescent probe design and its successful applications in detecting various targets, such as ssDNA, miRNA, and metal ion and for cellular labeling or imaging [18, 19].

Herein, we used oligonucleotides comprised of two functional regions: a specific DNA sequence [20, 21] for recognition of the target miRNA and a scaffold (C_{12}) for the preparation of DNA-AgNCs (Scheme 1) for the reason that DNA sequences rich in cytosine have been proven to work in the synthesis of fluorescent AgNCs through anchoring of the silver atoms . Furthermore, we reported an AgNCs-based fluorescent probe which could be used for the label-free and selective detection of the mirRNA 155 gene sequence based on the fluorescence quenching of DNA-AgNCs resulting from the specific hybridization of a complementary pair to form a double stranded DNA with minimal background and relatively high signal-to-background ratio.

Materials and Methods

Reagents and Materials

Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Merck and all other commercially available substances were purchased from Aldrich, Merck and Acros and used without further purification. The following oligonucleotides were synthesized by Shanghai Generay Biotech Co (Shanghai, China):

Probe: 5-CACGATTAGCATTAACAGCCCCCCCCCCCC-3
Target: 5- CUGUUAAUGCUAAUCGUG-3

Two-base mismatched target: 5- CUGUUAAAGCUAAUGGUG-3
Non complementary target: 5- CACGAUUAGCAUUAACAG-3

All other reagents were of analytical reagent grade and ultra-pure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout the reactions.

Apparatus

All fluorescence measurements were carried out on a Perkin-Elmer LS50 luminescence spectrometer with a xenon lamp as source of excitation while the spectral bandwidths of monochromators for excitation and emission were 10 nm. The sizes of DNA–AgNCs were measured by Transmission electron microscope (TEM) (Zeiss, EM10C, 80 KV, Germany). All of experiments were carried out at room temperature.

The Synthesis of DNA-AgNCs

DNA-AgNCs were synthesized according to a previous literature report with minor modification [20, 22]. Briefly, $AgNO_3$ solution (88.8 mM) was added to DNA solution (14.8 mM) prepared in 20 mM phosphate buffer (pH 7.0) by vigorous shaking for 3 min. After incubation for 30 min at room temperature, this mixture was reduced by adding freshly prepared $NaBH_4$ (88.8 mM) with vigorous shaking. The molar ratio of Ag-DNA-$NaBH_4$ in the solution was 6: 1: 6. The reaction mixture was kept in the dark at room temperature for 12 h before use. The AgNCs exhibited a fluorescence emission peak at 625 nm when excited at 550 nm.

Hybridization and Fluorescence Detection

In order to determine the hybridization effects of miRNA-155 on fluorescence intensities, different concentration of target miRNA added to DNA-AgNCs reaction. The hybridization was carried out by incubation for 1 h while gently stirred at 37 C°. All fluorescence experiments were carried out at room temperature.

Results and Discussion

The Characterization of Fluorescent Silver Nanoclusters

The DNA-AgNCs were prepared initially by sequestering AgNO3 with the DNA and then reducing Ag^+ to Ag^0 clusters with $NaBH_4$ [20, 21]. The high affinity of Ag^+ for Cytosine bases has enabled the production of DNA-AgNCs which exhibit excellent photophysical properties. The optical properties of the DNA-AgNCs are largely dependent on the length

and sequence of the DNA [20, 22]. To confirm the formation of AgNCs, the fluorescence emission was investigated. As shown in Fig. 1 A, upon excitation at 550 nm, DNA-AgNCs showed an emission band centered at 625 nm. The size of the AgNCs was charachterized by transmission electron microscopy (TEM) and as it is shown in Fig. 1 B, the AgNCs were well-dispersed with the average diameter of 2.0 nm.

We also investigated the time effect of synthesis of DNA-AgNCs on the fluorescence change of the DNA-AgNCs (Fig. 2). As illustrated in Fig. 2, with increasing time, the highest fluorescence intensity was obtained at 210 min. So, this time was chosen in the preparation of DNA- Ag NCs.

Also, we have investigated effects of excitation wavelength on the intensity of the DNA- Ag NCs. Figure 3 shows the effects of different excitation wavelength on the intensity of the nanoclusters. As it is shown, excitation at 550, 565, 570, 575 and 580 nm results in emission bands with maxima at 625 nm. It is notify, these bands with different excitation have a common emission wavelength, which suggests that they arise from a common electronic state of one species [21].

Under continuous illumination at 580 nm for 30 min, the fluorescence intensity of DNA-AgNCs was only slightly changed as is shown Fig. S1. The results displayed good light stability of the DNA-AgNCs.

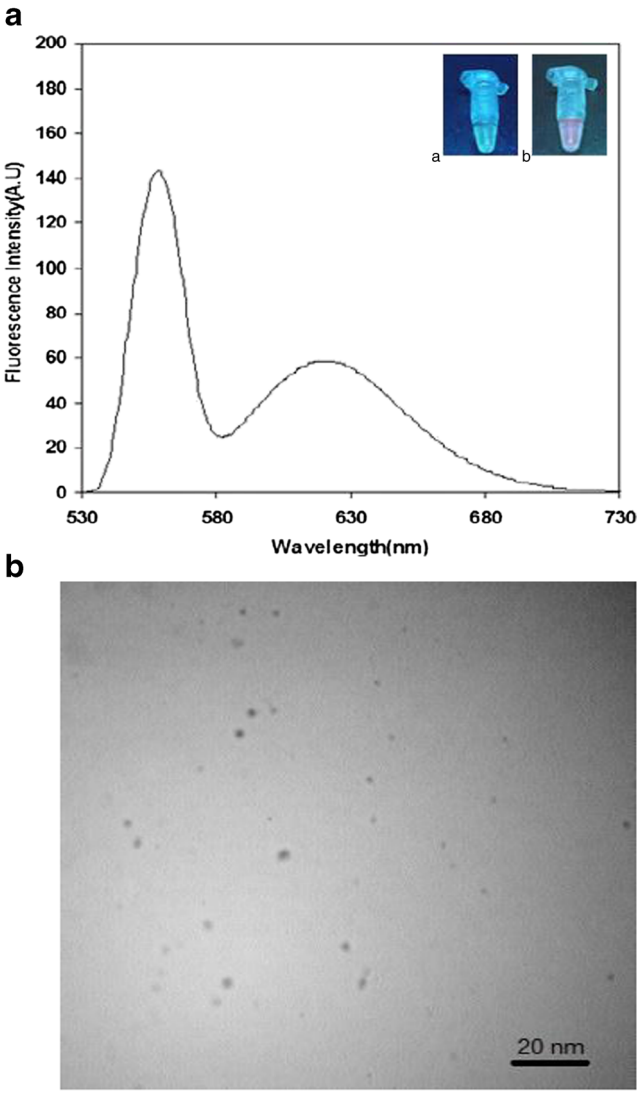

Fig. 1 **A** The fluorescence intensity of the synthesized DNA-AgNCs, Color change under UV radiation in initial time synthesis (*a*) and after 210 min (*b*) **B** TEM image of DNA-AgNCs

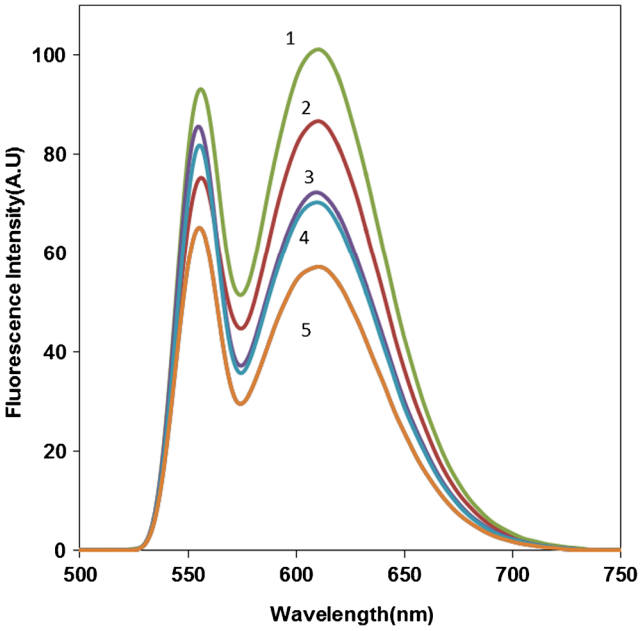

Fig. 2 The fluorescence intensity as a function of time of the synthesized DNA-AgNCs **1** 210 min, **2** 150 min, **3** 80 min, **4** 290 min, **5** 320 min

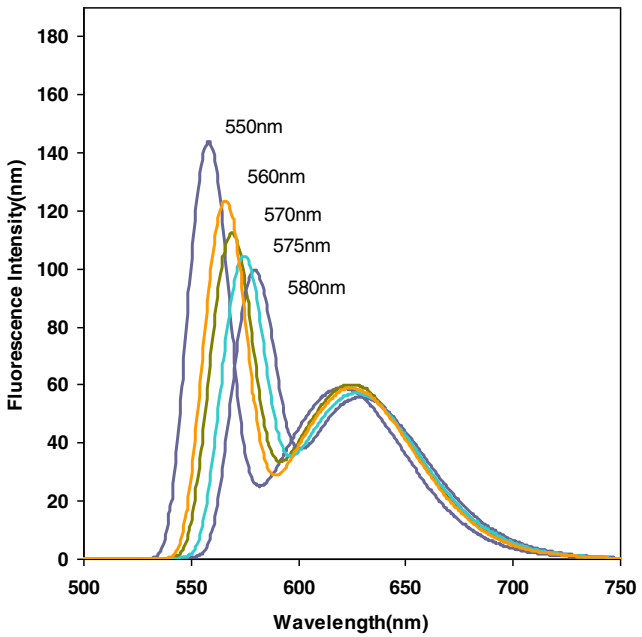

Fig. 3 Fluorescence emission spectra as a function of the excitation wavelengths, which were changed by 20 nm

The influence of solution pH on the fluorescence intensity of DNA-AgNCs is investigated in pH of range 5.0–10.0. As is shown in Fig. S2, maximum fluorescence occurs at pH 7. Therefore, in subsequent experiments, a solution of pH 7.0 adjusted by a buffer solution, was used further studied.

Performance of the Biosensor Detecting for miRNA-155

The performance of the present biosensor was evaluated by detecting miRNA-155 through the hybridization method under the optimal conditions. As shown in Fig. 4, the hybridization with increasing amounts of target DNA can specifically decrease the fluorescence intensity of the DNA-AgNCs. The decrease fluorescence intensities are linearly proportional to the concentrations of target DNA, which can be best described by a Stern-Volmer equation:

$$I_0/I = 1 + K_{Sv}[Q]$$

Where I_0 and I are the fluorescence intensity of DNA-AgNCs in the absence and presence of miRNA-155 respectively, [Q] is the miRNA-155 concentration, and K_{SV} is the Stern-Volmer plot, K_{SV} is calculated to be 5.0×10^4 M^{-1}. The calibration plot of I_0/I versus concentration of miRNA-155 was linear in the range from 0.2 nM to 30 nM (See the inset in Fig. 4). The regression equation was $I_0/I=1.068+ 5.0\times10^7$ C and this equation had a correlation coefficient of 0.999, which indicated that there was a good linear relationship between I_0/I and

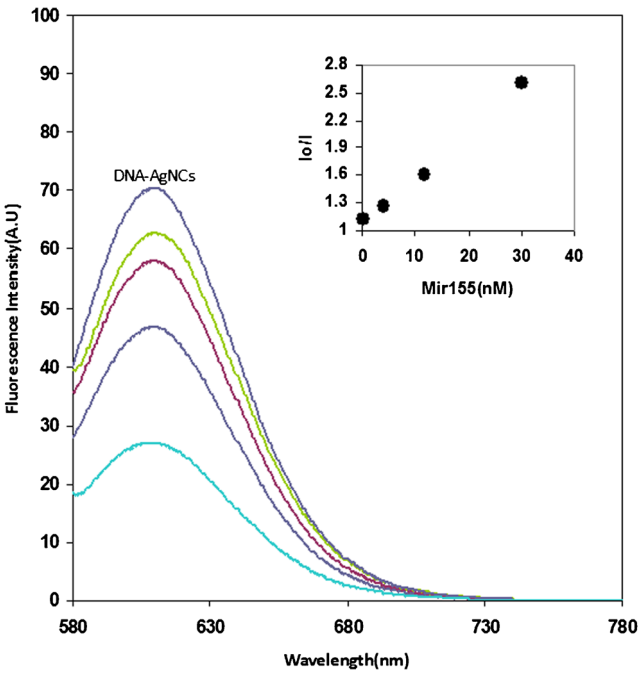

Fig. 4 Fluorescence intensity of DNA-AgNCs in 20 mM phosphate buffer (pH=7) with increasing concentration of miRNA-155. (inset) Stern-Volmer curve in the

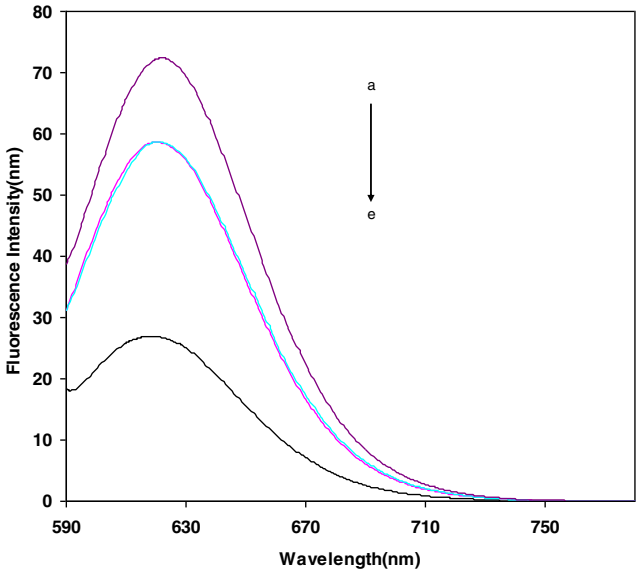

Fig. 5 Selectivity of the method for miRNA-155 in phosphate buffer (pH 7.0): **a** control; **b** Non-complementary; **c** TWo- base mismatched sequence; **d** Complete complementary sequence

miRNA-155 concentration. The detection limit (S/N=3) for miRNA-155 determined at 0.1 nM.

Selectivity

We further investigated the selectivity of the sensing platform described here by examining the fluorescence responses of the probe DNA toward perfectly matched target DNA, two-base mismatched target DNA and non- complementary target DNA at the same concentration of 20 nM. As the results are shown in Fig. 5, the fluorescence intensity of the DNA-AgNCs toward the perfectly matched target DNA was much stronger than that toward the mismatched DNA. All of these results indicated the high selectivity for DNA detection in this system. Therefore, this method with its simple, sensitive and quantitative capacity for miRNA-155 sequence detection could be of great potential for miRNA-155 diagnosis in clinical applications.

Conclusions

In conclusion, we have successfully constructed a novel and efficient method for the label-free and fluorescent detection of the miRNA-155 sequence based on quenching of DNA-AgNCs intensity. Our strategy relies on the notable fluorescence quenching of DNA-AgNCs resulting from the specific binding of DNA-AgNCs with target miRNA-155. Under the optimal conditions, the decrease fluorescence intensities were linearly proportional to the concentrations of target DNA from 0.2 nM to 30 nM. The proposed method was not only suitable

for determining miRNA-155 as shown above but also other interesting tumor markers.

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