Ratiometric Fluorescence Detection of DNA Based on the Inner Filter Effect of Ru(bpy)$_2$(dppx)$_2^{2+}$ toward Silicon Nanodots

Yanan Zhang,* Dajun Hou, Bingshan Zhao, Chunyin Li, Xiaoyan Wang, Lanying Xu, and Tao Long

ABSTRACT: A ratiometric DNA sensor was developed based on fluorescent silicon nanodots (SiNDs) and Ru(bpy)$_2$(dppx)$_2^{2+}$. The absorption spectrum of Ru(bpy)$_2$(dppx)$_2^{2+}$ has significant overlap with both the excitation and emission spectra of SiNDs. Therefore, fluorescence quenching of Ru(bpy)$_2$(dppx)$_2^{2+}$ toward SiNDs can occur on account of the strong inner filter effect. The effect of quenching is not influenced by the specific binding between Ru(bpy)$_2$(dppx)$_2^{2+}$ and DNA. Fluorescence turn-on detection of DNA can be performed employing Ru(bpy)$_2$(dppx)$_2^{2+}$ and SiNDs as the response and reference signals, respectively. Using SiND−Ru(bpy)$_2$(dppx)$_2^{2+}$, a convenient, sensitive, rapid, and precise method could be developed for DNA detection. In aqueous solutions, the $I_{448}/I_{648}$ fluorescence intensity ratio of SiND−Ru(bpy)$_2$(dppx)$_2^{2+}$ increases linearly in the DNA concentration range of 20−1500 nM. The limit of detection and precision of the method is 4.3 nM and 3.5% (50 nM, $n = 13$), respectively. The ratiometric sensor was tested for visual detection of trace DNA. Moreover, this method was found suitable for the ratiometric detection of DNA in a simulated sample and a human serum sample, and the recoveries were in the range of 98−119%.

1. INTRODUCTION

Deoxyribonucleic acid (DNA) is one of the most basic substances in life, which plays an extremely important role in genetic information storage, protein synthesis, and other life activities. DNA abnormality is a significant contributor to many hereditary diseases, including cancer. 1,2 In addition, some highly infectious viruses such as human immunodeficiency virus 1 have caused deadly diseases in humans. 3 Hence, the development of new methods for quantitative detection of disease-associated DNA sequences is of great significance for clinical diagnosis, pathogenesis research, and gene therapy. 4

To date, the methods for DNA determination include colorimetric, 5 surface-enhanced Raman scattering, 6 and fluorescence methods. 7 Among them, ratiometric fluorescence techniques have garnered increasing attention because the feature of self-calibration can improve accuracy. 8 With the advance of nanotechnology, new nanomaterials (e.g., silver nanoclusters, II−VI quantum dots, and carbon dots) have been applied in the construction of ratiometric sensors. 9−12 Indeed, the development of a simple, sensitive, rapid, and precise strategy for DNA detection is a research focus.

Fluorescent silicon nanodots (SiNDs) have been used in a variety of applications, including bioanalysis, 13 fluorescence imaging, 14 enantiomer recognition, 15 and anticounterfeiting, 16 which is attributable to advantages such as green preparation, outstanding optical properties, and good biocompatibility. 17−19 The fluorescence of SiNDs synthesized using different methods can be selectively quenched by certain ions or fluorescent dyes, and the turn-off/on strategies have been applied for the determination of target analytes (e.g., Cr$^{6+}$, Hg$^{2+}$, and NO$_2^-$). 20−23 Yet, there are only a few reports on the deployment of the quenching effect on SiND fluorescence for analyte detection.

As a molecular “light switch” complex for selective recognition of double-stranded DNA, Ru(bpy)$_2$(dppx)$_2^{2+}$ by itself has no fluorescence in aqueous solution but emits red fluorescence in the presence of DNA, 24 hence possessing the merit of low background for DNA detection. The fluorescence of Ru(bpy)$_2$(dppx)$_2^{2+}$ can be thoroughly quenched by water using a proton transfer mechanism. 25 DNA can protect Ru(bpy)$_2$(dppx)$_2^{2+}$ from water due to the intercalation binding mode. 24 The double helix of DNA can be intercalated by the dppx ligand of the Ru complex. 24,26 Such specific binding with a high combination constant ($K_b = 1.5 \times 10^4$ M$^{-1}$) is increasingly exploited in the development of turn-on sensors. 27−29 Herein, Ru(bpy)$_2$(dppx)$_2^{2+}$ is utilized for the...
reason that it can efficiently quench the blue fluorescence of SiNDs as a result of the inner filter effect (IFE). Using (3-aminopropyl)trimethoxysilane and trisodium citrate, blue-emitting SiNDs (spherical, about 2.5 nm, TEM characterization) can be easily prepared in a large scale (14 g) via a facile one-step hydrothermal method. By simply mixing desired amounts of SiNDs and Ru(bpy)$_2$(dppx)$_2$+, a ratiometric sensor can be fabricated for DNA detection. In the presence of DNA, Ru(bpy)$_2$(dppx)$_2$ shows red fluorescence and the blue fluorescence of the quenched SiNDs remains unchanged (Scheme 1). For the first time, a SiND−Ru(bpy)$_2$(dppx)$_2$+ sensor was prepared and utilized for the visual detection of DNA. Furthermore, a quantitative analysis of analytes in a simulated sample and a human serum sample was performed to demonstrate the applicability of this method.

2. RESULTS AND DISCUSSION

2.1. Feasibility for Ratiometric Detection of DNA. UV−vis spectroscopy was applied to characterize SiNDs and Ru(bpy)$_2$(dppx)$_2$+. As shown in Figure 1A, the excitation and emission spectra of SiNDs have significant overlap with the absorption spectrum of Ru(bpy)$_2$(dppx)$_2$+. Thus, the fluorescence of SiNDs can be efficiently quenched by Ru(bpy)$_2$(dppx)$_2$ due to the strong IFE. Accordingly, the fluorescence spectra of SiND−Ru(bpy)$_2$(dppx)$_2$ can be recorded by single-wavelength excitation. The quenching effect of Ru(bpy)$_2$(dppx)$_2$ toward SiNDs was investigated. It was revealed that the quenching efficiency reaches 97% at a relatively low concentration of the Ru complex (Figure 1B). To facilitate visual detection of DNA, 20 μM Ru(bpy)$_2$(dppx)$_2$ was used to quench SiNDs in the subsequent experiments.

In the presence of DNA of various concentrations, the fluorescence of Ru(bpy)$_2$(dppx)$_2$ at 601 nm increases with the increasing DNA concentration and the fluorescence of SiNDs at 448 nm remains unchanged (Figure 2). It is hence demonstrated that the specific binding of DNA with Ru(bpy)$_2$(dppx)$_2$ has no influence on the IFE of Ru(bpy)$_2$(dppx)$_2$ toward SiNDs. Based on the result, it is envisaged that the SiNDs can act as a fluorescence reference in a ratiometric sensor for DNA detection.

2.2. Optimization of Experimental Conditions. To achieve an optimal analytical performance for DNA detection, several experimental parameters that affect the signal ratio of SiND−Ru(bpy)$_2$(dppx)$_2$ were investigated, including SiND concentration, pH, as well as the salt concentration of the PBS buffer solution.

The relation between SiND concentration and fluorescence intensity was investigated. As shown in Figure S1, there is a good linear relationship between the two in the range of 40−400 μg/mL. The result demonstrates that the self-fluorescence quenching of SiNDs does not occur at a SiND concentration of 400 μg/mL. It was reported that the fluorescence intensity of the amino-terminated (FTIR characterization) SiNDs was influenced by

Figure 1. (A) UV−vis spectrum of Ru(bpy)$_2$(dppx)$_2$ (black line), fluorescence excitation spectrum (red line), and emission spectrum (blue line) of SiNDs. (B) Fluorescence spectra of SiNDs (400 μg/mL) upon increasing concentration of Ru(bpy)$_2$(dppx)$_2$: 0, 7.5, 15, 20, 37.5, 36.2, 75, 93.7, and 112.5 μM (from top to bottom). The inset shows the fluorescence intensity of SiNDs at 448 nm versus the concentration of Ru(bpy)$_2$(dppx)$_2$. Concentration of SiNDs: 400 μg/mL.

Figure 2. Fluorescence spectra of (a) SiNDs, (b) SiNDs + Ru(bpy)$_2$(dppx)$_2$, (c) SiNDs + Ru(bpy)$_2$(dppx)$_2$ + 65 nM DNA, (d) SiNDs + Ru(bpy)$_2$(dppx)$_2$ + 500 nM DNA, and (e) SiNDs + Ru(bpy)$_2$(dppx)$_2$ + 1000 nM DNA in 50 mM PBS buffer (pH 7.4, 100 mM NaCl).
the change in pH values. Therefore, we investigated the effect of pH on the quenching efficiency. As shown in Figure S2A, the fluorescence intensity of SiNDs gradually increases with increasing pH from 5.4 to 8.0. After combining with Ru(bpy)$_2$(dppx)$_2^+$, their fluorescence intensity do not change significantly with the increase of pH, which is probably due to the H-bond formation between the dppx ligand and amino groups. However, the quenching efficiencies of Ru(bpy)$_2$(dppx)$_2^+$ toward SiNDs are all around 40% in the studied pH range (Figure S2B). Considering the bioapplication potential of SiND–Ru(bpy)$_2$(dppx)$_2^+$, further experiments were performed in a pH 7.4 PBS buffer solution.

The concentration of NaCl in solution is a key factor for DNA stabilization. Thus, the effect of NaCl concentration on DNA detection was explored. As shown in Figure 3A, in the presence of 1 μM DNA, the fluorescence of Ru(bpy)$_2$(dppx)$_2^+$ decreases with the increasing concentration of NaCl in a pH 7.4 PBS buffer solution. The main reason is probably that the interaction between the dppx ligand and amino groups of SiNDs increases with the increasing ionic strength. The signal ratio of SiND–Ru(bpy)$_2$(dppx)$_2^+$ remains constant with the change in NaCl concentrations from 80 to 180 mM (Figure 3B). For the system, it is apparent that a high concentration of NaCl in the solution probably does not facilitate the intercalation between DNA and Ru(bpy)$_2$(dppx)$_2^+$. Consequently, we adopted a PBS buffer solution containing 100 mM NaCl in the following experiments.

2.3. Interference of Coexisting Substances. Under the optimized conditions, the selectivity and anti-interference ability of SiND–Ru(bpy)$_2$(dppx)$_2^+$ for the detection of DNA (1 μM) were assessed using substances such as 4 μM Fe$^{3+}$, 20 μM of monovalent or divalent ions, amino acids, Na$_2$EDTA, glucose, adenosine triphosphate (ATP), Al$^{3+}$, and 10 μM BSA that are possibly present in real conditions (Figure 4). It was found that only DNA can cause Ru(bpy)$_2$(dppx)$_2^+$ fluorescence and the coexistence of the other substances has a negligible effect on the signal ratio. The good selectivity and anti-interference ability of the SiND–Ru(bpy)$_2$(dppx)$_2^+$ sensor indicates its application potential.

2.4. Visual Detection of DNA. The SiND–Ru(bpy)$_2$(dppx)$_2^+$ sensor was explored for the visual detection of DNA in the pH 7.4 PBS buffer solution. DNA samples of various concentrations (0–750 nM) were separately added into the SiND–Ru(bpy)$_2$(dppx)$_2^+$ and Ru(bpy)$_2$(dppx)$_2^+$ solutions. In comparison to the case of Ru(bpy)$_2$(dppx)$_2^+$ alone (Figure 5A), a series of colors (from blue to red) can be observed for SiND–Ru(bpy)$_2$(dppx)$_2^+$ with the increasing DNA concentration under Xe light irradiation, and 20 nM DNA induced an obvious color change in comparison to the blank (Figure 5B). The results reveal that the ratiometric fluorescence method and 50 mM PBS buffer (pH 7.4). Concentration of SiNDs and Ru(bpy)$_2$(dppx)$_2^+$: 400 μg/mL and 20 μM, respectively.

**Figure 3.** (A) Fluorescence spectra of SiND–Ru(bpy)$_2$(dppx)$_2^+$ for analyte detection upon increasing concentrations of NaCl: 80, 100, 180, 280, and 380 mM (from top to bottom) in 50 mM PBS buffer (pH 7.4). (B) Fluorescence intensity ratios ($I_{flu}$/$I_{base}$) of SiND–Ru(bpy)$_2$(dppx)$_2^+$ for analyte detection with various concentrations of NaCl (80–380 mM) in 50 mM PBS buffer (pH 7.4). Concentration of SiNDs and Ru(bpy)$_2$(dppx)$_2^+$: 400 μg/mL and 20 μM, respectively.

**Figure 4.** (A) Selectivity ((1) DNA, (2) Fe$^{3+}$, (3) K$^+$, (4) Mg$^{2+}$, (5) Ca$^{2+}$, (6) Zn$^{2+}$, (7) Cu$^{2+}$, (8) Ni$^{2+}$, (9) Co$^{2+}$, (10) histidine, (11) cysteine, (12) glycine, (13) Na$_2$EDTA, (14) glucose, (15) ATP, (16) BSA, and (17) Al$^{3+}$) and (B) anti-interference ((1) DNA, (2) DNA + Fe$^{3+}$, (3) DNA + K$^+$, (4) DNA + Mg$^{2+}$, (5) DNA + Ca$^{2+}$, (6) DNA + Zn$^{2+}$, (7) DNA + Cu$^{2+}$, (8) DNA + Ni$^{2+}$, (9) DNA + Co$^{2+}$, (10) DNA + histidine, (11) DNA + cysteine, (12) DNA + glycine, (13) DNA + Na$_2$EDTA, (14) DNA + glucose, (15) DNA + ATP, (16) DNA + BSA, and (17) DNA + Al$^{3+}$) of SiND–Ru(bpy)$_2$(dppx)$_2^+$ in the presence of different substances in 50 mM PBS buffer (pH 7.4, 100 mM NaCl). Concentration of SiNDs and Ru(bpy)$_2$(dppx)$_2^+$: 400 μg/mL and 20 μM, respectively.

**Figure 5.** Photos of (A) Ru(bpy)$_2$(dppx)$_2^+$ and (B) SiND–Ru(bpy)$_2$(dppx)$_2^+$ in the presence of various concentrations of DNA (from left to right: 0–750 nM) in the buffer solution under Xe light irradiation. Concentration of SiNDs and Ru(bpy)$_2$(dppx)$_2^+$: 400 μg/mL and 20 μM, respectively.
sensor can be well applied for the visual detection of DNA in nanomolar concentrations.

The SiND–Ru(bpy)_2(dppx)_2^2+ sensor was further tested for the visual detection of DNA in a human serum sample. In the presence of an analyte, the fluorescence of SiND–Ru(bpy)_2(dppx)_2^2+ is susceptible to the serum matrix (Figure S3A). The I_{601}/I_{448} fluorescence intensity ratio of SiND–Ru(bpy)_2(dppx)_2^2+ in a 0.5–1% human serum sample is almost the same as that in the buffer solution (Figure S3B). The visual detection of DNA in a sample of 1% human serum was therefore performed with the sensor subsequently. As shown in Figure S4, the sensor can be used for the visual detection of DNA at the nanomolar level in the sample of 1% human serum.

2.5. Analytical Performance. The analytical performance of SiND–Ru(bpy)_2(dppx)_2^2+ for DNA detection was evaluated under the optimum conditions. As shown in Figure 6, a good linear relationship between the signal ratio and the DNA concentration can be achieved in the range of 20–1500 nM. The limit of detection (LOD) for DNA is 4.3 nM (S/N = 3, n = 11), and the relative standard deviation of the method is 3.5% (C = 50 nM, n = 13). A comparison of this method with other nanomaterial-related fluorescence methods for DNA detection is shown in Table S1. It can be seen that the linear range and LOD of this method are comparable to those of some reported methods.

2.6. Sample Analysis. To evaluate the applicability of SiND–Ru(bpy)_2(dppx)_2^2+, we applied it for the ratiometric detection of DNA in a simulated sample and a sample of 1% human serum. The results are compiled in Table 1, and the recoveries of target DNA in the samples are in the range of 98–119%. Based on these results, it is reasonable to infer that SiND–Ru(bpy)_2(dppx)_2^2+ has applicability for DNA detection even in a matrix that is relatively complicated.

Table 1. Analytical Results of DNA in a Simulated Sample and Human Serum

| samples               | added (nM) | found (nM) | recovery (%) |
|-----------------------|------------|------------|--------------|
| simulated sample      | 50         | 59 ± 10.5  | 118          |
|                       | 500        | 490 ± 1.4  | 98           |
|                       | 1000       | 1191 ± 43.7| 119          |
| 1% human serum        | 50         | 52 ± 6.8   | 104          |
|                       | 500        | 544 ± 32.7 | 109          |
|                       | 1000       | 984 ± 73.3 | 98           |

3. CONCLUSIONS

In this work, a ratiometric sensor was developed for fluorescence turn-on detection of DNA by simply mixing SiNDs and Ru(bpy)_2(dppx)_2^2+. The IFE of Ru(bpy)_2(dppx)_2^2+ toward SiNDs is not influenced by its intercalation into DNA. In the presence of DNA, dual-emission spectra of SiND–Ru(bpy)_2(dppx)_2^2+ can be conveniently recorded by single-wavelength excitation that is attributable to IFE. The SiND–Ru(bpy)_2(dppx)_2^2+ sensor exhibits high sensitivity and selectivity as well as good anti-interference ability. The visual detection of DNA at the nanomolar level has been realized using the sensor. Moreover, the ratiometric sensor has been applied for the detection of DNA in a simulated sample as well as in a sample of human serum.

4. EXPERIMENTAL SECTION

4.1. Materials and Reagents. (3-Aminopropyl)-trimethoxysilane (97%), ATP, FeCl₃·6H₂O, Na₂EDTA·2H₂O, histidine, cysteine, and glycine were purchased from Aladdin (China). Trisodium citrate (99%) was purchased from Sigma-Aldrich (USA). NaCl, NaH₂PO₄·H₂O, Na₂HPO₄ and other analytical grade reagents were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). HIV double-stranded DNA (5′-CGAGTTAAGAAAAGAAAAAGATGGAGC-3′/5′-GCTCAATCTTTTTTCTTAACTCGC-3′) was obtained from Shanghai Sangon Biotechnology Co. (China). The DNA was dissolved in 50 mM PBS buffer solution (pH 7.4, 100 mM NaCl). The Ru(bpy)_2(dppx)_2^2+ complex and SiNDs were synthesized as reported elsewhere (for details, see the Supporting Information). Human serum was supplied by Beijing Chengwen Immunochemistry Laboratory (China). High-purity water (18.2 MΩ·cm) was employed for all the experiments.

4.2. Apparatus. Fluorescence spectra were recorded using an RF-5301 fluorescence spectrophotometer (Shimadzu, Japan). UV–vis absorption spectra were recorded using a UV-2600 spectrophotometer (Shimadzu, Japan). The pH values of the solutions were measured with a PHS-25 pH meter (INESA Scientific Instrument Co. Ltd., China). The solutions were mixed homogeneously using a vortex mixer (Essenscien V6, USA).

4.3. General Experimental Procedure. Ru(bpy)_2(dppx)_2^2+ and SiNDs were uniformly mixed in a 50 mM PBS buffer solution (pH 7.4, 100 mM NaCl) to ensure a stable fluorescence intensity. Next, DNA of an appropriate concentration was added into the above SiND–Ru(bpy)_2(dppx)_2^2+ solution. The measurement of the emission...
The excitation and emission slits were 10 nm and 5 nm, respectively. The $I_{601}/I_{488}$ signal ratio was calculated on the basis of the fluorescence intensities at 601 and 448 nm. All optical measurements were performed three times in parallel at room temperature.

4.4. DNA Detection in Samples. The SiND–Ru(bpy)$_2$(dppx)$_2$ composite was employed for the ratiometric detection of DNA in a simulated sample, containing 4 μM Fe$^{3+}$, 20 μM K$^+$, Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$, amino acids, Na$_2$EDTA, glucose, and ATP as well as 10 μM BSA. Moreover, the sensor was also used for the ratiometric detection of DNA in a 1% human serum sample.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05434.

Synthesis of SiNDs and the Ru complex, fluorescence spectra of different concentrations of SiNDs, effect of pH on the fluorescence quenching of Ru(bpy)$_2$(dppx)$_2$ toward SiNDs, effect of the human serum matrix on analyte detection, and comparison of fluorescence methods for DNA detection (PDF)

## AUTHOR INFORMATION

### Corresponding Author

Yanan Zhang  –  Hubei Key Laboratory for Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China; orcid.org/0000-0001-7784-6694; Phone: +86-713-8833611; Email: ynzhang@hgnu.edu.cn

### Authors

Dajun Hou  –  School of Materials Science and Engineering, Wuhan University of Technology, Wuhan 430070, China

Bingshan Zhao  –  Hubei Key Laboratory for Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China

Chunyi Li  –  Hubei Key Laboratory for Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China

Xiaoyan Wang  –  Hubei Key Laboratory for Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China

Lanying Xu  –  Hubei Key Laboratory for Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China

Tao Long  –  Hubei Key Laboratory for Processing and Application of Catalytic Materials, College of Chemistry and Chemical Engineering, Huanggang Normal University, Huanggang 438000, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c05434

## Notes

The authors declare no competing financial interest.

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