Rectangular Coordination Polymer Nanoplates: Large-Scale, Rapid Synthesis and Their Application as a Fluorescent Sensing Platform for DNA Detection

Yingwei Zhang¹, Yonglan Luo¹, Jingqi Tian¹,², Abdullah M. Asiri³,⁴, Abdulrahman O. Al-Youbi³,⁴, Xuping Sun¹,³,⁴*

¹ State Key Lab of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Changchun, Jilin, People’s Republic of China, ² Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Beijing, People’s Republic of China, ³ Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia, ⁴ Center of Excellence for Advanced Materials Research, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

In this paper, we report on the large-scale, rapid synthesis of uniform rectangular coordination polymer nanoplates (RCPNs) assembled from Cu(II) and 4,4′-bipyridine for the first time. We further demonstrate that such RCPNs can be used as a very effective fluorescent sensing platform for multiple DNA detection with a detection limit as low as 30 pM and a high selectivity down to single-base mismatch. The DNA detection is accomplished by the following two steps: (1) RCPN binds dye-labeled single-stranded DNA (ssDNA) probe, which brings dye and RCPN into close proximity, leading to fluorescence quenching; (2) Specific hybridization of the probe with its target generates a double-stranded DNA (dsDNA) which detaches from RCPN, leading to fluorescence recovery. It suggests that this sensing system can well discriminate complementary and mismatched DNA sequences. The exact mechanism of fluorescence quenching involved is elucidated experimentally and its use in a human blood serum system is also demonstrated successfully.

Citation: Zhang Y, Luo Y, Tian J, Asiri AM, Al-Youbi AO, et al. (2012) Rectangular Coordination Polymer Nanoplates: Large-Scale, Rapid Synthesis and Their Application as a Fluorescent Sensing Platform for DNA Detection. PLoS ONE 7(1): e30426. doi:10.1371/journal.pone.0030426

Editor: Maxim Antopolovsky, University of Helsinki, Finland
Received November 3, 2011; Accepted December 16, 2011; Published January 18, 2012
Copyright: © 2012 Zhang et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by National Basic Research Program of China (No. 2011CB935800) and the National Natural Science Foundation of China (No. 21175129). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: sunxp@ciac.jil.cn

Introduction

Simple, fast, cost-effective, and sensitive detection of specific DNA sequences is crucial to molecular diagnostics for pathogen detection and biomedical research. The past years have witnessed the growing importance in developing specific methods for DNA detection, which has various applications in gene expression profiling, clinical disease diagnostics and treatment, fast detection of biological warfare agents, and forensic applications etc [1]. Detecting genetic mutations at the molecular level opens up the possibility of performing reliable disease diagnostics in clinical practice even before any symptom of a disease appears. Polymerase chain reaction (PCR) as a technique for DNA amplification and sequencing has found extensive application in modern biological and medical sciences; however, it has the disadvantages of high cost, risk of contamination, and false-negative results [2,3]. Gene chip is a widely used high-throughput DNA detection technique, but it requires highly precise and expensive instrumentation for fluorescent signal readout and needs sophisticated numerical algorithms to interpret the data [4]. Thus, new DNA detection methods need to be developed. Many efforts have recently been made to develop homogeneous fluorescence assays based on FRET (fluorescence resonance energy transfer) or quenching mechanism for DNA sequence detection [5]. It is shown that nanostructures can be used as a quencher in this assay with the advantage of eliminating the selection issue of fluorophore-quencher because they can quench dyes of different emission frequencies [5,6]. Until now, we and other researchers have successfully demonstrated that versatile structures can serve as an effective quencher for fluorescence-enhanced DNA detection, including gold nanoparticles [7–11], single-walled carbon nanotubes (SWCNTs) [12], carbon nanoparticles [13], nano-C₆₀ [14], graphene oxide (GO) [15,16], poly(o-phenylenediamine) (PMPD) nanorods [17], poly(m-phenylenediamine) (PMDP) nanorods [18], Ag@poly(m-phenylenediamine) core–shell nanoparticles [19], polyaniline nanofibres [20], poly(o-phenylenediamine) colloids [21], supramolecular microparticles [22], etc. However, all the above systems have their inherent drawbacks which limit their practical use. For example, the SWCNT or GO system suffers from the high cost that both SWCNT and graphite powder used for producing GO are usually purchased from some manufacturers and suppliers, and on the other hand, an organic solvent like N,N-dimethylformamide (DMF) is used to disperse SWCNT by a period of several hours sonication or the GO preparation by the Hummer’s method is time-consuming and labor-intensive [12,23]. Our PN system has the disadvantage that the nanobelts are tens of micrometers in length and tend to sink in the aqueous solution due to the gravity, causing the probability of stability in detection [17]. An additional limitation is that its discrimination ability toward complementary and single-base mismatched target sequences is very poor.

Coordination polymers (CPs) are a class of organic–inorganic hybrid materials, in which metal ions are linked together by...
organic bridging ligands, and have been developed extremely rapidly due to their versatile properties provoked by combining the merits of two sources and may find applications in many fields [24–29]. Only until recently, however, have CPs been used for DNA detection where coordination polymer colloids and nanobelts were used as a quencher [30–32], but these systems still suffer from more or less severe drawbacks, including (1) the H₂PtCl₆ precursor is expensive and monodispersed structures can’t be prepared on a large scale [30–32]; (2) a period of several hours is required to prepare the colloids [30]; (3) the dye fluorescence can’t be completely quenched by these quenchers, leading to strong background fluorescence [30–32]; (4) the gradual reduction of Ag[I] by 4,4’-bipyridine with elapsed time leads to Ag nanoparticle-decorated nanobelts [31,33]. Accordingly, the development of new fluorescent sensing platform overcoming all the above-mentioned shortcomings is highly desirable.

In this paper, for the first time, we report on the large-scale, rapid, and economic synthesis of uniform rectangular coordination polymer nanoplates (RCPNs) assembled from Cu(II) and 4,4’-bipyridine, carried out by directly mixing aqueous CuCl₂ solution and ethanol solution of 4,4’-bipyridine at room temperature. We further demonstrate that such RCPNs can be used as a very effective fluorescent sensing platform for multiple DNA detection with a detection limit as low as 30 pM and a high selectivity down to single-base mismatch. The DNA detection is accomplished by the following two steps: (1) RCPN binds dye-labeled single-stranded DNA (ssDNA) probe, which brings dye and RCPN into close proximity, leading to fluorescence quenching. (2) Specific hybridization of the probe with its target generates a double-stranded DNA (dsDNA) which detaches from RCPN, leading to fluorescence recovery. It suggests that this sensing system can well discriminate complementary and mismatched DNA sequences. The exact mechanism of fluorescence quenching involved is elucidated experimentally and its use human blood serum system is also demonstrated successfully.

### Results and Discussion

The mixing of aqueous CuCl₂ solution and ethanol solution of 4,4’-bipyridine results in the immediate formation of a large amount of blue precipitates (see Materials and Methods for preparation details). Figure 1A shows the low magnification SEM image of the precipitates thus formed, indicating that the products consist exclusively of a large quantity of rectangular coordination nanoplates. The high magnification image further reveals that these plates are nanoplates with typical dimensions of 800 nm in length, 500 nm in width, and 100 nm in thickness (inset), as shown in Figure 1B. Figure 1B gives the histogram of sizes of these nanoplates with standard deviation. The chemical composition of these nanoplates was determined by energy-dispersive spectrum (EDS) shown in Figure S2. The peaks of C, N, Cu, and Cl elements are observed, indicating that these nanoplates are products of 4,4’-bipyridine and CuCl₂. It is well-documented that nitrogen-contained ligand can coordinate with Cu(II) [34]. We can suggest that the formation of such rectangular coordination polymer nanoplates in our present study is attributed to coordination-induced assembly from Cu(II) and 4,4’-bipyridine. A possible formation mechanism of the nanoplates is briefly presented as following. When Cu(II) and 4,4’-bipyridine are mixed together, the two nitrogen atoms on the para positions of one 4,4’-bipyridine molecule can coordinate to two different Cu(II) cations leading to 4,4’-bipyridine-bridged structure, and the Cu species contained in as-formed structure can further capture other 4,4’-bipyridine molecules by coordination interactions along different directions. This coordination-induced assembly process can proceed repeatedly until the depletion of reactants in the solution, resulting in the formation of large coordination polymers, finally. The formation of such RCPNs is complete within seconds and thus it is impossible for us to trace the time-dependent growth process. We can only speculate the possible formation mechanism involved and the detailed formation mechanism is far beyond our understanding. It is of importance to note that the yield of RCPNs is estimated to be about 90% based on the weight difference between the reactants and products.

To test the feasibility of using RCPNs as a novel effective fluorescent sensing platform for nucleic acid detection, an oligonucleotide sequence associated with human immunodeficiency virus (HIV) was chosen as a model system. Figure 2 shows the fluorescence emission spectra of P₇HV at different conditions. The fluorescence spectrum of P₇HV, the FAM-labeled probe, in the absence of RCPNs, exhibits strong fluorescence emission due to the presence of the fluorescein-based dye (curve a). However, in the presence of RCPNs, we failed to see the fluorescence emission

---

**Figure 1. Instrumental analysis of the precipitate thus formed.**
(A) Low and (B) high magnification SEM images of the precipitates thus formed. Inset shows that the nanoplate is about 100 nm in thickness. doi:10.1371/journal.pone.0030426.g001

**Figure 2. Performance of target DNA detection.** Fluorescence emission spectra of P₇HV (50 nM) at different conditions: (a) P₇HV; (b) P₇HV + 300 nM T₁; (c) P₇HV + RCPNs; (d) P₇HV + RCPNs + 300 nM T₁. Inset: fluorescence intensity ratio of P₇HV-RCPN complex with F/F₀=1 F₀ and F are the fluorescence intensity without and with the presence of T₁, respectively plotted against the logarithm of the concentration of T₁ (nM). Excitation was at 480 nm, and the emission was monitored at 518 nm. All measurements were done in Tris-HCl buffer in the presence of 15 mM MgCl₂ (pH: 7.4).

doi:10.1371/journal.pone.0030426.g002
peak of the FAM dye (curve c), indicating that RCPNs can adsorb ssDNA and quench the fluorescent dye very effectively. On the other hand, the PHIV-RCPN complex shows significant fluorescence enhancement upon its incubation with complementary target T1 over a period of 1 h, leading to about 83% fluorescence recovery (curve d). It should be noted that the fluorescence intensity of the free PHIV was, however, scarcely influenced by the addition of T1, in the absence of RCPNs (curve b). Figure 2 inset illustrates the fluorescence intensity changes \((F/F_0-1)\) of PHIV-RCPN complex upon addition of different concentrations of T1, where \(F_0\) and \(F\) are FAM fluorescence intensities at 518 nm in the absence and the presence of T1, respectively. In the DNA concentration range of 5–300 nM, a dramatic increase of FAM fluorescence intensity was observed, suggesting that the RCPN/DNA assembly approach is effective in probing biomolecular interactions.

It was found that the amount of RCPNs involved in this system has profound effect on the efficiency of the fluorescence quenching and the subsequent recovery. The increase of RCPNs in volume from 0 to 100-μL leads to increased quenching efficiency but a decrease in recovery efficiency (Figure 3). Thus, an optimal volume of 60-μL was chosen in our present study if not specified. It is also important to note that optimal signal-to-noise ratio and hence lower detection limit can be obtained by decreasing the amount of RCPN and PHIV used. For instance, a detection limit as low as 30 pM was achieved when the experiment was carried out using 0.6-μL RCPNs and 500 pM PHIV in the system (Figure S3).

It is of importance to note that such RCPNs can be well-dispersed in water by shaking and kept stable during our measurements.

Such RCPN is a π-rich structure and thus there should be strong π-π stacking interactions between ssDNA bases and RCPN [35]. Indeed, we have found that fluorescence quenching was suppressed by introducing N,N-dimethylformamide (DMF) to change the solvent polarity in assay solution (Figure S4), which can be attributed to that this π-π interaction is weaken by DMF molecules leading to decreased adsorption of ssDNA on RCPNs [19]. The zeta potential of the nanoplate was measured to be about 24.1 mV in pure water, meaning the nanoplate has a positively charged surface due to the presence of Cu(II) cations in the plate. However, the electrostatic attractive interactions between RCPN and negatively charged backbone of ssDNA is greatly weakened due to the presence of a large amount of salt in buffer [19]. In contrast, RCPN should have weak or no binding with dsDNA due to the absence of unpaired DNA bases and the rigid conformation of dsDNA. Scheme S1A presents a schematic to illustrate the fluorescence-enhanced nucleic acid detection using RCPN as a sensing platform. The DNA detection is accomplished by the following two steps: In the first step, the adsorption of fluorescent FAM-labeled ssDNA onto the nanoplate via π-π stacking leads to fluorescence quenching due to their close proximity. In the second step, the specific hybridization of the dye-labeled ssDNA with its target leads to fluorescence recovery because the hybridization will disturb the interaction between the dye-labeled ssDNA and nanoplate, producing a dsDNA which detaches from RCPN. The release of the dsDNA from RCPNs is evidenced by the following experimental fact that no obvious fluorescence change was observed after the removal of the RCPNs from the solution by centrifugation, as shown in Figure S5. The absorption spectrum of RCPNs dispersed in Tris-HCl buffer (pH 7.4) shown in Figure S6 exhibits an absorption peak at 238 nm, suggesting that there is no spectra overlap and thus no FRET occurs between RCPN and the fluorescent dye FAM. We can attribute the observed fluorescence quenching in our present study to photoinduced electron transfer (PET) from nitrogen atom in RCPN to excited fluorophore due to their close proximity [36]. Scheme S1B presents a schematic to illustrate the fluorescence quenching mechanism involved. When the fluorophore is excited, an electron from the highest occupied molecular orbital (HOMO) is promoted to the lowest unoccupied molecular orbital (LUMO), leaving an electronic vacancy in the fluorophore HOMO, which is filled by transfer of an electron from the higher energy HOMO of the nitrogen atom in RCPN serving as a donor. The overall effect of PET process is that the excited state lifetime is shortened and little fluorescence occurs. Note that although the pyridine binds to Cu(II) via coordination of its N atom to Cu center, given that 4,4’-bipyridine has two N atoms at opposite positions, it is expected that there are still some N atoms available on the RCPN surface for protonation. Upon protonation of the donor, its redox potential is raised and its HOMO becomes lower in energy than that of the fluorophore. Consequently, electron transfer from donor to fluorophore is hindered and the fluorescence quenching is thus suppressed. Although fluorescence intensity of dye-labeled probe is sensitive to pH value, it was observed that fluorescence quenching is suppressed with the increase of protonation degree of donor by decreasing the pH value of the system (Figure S7).

We also studied the kinetic behaviors of PHIV and RCPNs, as well as of the PHIV–RCPN complex with T1 by collecting the time-dependent fluorescence emission spectra (Figure 4). Figure 4A shows the fluorescence quenching of PHIV in the presence of RCPNs as a function of incubation time. In the absence of the target, the curve exhibits a rapid reduction in the first 1 min and reaches equilibrium within the following 4 min, indicating that ssDNA adsorption on RCPN is much faster than on SWCNT but similar to on GO [6,12,14,15]. Figure 4B shows the fluorescence recovery of PHIV-RCPN by T1 as a function of time. In the presence of the target T1, the curve shows a fast increase in the first 1 min, followed by a slow fluorescence enhancement. The best fluorescence response was obtained after about 70 min of incubation time. It suggests that the kinetics of the hybridization of the probe adsorbed on RCPN to its target and the subsequent release of the dsDNA thus formed from RCPN resembles that on

**Figure 3. Investigation of the influence of the amount of RCPNs on the system.** Fluorescence intensity histograms of PHIV + RCPNs and PHIV + RCPNs + T1 with the use of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μL of RCPNs in this system [P_{PHIV}]=50 nM; [T_1]=300 nM. Excitation was at 480 nm, and the emission was monitored at 518 nm. All measurements were done in Tris-HCl buffer in the presence of 15 mM Mg^{2+} (pH 7.4).

doi:10.1371/journal.pone.0030426.g003
dependent quenching study to calculate $K_s$ sensitivity. Hence, we have performed a quencher concentration-base mismatched target $T_2$, two-base mismatched target $T_3$, and further investigated. Figure 5A shows the fluorescence responses of PHIV toward $T_1$ and $T_2$ in the presence of RCPNs at room temperature. The $F/F_0$ value obtained upon addition of 300 nM of $T_3$ is about 56% of the value obtained upon addition of $T_2$ is about 56% of the value obtained upon addition of $T_1$ into PHIV-RCPN complex. Figure 5B compares the fluorescence signal enhancement of PHIV-RCPN complex upon incubation with $T_1$ and $T_2$ at 25 and 50°C, respectively. All the above observations indicate that the present sensing system can well discriminate complementary and mismatched DNA sequences with good reproducibility and its discrimination ability increases with increased hybridization temperature which makes the hybridization harder for probe and mismatched target.

It was found that the use of shorter oligonucleotide can further improve the ability of this sensing system to distinguish mismatch. Figure 6 shows the fluorescence responses of FAM-labeled, 9-nucleotide ssDNA probe $P_5$ (50 nM) toward complementary target $T_1$, and single-base mismatched target $T_2$, and double-base mismatched target $T_3$, respectively. All the above observations indicate that the present sensing system can well discriminate complementary and mismatched DNA sequences with good reproducibility and its discrimination ability increases with increased hybridization temperature which makes the hybridization harder for probe and mismatched target.

The discrimination ability of this sensing platform was also evaluated by detecting specific sequences on much longer DNA targets than probes. For this purpose, two long DNA strands were chosen as targets: $T_{L1}$, the middle part of which is complementary sequence to PHIV; $T_{L2}$, the middle part of which is single-base mismatched sequence to PHIV. Figure 7 shows the fluorescence responses of PHIV toward $T_{L1}$ and $T_{L2}$ in the presence of RCPNs at room temperature. The $F/F_0$ value obtained upon addition of 300 nM of $T_{L2}$ is about 54% of the value obtained upon addition of 300 nM of $T_{L1}$ into PHIV-RCPN complex.

The discrimination ability of this sensing platform was also evaluated by detecting specific sequences on much longer DNA targets than probes. For this purpose, two long DNA strands were chosen as targets: $T_{L1}$, the middle part of which is complementary sequence to PHIV; $T_{L2}$, the middle part of which is single-base mismatched sequence to PHIV. Figure 7 shows the fluorescence responses of PHIV toward $T_{L1}$ and $T_{L2}$ in the presence of RCPNs at room temperature. The $F/F_0$ value obtained upon addition of 300 nM of $T_{L2}$ is about 54% of the value obtained upon addition of 300 nM of $T_{L1}$ into PHIV-RCPN complex.

The discrimination ability of this sensing platform was also evaluated by detecting specific sequences on much longer DNA targets than probes. For this purpose, two long DNA strands were chosen as targets: $T_{L1}$, the middle part of which is complementary sequence to PHIV; $T_{L2}$, the middle part of which is single-base mismatched sequence to PHIV. Figure 7 shows the fluorescence responses of PHIV toward $T_{L1}$ and $T_{L2}$ in the presence of RCPNs at room temperature. The $F/F_0$ value obtained upon addition of 300 nM of $T_{L2}$ is about 54% of the value obtained upon addition of 300 nM of $T_{L1}$ into PHIV-RCPN complex.

The discrimination ability of the present sensing platform toward complementary and mismatched target sequences was further investigated. Figure 5A shows the fluorescence responses of PHIV-RCPN complex toward complementary target $T_1$, single-base mismatched target $T_2$, and non-complementary target $T_4$. It is observed that the $F/F_0$ value ($F_0$ and $F$ are the fluorescence intensities without and with the presence of target, respectively) obtained upon addition of 300 nM of $T_2$ and $T_4$ is about 79% and 33% of the value obtained upon addition of 300 nM of $T_1$ into the PHIV-RCPN complex, respectively. In contrast, only very small fluorescence change was observed for the PHIV-RCPN upon addition of 300 nM $T_4$, indicating that the fluorescence enhancement in our present system is indeed due to the base pairing between probe and its target other than competitive binding. Compared to the complementary target, the mismatched target should have lower hybridization ability toward the ssDNA probe. As a result, a decreased hybridization and thus fluorescence recovery efficiency is observed. Figure 5A inset shows the corresponding fluorescence intensity histograms with error bar. We also carried out hybridization experiments at an elevated temperature of 50°C. It should be noted that FAM-ssDNA only exhibits slight fluorescence decrease at elevated temperature. The observed decrease of FAM fluorescence intensity at elevated temperature in our present study can be attributed to that hybridization stringency conditions do not favor duplex formation between short single strands [17,38], leading to decreased hybridization and thus fluorescence recovery efficiency. It is found that the $F/F_0$ value obtained upon addition of $T_4$ is about 56% of the value obtained upon addition of $T_2$ into PHIV-RCPN complex. Figure 5B compares the fluorescence signal enhancement of PHIV-RCPN complex upon incubation with $T_1$ and $T_2$ at 25 and 50°C, respectively. All the above observations indicate that the present sensing system can well discriminate complementary and mismatched DNA sequences with good reproducibility and its discrimination ability increases with increased hybridization temperature which makes the hybridization harder for probe and mismatched target.

The present sensing platform has excellent sensitivity and selectivity in purue buffer systems. In further experiments, the potential application of this sensing platform for real sample analysis was challenged with human blood serum samples. Figure 8 shows the fluorescence emission spectra of PHIV in the presence of 10% blood serum (volume ratio) in Tris-HCl buffer at different
conditions. The $F/F_0$ value obtained upon addition of 300 nM of $T_2$ is about 70% of the value obtained upon addition of 300 nM of $T_1$ into $P_{HV}$-RCPN complex, indicating that this system is still capable of distinguishing complementary and mismatched sequences with good reproducibility in the presence of blood serum. These observations show that our measurements were not seriously affected by blood serum components and hence this sensing system is likely to be capable of practically useful mismatch detection upon further development.

Finally, the feasibility of using the platform described herein to detect multiple DNA targets simultaneously was explored. To this end, we chose FAM-labeled $P_{HV}$ and another two probes $P_{HBV}$ and $P_{K167}$ labeled with ROX and Cy5 (cyanine 5), respectively, as model systems. Because these three dyes are individually excited at 480, 587, and 643 nm to emit at 518, 615, and 660 nm, respectively, significant dye-to-dye energy transfer is avoided. In the presence of RCPNs, the fluorescence of all dyes in the probe mixture was heavily quenched, suggesting that RCPN can effectively quench dyes of different emission frequencies. Figure 9 shows the fluorescence intensity histograms of the probe mixture toward different target combinations in the presence of RCPNs under excitation/emission wavelengths of 480/518, 587/615, and 643/660 nm. It is clearly seen that the addition of $T_1$ gives only one strong emission peak at 518 nm when excited at 480 nm. However, the target combination of $T_1+T_5$ gives two strong emission peaks at 518 and 615 nm when excited at 480 and 587 nm, respectively. Three strong emission peaks are observed for the $T_1+T_5+T_6$ target combination at 518, 615, and 660 nm when excited at 480, 587, and 643 nm, respectively. Based on all
In summary, uniform RCPNs have been rapidly prepared on a large scale at room temperature and further used as a very effective fluorescent sensing platform for multiple DNA detection with high sensitivity and selectivity. The mechanism of fluorescence quenching is discussed and the application of this sensing platform in human blood serum system is also demonstrated successfully. This RCPN-based assay is homogenous, "mix and read" and requires no wash or complex sample preparation steps. Our present observations are significant for the following three reasons: (1) it provides us a facile method for the rapid, economic synthesis of RCPNs for DNA detection; (2) such RCPNs are obviously easier to produce and thus the RCPN-based DNA sensing platform is practically more important than those based on other structures [5–12]; (3) this RCPN-based assay holds great promise for practical application in clinical sample analysis.

**Materials and Methods**

All chemically synthesized oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). DNA concentration was estimated by measuring the absorbance at 260 nm. All the other chemicals were purchased from Aladin Ltd. (Shanghai, China) and used as received without further purification. The water used throughout all experiments was purified through a Millipore system. Human blood serum was obtained from Institute of Virology and AIDS Research, First Affiliated Hospital, Jilin University, Changchun, Jilin, People’s Republic of China.

Oligonucleotide sequences used are listed below (mismatch underlined):

1. PHIV (FAM dye-labeled ssDNA):
   - 5’-FAM-AGT CAG TGT GGA AAA TCT CTA GC-3’

2. T1 (complementary target to PHIV):
   - 5’-GCT AGA GAT TTT CCA CAC TGA CT-3’

3. T2 (single-base mismatched target to PHIV):
   - 5’-GCT AGA GAT TGT CCA CAC TGA CT-3’

4. T3 (two-base mismatched target to PHIV):
   - 5’-GCT AGA GAT TGT ACA CAC TGA CT-3’

5. T5 (non-complementary target to PHIV):
   - 5’-TTT TTT TTT TTT TTT TTT TT-3’

6. P (FAM dye-labeled shorter ssDNA):
   - 5’-TGG AAA ATC-3’

7. T11 (complementary target to P):
   - 5’-GAT TTT CCA-3’

8. T12 (single-base mismatched target to P):
   - 5’-GAT TGT CCA-3’

9. PHBV (ROX dye-labeled ssDNA):
   - 5’-ROX-TAC CAC ATC ATC CAT ATA ACT GA-3’

10. T5 (complementary target to PHBV):
    - 5’-TCA GTT ATA TGG ATG ATG TGG TA-3’

11. PK16 (Cy5 dye-labeled ssDNA):
    - 5’-Cy5-TCT GCA CAC CTC TTG ACA CTC CG-3’

12. T6 (complementary target to PK167):
    - 5’-CGG AGT GTG AAG AGG TGT GCA GA-3’

13. T1 (The middle part of a long strand as a target complementary to PHIV):

![Figure 8. Performance of single-base mismatch discrimination in the presence of blood serum. Fluorescence emission spectra of PHIV (50 nM) in 40-μL blood serum and 360-μL Tris-HCl buffer containing 15 mM Mg2+ (pH: 7.4) at different conditions: (a) PHIV + RCPNs; (b) PHIV + RCPNs + 300 nM T1; (c) PHIV + RCPNs + 300 nM T2. Inset: fluorescence intensity histograms with error bar. Excitation was at 480 nm, and the emission was monitored at 518 nm. The error bar represents the standard deviation of three measurements.](Image)

![Figure 9. Multiplex DNA detection. Fluorescence intensity histograms of the probe mixture toward different target combinations in the presence of RCPNs under excitation/emission wavelengths of 480/518, 587/615, and 643/660 nm/nm. All measurements were done in Tris-HCl buffer in the presence of 15 mM Mg2+ (pH: 7.4). The error bar represents the standard deviation of three measurements.](Image)
CuCl₂ aqueous solution at room temperature under stirring. After fluorescence intensity histograms with error bar. Excitation was 4,4′100 mM NaCl, 5 mM KCl, and 15 mM MgCl₂ (pH: 7.4). All the measurements were done in Tris-HCl buffer in the presence of 15 mM Mg²⁺ (pH: 7.4).

Figure S4 Investigation of the influence of the solvent polarity on the system. Fluorescence intensity histograms of P HIV (50 nM) in the presence of RCPNs at different solvent conditions: (a) 300 μL Tris-HCl buffer and (b) 150 μL Tris-HCl buffer + 150 μL DMF.

Figure S5 Confirmation of the release of the dsDNA from RCPNs. Fluorescence emission spectra of (a) P HIV–RCPN complex + T₁ and (b) the supernatant of (a) after removing RCPNs by centrifugation. ([P HIV] = 50 nM; [T₁] = 300 nM; λex = 480 nm). All measurements were done in Tris-HCl buffer in the presence of 15 mM Mg²⁺ (pH: 7.4).

Figure S6 UV-Vis absorption of RCPNs. Absorption spectrum of RCPNs dispersed in Tris-HCl buffer in the presence of 15 mM Mg²⁺ (pH 7.4).

Figure S7 Investigation of the influence of pH value on the fluorescence quenching. The histograms of fluorescence intensity changes (I/I₀) of FAM-labeled ssDNA at different pH values, where I₀ and I are fluorescence intensities at 518 nm in the absence and presence of RCPNs, respectively.

Figure S8 Stern–Volmer quenching constant (KSV) determination of different quenchers. Stern-Volmer plot for quenching of the FAM fluorescence by different quenchers at room temperature: (a) graphene oxide (GO); (b) multi-walled carbon nanotubes (MWNT); (c) poly(β-phenylenediamine) nanobelt (PN); (d) RCPNs.

Table S1 Stern-Volmer quenching constant KSV of FAM fluorescence by different quenchers at room temperature.

Author Contributions
Conceived and designed the experiments: XS. Performed the experiments: YZ. Analyzed the data: JT YL AMA AOA. Wrote the paper: XS.
12. Yang R, Jin J, Chen Y, Shao N, Kang H, et al. (2008) Carbon nanotube-quenched fluorescent oligonucleotides: probes that fluoresce upon hybridization. J Am Chem Soc 130: 8351–8358.

13. Li H, Zhang Y, Wang L, Tian J, Sun X (2011) Nucleic acid detection using carbon nanoparticles as an fluorescent sensing platform. Chem Commun 47: 961–963.

14. Li H, Zhang Y, Luo Y, Sun X (2011) Nano-C60 as a novel, effective fluorescent sensing platform for biomolecular detection. Small 7092(2011):00068.

15. Lu C, Yang H, Zhu C, Chen X, Chen G (2009) A graphene platform for sensing biomolecules. Angew Chem Int Ed 48: 4785–4787.

16. He S, Song B, Li D, Zhu C, Qi W, et al. (2010) A graphene nanoprobe for rapid, sensitive, and multicolor fluorescent DNA analysis. Adv Funct Mater 20: 453–459.

17. Wang L, Zhang Y, Tian J, Li H, Sun X (2011) Conjugation polymer nanobelts: a novel fluorescent sensing platform for nucleic acid detection. Nucleic Acids Res 39: e37–42.

18. Zhang Y, Li H, Luo Y, Shi X, Tian J, et al. (2011) Poly(m-Phenylenediamine) Nanospheres and Nanorods: Selective Synthesis and Their Application for Multiplex Nucleic Acid Detection. PLoS ONE 6: e20569.

19. Zhang Y, Wang L, Tian J, Li H, Luo Y, et al. (2011) Ag(poly(m-phenylenediamine)) core–shell nanoparticles for highly selective, multiplex nucleic acid detection. Langmuir 27: 2170–2175.

20. Liu S, Wang L, Luo Y, Tian J, Li H, et al. (2011) Polyamine nanotubes for fluorescent nucleic acid detection. Nanoscale 3: 967–969.

21. Tian J, Li H, Luo Y, Wang L, Zhang Y, et al. (2011) Poly[phenylenediamine] colloid-quenched fluorescent oligonucleotide as a probe for fluorescence-enhanced nucleic acid detection. Langmuir 27: 874–877.

22. Li H, Zhai J, Sun X (2011) Electrostatic-assembly-driven formation of supramolecular rhombus microparticles and their application for fluorescent nucleic acid detection. PLoS ONE 6: e18958.

23. Hummers WS, Offeman RE (1958) Preparation of graphitic oxide. J Am Chem Soc 80: 1339–1339.

24. James SL (2003) Metal-organic frameworks. Chem Soc Rev 22: 276–288.

25. Ertlhoudt M, Moler DR, Li H, Chen B, Reineke TM, et al. (2001) Modular Chemistry: Secondary Building Units as a Basis for the Design of Highly Porous and Robust Metal–Organic Carboxylate Frameworks. Acc Chem Res 34: 319–330.

26. Mederos A, Domínguez S, Hernández-Molina R, Sanchiz J, Brito F (1999) Coordinating ability of ligands derived from phenylenediamines. Coord Chem Rev 193–195: 857–911.

27. Mederos A, Domínguez S, Hernández-Molina R, Sanchiz J, Brito F (1999) Coordinating ability of phenylenediamines. Coord Chem Rev 193–195: 913–939.

28. Spokony AM, Kim D, Sumrein A, Mirkin CA (2009) Infinite coordination polymer nano- and microparticle structures. Chem Soc Rev 38: 1218–1227.

29. Sun X, Dong S, Wang E (2005) Coordination-Induced Formation of Submicrometer-Scale, Monodisperse, Spherical Colloids of Organofluorinated Materials at Room Temperature. J Am Chem Soc 127: 13102–13103.

30. Li H, Sun X (2011) Fluorescence-enhanced nucleic acid detection using coordination polymer colloids as a sensing platform. Chem Commun 47: 2625–2627.

31. Luo Y, Liao F, Lu W, Chang G, Sun X (2011) Coordination polymer nanobelts for nucleic acid detection. Nanotechnol 22: 195502–195506.

32. Li H, Wang L, Zhang Y, Tian J, Sun X (2011) Coordination polymer nanobelts as an effective sensing platform for fluorescence-enhanced nucleic acid detection. Macromol Rapid Commun 32: 889–904.

33. Luo Y, Lu W, Chang G, Liao F, Sun X (2011) One-step preparation of Ag nanoparticle–decorated coordination polymer nanobelts and their application for enzymeless H2O2 detection. Electrochim. Acta 56: 8371–8374.

34. Maslak P, Szczepanski JJ, Parvez M (1991) Complexation through nitrogen in copper and nickel complexes of substituted ureas. J Am Chem Soc 113: 1062–1063.

35. Varghese N, Mogera U, Govindaraj A, Das A, Maiti PK, et al. (2009) Binding of DNA nucleobases and nucleosides with graphene. ChemPhysChem 10: 206–210.

36. Bernard V (2001) Molecular fluorescence: principles and applications. Wiley-VCH.

37. Lakowicz JR (1999) Principles of Fluorescence Spectroscopy, second ed. New York: Plenum Press.

38. Burr HE, Schanze KT (1982) Reduced-stringency DNA reassociation: sequence specific duplex formation. Nucleic Acids Res 10: 719–733.