One-pot fluorescence detection of multiple analytes in homogenous solution based on noncovalent assembly of single-walled carbon nanotubes and aptamers

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1. Introduction

Over the past decade, the rapid development in nanoscience and nanotechnology has resulted in the successful synthesis and characterization of various nanomaterials including metallic nanoparticles, semiconductor nanocrystals, SiO2 nanoparticles, carbon nanotubes, and so on (Burdà et al., 2005). These nanomaterials have been shown to possess unique optical, electronic, magnetic and catalytic properties, making them ideal candidates for signal generation and transduction in sensing (Jeon et al., 2009; Wang et al., 2009, 2010; Wang and Lu, 2009; Zhao et al., 2008). Aptamers are single-strand DNA or RNA selected in vitro that bind specifically with a broad range of targets from metal ions, organic molecules, to proteins, cells and microorganisms (Mayer, 2009). Because of their high affinity and high specificity, aptamers have been widely used as ideal recognition elements for detecting various targets in recent years (Famulok et al., 2007; Liu et al., 2009; Tombelli et al., 2007). Therefore, the integration of aptamers with nanomaterials provides new sensing platform that combine the specific molecular recognition of aptamers with the diverse and strong signal transduction of nanomaterials (Baron et al., 2007; Chiu and Huang, 2009; Wang et al., 2009, 2010; Wang and Lu, 2009; Zhao et al., 2008). Gold nanoparticles (AuNPs) have been widely used in biosensor due to their easy preparation and their attractive electronic, optical, and thermal properties as well catalytic properties (Guo and Wang, 2007; Zhao et al., 2008). The thiol-modified aptamers can be easily attached the AuNPs through gold–sulfur bond, resulting in the formation of AuNPs–aptamer hybrid system for biosensing (Zhao et al., 2008; Zhang et al., 2010). However, the poor stability of the AuNPs–aptamer sensing systems would limit their wide application because the thiol layer is easily oxidized and gradually degraded in air or in buffer solution (Sun et al., 2010).

Single-walled carbon nanotubes (SWNTs), as quasi one-dimensional functional quantum wires, are another important type of nanomaterials for biosensing due to their unique mechanical, electrical and optical characteristics (Lu et al., 2009; Zhu et al., 2010a). Interestingly, single-stranded DNA (ssDNA) can be adsorbed noncovalently onto the side walls of SWNTs by means of π-stacking interaction between nucleotide bases and the side walls of SWNTs (Tang et al., 2006). In addition, SWNTs have been demonstrated to be superior and universal quenchers for a variety of organic fluorophores (Zhu et al., 2010a). Thus, Tan et al. developed a new class of fluorescent biosensors based on noncovalent assembly of SWNTs and ssDNA (Yang et al., 2008a). The fluorophore-labeled ssDNA can wrapped onto the SWNTs surface to quench the fluorescence. Hybridization of a complimentary DNA strand or binding of target could cause the fluorophore-labeled ssDNA to be released from the SWNTs, resulting in an increase of fluorescence emission comparable to the fluorescence of the ssDNA/SWNTs assembled complex (Yang et al., 2008a). Based on the above scheme, the ssDNA/SWNTs assembled fluorescence system has been used to detection of DNA (Yang et al., 2008b), Hg2+...
2.1. Reagents

In the sensing platform developed by Tan et al. (Yang et al., 2008a,b), the aggregates and undispersed SWNTs in the incubated solution needed to be removed by ultracentrifugation, and the supernatant was collected for fluorescence measurement. This ultracentrifugation step makes this detection method more complex and time-consuming.

One major challenge in analytical chemistry is multiplex sensing of number of analytes with each analyte displaying a different signal (Liu et al., 2007). Compared with the single-target assay, multiplex assay has the ability to detect for multiple analytes in a single solution (Cao et al., 2002; Geng et al., 2010; Liu et al., 2007; Miao et al., 2008; Xie and Walton, 2010; Zhang et al., 2010a,b; Zhang and Hu, 2010; Zhu et al., 2010b), which has the significant advantage for rapid, simple, low sample and reagent consumption. In this work, we describe proof-of-concept development of the ssDNA/SWNTs assembled fluorescence sensing for multiplex assay that relies on dual-aptamer recognition. Fig. 1 shows the process for multiplex sensing. Thrombin and ATP are chosen as model analytes. The anti-thrombin aptamer is labeled with 6-carboxy-X-rhodamine (ROX). These dye-labeled aptamers are closely to the nanotube, thus effectively quenching the dyes' fluorescence. Upon complex formation, the dye molecules are close proximity to the nanotubes, as shown in Fig. 1. In the sensing platform developed by Tan et al. (Yang et al., 2008a,b), the aggregates and undispersed SWNTs in the incubated solution needed to be removed by ultracentrifugation, and the supernatant was collected for fluorescence measurement. This ultracentrifugation step makes this detection method more complex and time-consuming.

Fig. 1. Schematic of a multicolor aptamer-based fluorescent sensor for multiplex detection of thrombin and ATP.

2.2. Apparatus

All fluorescence measurements were made using Hitachi model F-4600 fluorescence spectrophotometer (Kyoto, Japan). UV–visible–NIR adsorption spectra were recorded on a PerkinElmer model Lambd 950 UV–visible–NIR spectrophotometer (California, USA) at room temperature. A Chirascan Model Circular Dichroism Spectrometer (Leatherhead, Surrey, U.K.) was utilized to collect the Circular Dichroism spectra of aptamer. Quanta 200 Environmental scanning electron microscope (FEI Company USA) and JEM-2100 transmission electron microscope (Japan Electronics Co., Ltd) were used to study the morphology of SWNTs.

2.3. Pretreatment of SWNTs

The SWNTs were pretreated according to the literature (Zhang et al., 2010a,b). Briefly, the SWNTs were treated by refluxing in 4.0 M HNO₃ for 24 h, then were filtered with a 220 nm millipore size membrane and washed with water. Subsequently, the 3:1 concentrated H₂SO₄:HNO₃ mixture was realized as the oxidizing acid in this cutting operation. The SWNTs were sonicated for 8 h in ice bath. They were washed with water to obtain a neutral and filtered with a 220 nm millipore size membrane. Finally, they were dried under vacuum at 60 °C overnight. The purified SWNTs were sonicated in DMF for 5 h. The pretreated SWNTs were water-dispersible due to the presence of suspended hydroxyl and carboxyl groups at the surface of sidewall. The photograph of transmission electron

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Quenching rate (%)</th>
<th>F/F₀</th>
</tr>
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<tbody>
<tr>
<td>P₁ 5-FAM-TCT CTC AGT CGG TGG TAG GGC</td>
<td>91.1</td>
<td>1.80</td>
</tr>
<tr>
<td>P₂ 5-FAM-GGT TGG TGT GGT TGG-3'</td>
<td>92.9</td>
<td>1.07</td>
</tr>
<tr>
<td>P₃ 5-FAM-TCT CTC GGT TGG TGT GGT-3'</td>
<td>90.3</td>
<td>1.53</td>
</tr>
<tr>
<td>P₄ 5-FAM-TCT CTC TCT CTC TCT CTC GGT</td>
<td>83.3</td>
<td>1.54</td>
</tr>
<tr>
<td>P₅ 5-ROX-TCT CTC ACC TGG GGG AGT AT</td>
<td>92.3</td>
<td>2.03</td>
</tr>
<tr>
<td>P₆ 5-ROX-AGA GAG ACC TGG GGG AGT AT</td>
<td>91.3</td>
<td>1.63</td>
</tr>
</tbody>
</table>

P₁, P₂, P₃ and P₄, see Table 1 for the sequences) and the 6-carboxy-X-rhodamine (ROX)-labeled anti-ATP aptamers (P₅ and P₆) were synthesized at Augct Biotechnology Co., Ltd. (Beijing, China). The aptamers were dissolved in Tris–HCl buffer (20 mM, pH 7.4), whose concentrations were quantified using UV–vis absorption spectroscopy with the corresponding extinction coefficients (ε₂₆₀=2×10⁴ M⁻¹ cm⁻¹). Human thrombin (1710 U mg⁻¹), adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP) were purchased from Sigma–Aldrich (St. Louis, MO, USA), N,N-dimethylformamide (DMF) and the used metal salts (KCl, NaCl, CaCl₂, etc.) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Single-walled carbon nanotubes were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Millipore Milli-Q water (18 MΩ cm⁻¹) was used in all experiments.

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2. Experimental

2.1. Reagents

The fluorescein amidite (FAM)-labeled anti-thrombin aptamers (P₁, P₂, P₃ and P₄, see Table 1 for the sequences) and the 6-carboxy-X-rhodamine (ROX)-labeled anti-ATP aptamers (P₅ and P₆) were synthesized at Augct Biotechnology Co., Ltd. (Beijing, China). The aptamers were dissolved in Tris–HCl buffer (20 mM, pH 7.4), whose concentrations were quantified using UV–vis absorption spectroscopy with the corresponding extinction coefficients (ε₂₆₀=2×10⁴ M⁻¹ cm⁻¹). Human thrombin (1710 U mg⁻¹), adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP) and uridine triphosphate (UTP) were purchased from Sigma–Aldrich (St. Louis, MO, USA), N,N-dimethylformamide (DMF) and the used metal salts (KCl, NaCl, CaCl₂, etc.) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Single-walled carbon nanotubes were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Millipore Milli-Q water (18 MΩ cm⁻¹) was used in all experiments.
spectra of the free P5 (0.5 M), SWNTs (15 μL, 50 μg/mL) and P1 (20 μL, 0.2 μM) + SWNTs (15 μL, 50 μg/mL) + thrombin (10 μL, 540 nM). The inset is the fluorescent photograph of P5 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) + thrombin (10 μL, 540 nM) (3). Experimental conditions: 80 min incubation time, room temperature and λ_ex = 470 nm. (B) Fluorescence emission spectra of P5 at different conditions: (a) P5 (20 μL, 0.2 μM); (b) P5 (20 μL, 0.2 μM) + ATP (10 μL, 280 nM); (c) P5 (20 μL, 0.2 μM) + SWNTs (20 μL, 50 μg/mL); and (d) P5 (20 μL, 0.2 μM) + SWNTs (20 μL, 50 μg/mL) + ATP (10 μL, 28 nM). The inset is the fluorescent photograph of P5 (20 μL, 2.6 μM) (1), P5 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) (2), and P5 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) + ATP (140 nM) (3). Experimental conditions: 80 min incubation time, room temperature and λ_ex = 530 nm.

microscopy (TEM) revealed that the outer diameter of SWNTs was about 4 nm (Fig. S1). The SWNTs solution was used in the following all studies.

2.4. Fluorescent assays

For thrombin assay, 20 μL of the P1 solution (0.20 μM), 15 μL of SWNTs solution (50 μg/mL), and 10 μL of appropriate concentrations of thrombin solution (20 mM Tris–HCl, 140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, pH 7.4) were mixed, and then the mixed solution was diluted with Tris–HCl buffer (20 mM Tris–HCl, 100 mM NaCl, 5.0 mM KCl, pH 7.4) to 250 μL. The above prepared solution was incubated for 80 min at room temperature. Finally, the fluorescence intensity of the incubated solution was measured at 520 nm with an excitation wavelength of 480 nm.

For ATP assay, 20 μL of the P5 solution (0.20 μM), 20 μL of SWNTs solution (50 μg/mL), and 10 μL of appropriate concentrations of ATP solution (20 mM Tris–HCl, 140 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, pH 7.4) were mixed, and then the mixed solution was diluted with Tris–HCl buffer to 250 μL. After the above prepared solution was incubated for 80 min at room temperature, fluorescence intensity of the incubated solution was recorded at 590 nm with an excitation wavelength of 530 nm.

For multiplex assay, 20 μL of FAM-labeled P1 probe (0.20 μM) and 20 μL of ROX-labeled P5 probe (0.25 μM) were mixed in a solution, next 40 μL SWNTs solution (50 μg/mL) were added, and then 10 μL of appropriate concentrations of ATP and thrombin solution were mixed. Finally, the mixed solution was diluted with Tris–HCl buffer to 250 μL. We employed different amounts of probes and SWNTs in order to optimize the detection performance. All other conditions were similar to those in the single target detection.

3. Results and discussion

3.1. Design of sensing strategy

Fig. 1 depicts the analytical process for thrombin and ATP. The important insight in our design comes from the interaction of SWNTs and DNA, which are based on the following: (1) dye-labeled single-stranded DNA (ssDNA) can be adsorbed noncovalently onto the side walls of SWNTs; (2) SWNTs have ultrahigh surface area for loading multiple molecules and quench all dyes; and (3) in the presence of a target, the target only binds its aptamer resulting in the fluorescent restoration of relative dye, because the aptamers have highly affinity and specificity recognition ability. To test the general feasibility of this approach, two DNA oligonucleotides (P1

Fig. 2. (A) Fluorescence emission spectra of P1 at different conditions: (a) P1 (20 μL, 0.2 μM); (b) P1 (20 μL, 0.2 μM) + throrhm (10 μL, 5.4 μM); (c) P1 (20 μL, 0.2 μM) + SWNTs (15 μL, 50 μg/mL); and (d) P1 (20 μL, 0.2 μM) + SWNTs (15 μL, 50 μg/mL) + throrhm (10 μL, 540 nM). The inset is the fluorescent photograph of P1 (20 μL, 2.0 μM) (1), P1 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) (2) and P1 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) + throrhm (10 μL, 540 nM) (3). Experimental conditions: 80 min incubation time, room temperature and λ_ex = 470 nm. (B) Fluorescence emission spectra of P5 at different conditions: (a) P5 (20 μL, 0.2 μM); (b) P5 (20 μL, 0.2 μM) + ATP (10 μL, 280 nM); (c) P5 (20 μL, 0.2 μM) + SWNTs (20 μL, 50 μg/mL); and (d) P5 (20 μL, 0.2 μM) + SWNTs (20 μL, 50 μg/mL) + ATP (10 μL, 28 nM). The inset is the fluorescent photograph of P5 (20 μL, 2.6 μM) (1), P5 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) (2), and P5 (20 μL, 2.0 μM) + SWNTs (50 μL, 100 μg/mL) + ATP (140 nM) (3). Experimental conditions: 80 min incubation time, room temperature and λ_ex = 530 nm.

Fig. 3. (A) UV–visible–NIR absorption spectra of the free P1 (0.5 μM), SWNTs (12.5 μg/mL), P1 + SWNTs, and P1 + SWNTs + thrombin (0.7 μM). (B) UV–visible–NIR absorption spectra of the free P5 (0.5 μM), SWNTs (12.5 μg/mL), P5 + SWNTs, and P5 + SWNTs + ATP (12.5 μM). Experimental conditions: incubation time 80 min; room temperature.
and P₅, which were labeled with FAM and ROX, respectively) were used. The DNA oligonucleotides sequence contained two parts: linker sequence and the anti-thrombin aptamer (or the anti-ATP aptamer). In the absence of targets (thrombin and ATP), the fluorescence of ROX and FAM was quenched due to the noncovalent assembly of SWNTs and dye-labeled DNA. In the presence of thrombin, the P₅ was bound to form G-quartets, and divorced from the surface of SWNTs, which lead to significant increase in the emission of FAM at 520 nm. From the photograph of Fig. 2(A), we could see the enhancement of yellow-green fluorescence. The restoration of fluorescence depended on the thrombin concentration, which was measured by fluorescence spectroscopy. Though thrombin (5.4 μM) can make the fluorescence of FAM increased, the fluorescence does not change while the concentration of thrombin was less than 200 nM. Similarly, though ATP (280 nM) made the fluorescence of ROX decreased, the fluorescence does not change while the concentration of ATP was less than 150 nM. And ATP bound P₅ to form the top G-quartet and double-stranded ribbonlike framework, resulting in the increase of the ROX fluorescence intensity at 590 nm (Fig. 2(B)). The concentration of ATP could be measured through the fluorescence enhancement at 590 nm.

The optical properties of the dispersed SWNT–aptamer solution were characterized by UV–vis–NIR absorption spectroscopy. Fig. 3(A) shows the UV–vis–NIR absorption spectra of the free state of P₁, P₁–SWNTs and P₁–SWNTs–thrombin. According to the extensive studies reported in the literature (Satake et al., 2005), three regions are identified in Fig. 3(A): UV absorption bands (260 nm) of DNA, Soret bands (486 nm) of FAM and NIR absorption bands (900–1200 nm) of SWNTs. While P₁ (0.5 μM) was mixed with the SWNTs (125 μg/ml), it became evident that the absorption band of FAM was red-shifted and the absorbance increased. The possible reason is that P₁ is wrapped on the SWNTs surface, indicating strong electronic interactions between the sapphyrin π surface and the nanotubes (Aminur et al., 2006; Ma et al., 2006; Zheng et al., 2003a,b). Simultaneity, the enhancement of absorbance in the NIR spectrum indicates that the electronic properties of SWNTs are retained after the DNA wrapping (Chen et al., 2009). After thrombin was incubated with the SWNTs–aptamer solution for 80 min, the absorbance decreased. It illustrated that the DNA disengages the sidewall of SWNTs and SWNTs gradually lost its aptamer-induced dispersion stability. Fig. 3(B) shows the UV–vis–NIR absorption spectra of the free state of P₁, P₁–SWNTs and P₁–SWNTs–ATP. The phenomenon of assay was similar with that of ATP–SWNTs–thrombin system. There was an absorption peak near 550 nm instead of 486 nm because P₁ was labeled by ROX.

Fluorescence anisotropy technique is commonly used to probe molecular interaction and molecular diffusion in solution (Yang et al., 2008a,b). To further understand interaction of between aptamer (DNA) and SWNTs or targets in solution, the fluorescence anisotropy of the aptamer–SWNTs–target system was measured (Table S1). The fluorescence anisotropy of the state of P₁–thrombin and P₁–SWNTs was 0.2668 and 0.2402, respectively. It was proved that P₁ more strongly interacted with thrombin than SWNTs. When thrombin was added to the solution, fluorescence anisotropy increased to 0.2638 compared with the state of P₁–SWNTs. It illustrated that both thrombin and SWNTs produced competition relationship and thrombin more easily bound with P₁, resulting in divorce of P₁ from SWNTs. Compared with thrombin–P₁–SWNTs system, the change (form 0.2016 to 0.2087) in the fluorescence anisotropy of ATP–P₁–SWNTs system was slight before and after the addition of ATP.

3.2. Optimization of assay condition

The SWNTs concentration is a key factor for this aptasensor, which decides on fluorescence quenching and restoration recovery. For P₁–SWNTs–thrombin aptasensor, while the SWNTs were 75 μg/ml (P₁ 0.2 μM), the ratio of F/F₀ (where F₀ and F are the fluorescence intensity without and with target, respectively) reached the largest value. For P₅–SWNTs–ATP aptasensor, the F/F₀ reached largest while the SWNTs were 100 μg/ml (P₅ 0.2 μM) (Fig. S2). In multiplex analyte experiments, 40 μL of 50 μg/ml SWNTs was recognized as the operational dosage. In addition, the quenching kinetics was fairly fast, with nearly 80% fluorescence quenching within only one min after adding SWNTs (Fig. S3). The fluorescence restoration was slow, especially to P₅–SWNTs–ATP system, in which it took 80 min to reach the top. The possible reason is that the release of the aptamer from SWNTs and binding of aptamer with target are relatively slow. So we chose 80 min as incubation time.

In the assay, there were two kinds of DNA including anti-thrombin aptamer (P₁, P₂, P₃ and P₄) and anti-ATP aptamer (P₅ and P₆) (see Table 1 for the sequence). For P₂, P₃ and P₄, which were all comprised of 15-mer anti-thrombin aptamer, we found that the longer linker-sequence led to the smaller quenching rate and the larger restoration. While the linker-sequence had six bases, the F/F₀ reached the largest. So we chose 6-mer linker sequence. The anti-thrombin aptamer has two different sequences: one is the 15-mer aptamer and the other is 29-mer aptamer (Macaya et al., 1993). P₁ is comprised with 29-mer aptamer, and P₃ is comprised with 15-mer aptamer. At the same time, P₁ and P₃ have the same linker sequence. The experimental results showed that the quenching rates of P₁ and P₃ were similar, but the restoration of P₁ was much larger than that of P₃. The possible reason is that P₁ may stack the top G-quartet with the two short stems in the presence of thrombin (Ikebukuro et al., 2005) and has larger steric hindrance, resulting in going farther away the SWNTs. On the other hand, we found that the types of base in the linker sequence had impact on fluorescent restoration. Through P₂ and P₃, we were identical except type of linker sequence, the fluorescent restoration of P₃ was much larger than that of P₂. The possible reason is that poly (T) and poly (C) are smaller free energies of binding to carbon nanotubes which was easier to disengage SWNTs into solution (Zheng et al., 2003a,b). In conclusion, the fluorescent quenching and restoration depend not only the length of DNA, but also the configuration of DNA.

To further study the conformations of DNA before and after SWNTs wrapping, circular dichroism (CD) was used. In Fig. S4(A), there are the positive and negative peak positions at about 265 nm and 240 nm because P₁ bound with thrombin to form the top G-quartet and the two short stems. The peak intensity of SWNTs–P₁ largely decreased at 265 nm compared with P₁. It is illuminated that P₁ wrapped on the surface of SWNTs. In the presence of thrombin, the peak intensity occurred to prominently increase. It is illuminated that thrombin bound with P₁ resulting in the more compact G-quartet formation. As shown in Fig. S4(B) and (C), there are a negative peak near 265 nm and a positive peak near 295 nm in the CD spectra, which is typical of an antiparallel quadruplex conformation. No appreciable change in their CD patterns is observed and the peak intensity only happened to appreciably increase at 295 nm, in the presence of thrombin. It is illuminated that the ability to disengage SWNTs of three sequences were weaker than P₁. This result accorded well with the above fluorescent assay. For the ATP system, the result was almost similar with that of P₁. There were the positive and negative peak positions at about 265 nm and 240 nm in the CD spectra (Fig. S4(E) and (F)), which indicated that P₁ form the top G-quartet and double-stranded ribbonlike framework.

3.3. Sensitivity and selectivity

We first constructed and tested the single-color aptasensor. In the absence of targets, the probes showed small background fluorescence, due to the strong quenching of SWNTs. In the presence
of thrombin, thrombin bound with its aptamer (P1), resulting in significant fluorescent increase of FAM. As shown in Fig. S5(A), the fluorescence intensity increased with increasing thrombin concentration, and the sensor for thrombin had a linear range of 5.4–270 nM with the detection limit of 1.6 nM (3σ). Similarly, for SWNTs–P5 (ATP’s aptamer) system, the addition of ATP resulted in the fluorescent increase of ROX (Fig. S5(B)). The sensor for ATP had a linear range of 0.28–28 nM with the detection limit of 0.1 nM (3σ).

The performance of the multicolor aptasensor was evaluated by excitation at 470 nm (maximum excitation wavelength of FAM), 530 nm (maximum excitation wavelength of ROX), respectively. Upon the addition of specific targets to the probe containing P1 and P5, we observed the emission from the corresponding wavelength. As shown in Fig. 4(A), in the presence of thrombin, the binding of thrombin with its aptamer (P1) led to the significant increase of FAM emission at 520 nm, whereas the emission of ROX was very small. As shown in Fig. 4(B), in the presence of ATP, the binding of ATP with its aptamer (P5) resulted in the significant increase in ROX emission at 590 nm. The detection limits were 1.5 nM for thrombin and 2.0 nM for ATP. Compared with the corresponding results for single detection, the detection limits of the multicolor aptasensor were inferior.

To evaluate the specificity of the multicolor aptasensor, several analogue molecules of the target were chosen to study. We found that the specific targets led to fluorescent recovery of their corresponding dyes, while all the analogue molecules led to only minimal fluorescent recovery. As shown in Fig. 5(A), a significant fluorescent increase is observed for thrombin (0.05 μM) and the change of fluorescence (F/F0 – 1) is 0.583, which is much higher than that of 0.033 for BSA (5 μM), 0.046 for IgG (5 μM), 0.019 for lysozyme (5 μM) and 0.013 for ATP (5 μM). Compare to thrombin aptasensor, ATP aptasensor also showed good specificity. However, because ATP and AMP respond to the anti-ATP aptamer, the F/F0 – 1 is much higher than others. These results demonstrate the high specificity of our aptasensor.

4. Conclusions

In this work, we combine the highly specific binding ability of aptamers with the ultrahigh quenching ability of SWNTs to develop a multicolor fluorescent sensing system. This multicolor fluorescent system is used to simultaneously detect thrombin and ATP in a single solution. This assay has several important features. First, use of water-soluble carboxyl-modified SWNTs offers a convenient “mix–and-detect” approach for the rapid detection in aqueous solutions at room temperature. Second, use of aptamer imparts high selectivity to this sensor. Third, compared with the single-target assay, this multiplex assay has the ability to detect for multiple analytes in a single solution, which has the significant advantage for rapid, simple, low sample and reagent consumption. Of
course, this strategy can be easily extended to designing aptamer-based system for simultaneous multianalysis of various protein and small-molecule analytes.

Acknowledgements

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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.bios.2011.01.035.

References