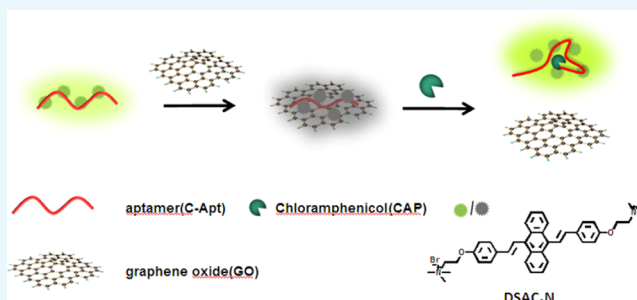


Label-Free Aptamer-Based Biosensor for Specific Detection of Chloramphenicol Using AIE Probe and Graphene Oxide

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ABSTRACT: A facile, sensitive, and label-free aptamer-based fluorescent biosensor (aptasensor) for chloramphenicol (CAP) detection was successfully developed based on an aggregation-induced emission (AIE) probe and graphene oxide (GO). In this aptasensor, the specific aptamer of CAP (C-Apt) is used as the recognition part, an AIE molecule, 9,10-distyrylanthracene (DSA) derivative with short alkyl chains (9,10-bis{4-[2-(*N,N,N*-trimethylammonium)-ethoxy]-styrene}anthracene dibromide, DSAC₂N), as the fluorescent probe, and GO with a low oxidation degree as the fluorescent quencher. Initially, the AIE probe DSAC₂N and C-Apt could be adsorbed on GO through π -stacking interactions, and the fluorescence of DSAC₂N could be efficiently quenched due to the energy transfer between DSAC₂N and GO. When CAP is added, C-Apt can preferentially bind with CAP and the newly formed complex (C-Apt–CAP) can be released from GO, resulting in the recovery of the fluorescence signal of DSAC₂N. Thus, with the aid of GO, turn-on detection of CAP can be readily realized by monitoring the fluorescence signal of DSAC₂N from “off” to “on”. Under the optimized conditions, the aptasensor exhibits a high sensitivity toward CAP with a limit of detection of 1.26 pg/mL. Besides, we have successfully applied this aptasensor to the detection of CAP in spiked milk.



1. INTRODUCTION

As a broad-spectrum antibiotic, chloramphenicol (CAP) has been widely used in the field of treatment of a variety of bacterial infections.^{1,2} However, CAP is easy to accumulate in liver once it is absorbed into the body from food and medicine, resulting in many serious side effects on human beings, such as leukemia, gray baby syndrome, and aplastic anemia.^{3,4} And the European Union has established the “minimum required performance limit” value of CAP as 0.3×10^{-6} g/kg for the detection of its residues in food products. Therefore, it is of considerable important to develop ultrasensitive approaches for CAP determination.

Traditionally, CAP residues were mainly detected by gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, high-performance liquid chromatography (HPLC) with excellent sensitivity and reliability.^{5–7} However, the above methods usually require expensive equipments and complicated operation procedures. Then, some detection methods based on immunoassay are developed with higher specificity, shorter analysis time, and simple sample processing.^{8–10} Nevertheless, these detection methods generally show some limitations such as poor sensitivity, accuracy, and stability. Therefore, it is great important to develop an efficient, simple, economic, and sensitive method for CAP detection.

In recent years, aptamer-based sensors, aptasensors, have been widely developed and used in analytical methods. Aptamers are short single-strand DNA or RNA molecules that are experimentally selected by an in vitro technique called

systematic evolution of ligands by exponential enrichment.^{11,12}

They can be used as recognition reagents and bind to various targets with high specificity and binding affinity. Owing to the superior characteristics of easier modification, low cost, and better stability over antibodies, numerous aptasensors for CAP detection including fluorescent biosensor, colorimetric assay, electrochemical analysis, and surface-enhanced Raman scattering are constructed.^{13–17} Among these methods, fluorescent biosensors have attracted increasing attention due to their characteristics of simplicity, high specificity, and high sensitivity. And various fluorophores, such as inorganic quantum dots and organic molecules, have been developed as fluorescent probes for sensing platform. However, most of the biosensors require dye-labeled or modified aptamers, which have several critical defects of complicated operation, low labeling efficiency, and expensive cost. Additionally, the fluorescence emission of most traditional fluorophores is often quenched when bound with the molecules, which can be called aggregation-caused quenching (ACQ),^{18,19} and compel the biosensors to operate in a fluorescent “turn-off” mode.

The appearance of aggregation-induced emission (AIE) fluorescent probes effectively solves the ACQ problem of traditional organic probes and provides new approaches and methods for the development of “turn-on” and label-free fluorescent biosensors.^{20–22} To be specific, AIE-active

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Scheme 1. Sensing Principle for CAP Detection

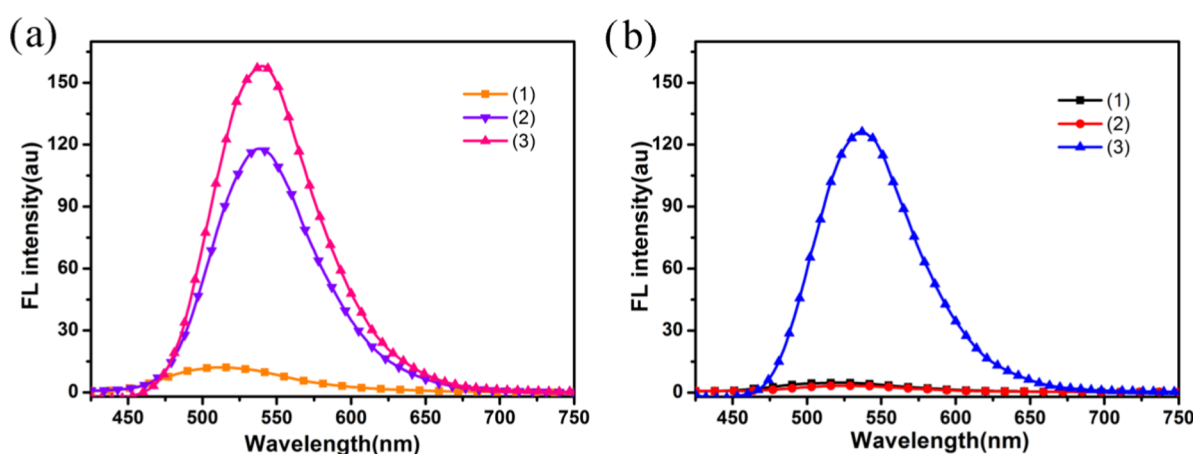
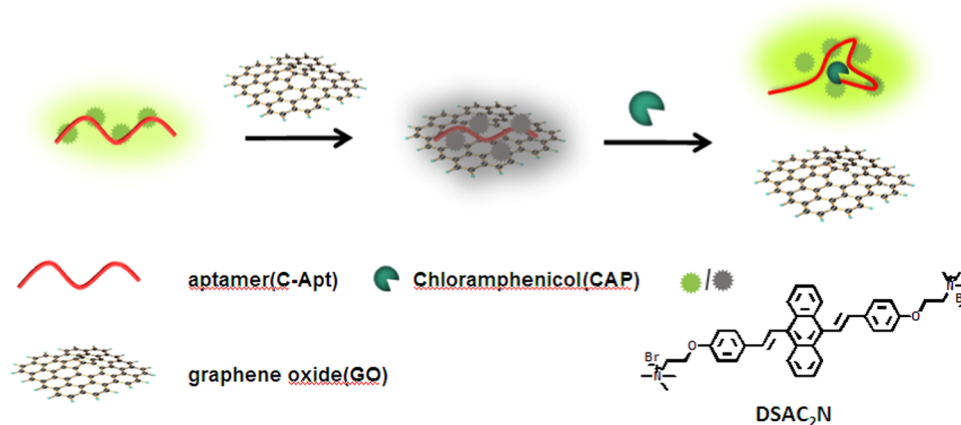


Figure 1. Fluorescence emission spectra of DSAC₂N (10 μM) in the absence (a) and presence (b) of GO. (1) DSAC₂N in buffer, (2) DSAC₂N + C-Apt, and (3) DSAC₂N + C-Apt + CAP.

molecules are almost nonemissive in dilute solution states but show strong fluorescence emission in the aggregated states, resulting in a turn-on fluorescent signal with better accuracy and higher sensitivity for the sensing platform.^{23,24} Based on the characteristic, a series of AIE fluorophores have been designed and utilized as fluorescent indicators in biosensors. However, it is a pity that most of the AIE-based sensors have no selectivity. Because the probe and target molecules are usually combined through electrostatic or hydrophobic interactions, AIE probes will also have the similar fluorescence response to other interferent in addition to the target molecules, which seriously affects the selectivity of the sensing platform.

Graphene oxide (GO), a novel two-dimensional carbon nanomaterial with unique physicochemical properties, shows an advantageous application prospect in the fabrication of electrochemical, colorimetric, and fluorescent aptasensors because of its excellent biocompatibility, water solubility, and fluorescent quenching ability. Especially, it can interact with various inorganic, organic, and biological molecules through covalent, noncovalent, or electrostatic interactions, resulting in higher-sensitivity and -selectivity aptasensors by introducing it to the sensing platform.^{25,26} Inspired by these studies, our group constructed several fluorescent aptasensors by integrating AIE probes and GO, and we found that the analytical performance of the assay can be significantly improved by

tuning the supramolecular interactions of AIE probe and GO with DNA.²⁷

Herein, we developed a sensitive, facile, and turn-on fluorescent aptasensor for CAP detection by combining an AIE DSA derivative with short alkyl chains (DSAC₂N) as the fluorescent probe, the highly specific aptamer as the recognition element and GO with low oxidation degree as the fluorescence quencher. Upon the addition of CAP, the fluorescence signal of the sensing platform can turn from “off” to “on”, and the quantification of CAP can be consequently realized with easy operation, low cost, favorable stability, and high selectivity and sensitivity. Moreover, the assay approach exhibited excellent analytical performance during the determination of CAP in milk samples.

2. RESULTS AND DISCUSSION

2.1. Sensing Strategy. The sensing strategy of the fluorescent biosensor based on probe DSAC₂N and GO is illustrated in Scheme 1. The fluorescent probe DSAC₂N has good water solubility and shows typical AIE properties. It has almost no fluorescence in the dispersion state. After adding the aptamer of CAP (C-Apt), the fluorescence of DSAC₂N can be enhanced due to the formation of the aggregation complex (DSAC₂N/C-Apt). The cation probe DSAC₂N can bind with C-Apt through electrostatic attraction and hydrophobic interaction, resulting in the formation of DSAC₂N/C-Apt

complex and the fluorescence enhancement because of the restricted intramolecular torsion between the 9,10-anthylene core and the vinylene segment of DSAC₂N in the aggregate state. When the GO is introduced, the complex DSAC₂N/C-Apt can be adsorbed on the surface of GO due to the hydrogen bond and the π - π stacking interaction with GO, resulting in efficient fluorescence quenching of DSAC₂N via the fluorescence resonance energy transfer (FRET) from DSAC₂N to GO. When the target molecule CAP is added, C-Apt can specifically bind with CAP and form the C-Apt-CAP complex, and the tertiary structure of C-Apt changes accordingly. Consequently, C-Apt-CAP can be released from GO due to the weak interaction between them. Meanwhile, DSAC₂N can be still adsorbed on C-Apt-CAP, and the fluorescence of the solution will be gradually recovered due to the aggregation of the probe. Therefore, by monitoring the changes in the fluorescence signal of DSAC₂N with CAP addition (from off to on), we can easily realize the supersensitive detection of CAP.

2.2. Fluorescent Response of the Probe. The fluorescent emission spectra of DSAC₂N (10.0 μ M) under different conditions are illustrated in Figure 1. In the case of without GO, the fluorescence intensity of DSAC₂N/C-Apt-CAP complex (spectrum (3)) is somewhat higher than that of DSAC₂N/C-Apt complex (spectrum (2)) (Figure 1a). The small fluorescence intensity difference indicates that the background noise of the system is too big, which severely limits the sensitivity of the sensing platform for the detection of CAP. Surprisingly, when introducing GO into the sensing platform, as shown in Figure 1b, the fluorescence of both DSAC₂N in dispersed state (spectrum (1)) and DSAC₂N/C-Apt complexes (spectrum (2)) is quenched by GO. After the addition of CAP, most of the C-Apt molecules detach from GO due to the formation of the C-Apt-CAP complex, leading to an obvious increase in the fluorescence of DSAC₂N (spectrum (3)). The sensing platform based on GO, aptamer, and AIE probe can successfully distinguish the target substance, CAP, due to the obvious difference in fluorescence intensity before and after adding the target substance.

2.3. Feasibility of the Fluorescent Aptasensor. To investigate the quenching ability of GO, we investigated the fluorescence quenching kinetics in the sensing platform. As shown in Figure 2, when DSAC₂N was combined with C-Apt, the solution had a strong fluorescence emission. With the continuous addition of GO, the fluorescence intensity of the solution dropped sharply and was quenched rapidly. The double logarithm regression curve of DSAC₂N and GO is shown in Figure 3. The binding constant K_a of DSAC₂N and GO was calculated to be 13.10 L/g according to the double logarithm regression equation.²⁸

On the other hand, the binding affinity of the formed C-Apt-CAP with DSAC₂N was investigated, so that the supramolecular interactions within the aptasensor were explored. The binding constant of DSAC₂N with C-Apt-CAP complex was evaluated through a fluorescence titration experiment. It can be found that the fluorescent intensity of DSAC₂N increases gradually with the increasing amounts of the C-Apt-CAP complex (Figure 4a). Figure 4b shows the Benesi-Hildebrand plot of DSAC₂N and the C-Apt-CAP complex, and the binding constant K_b of DSAC₂N and C-Apt-CAP was calculated to be 18.40 L/g.²⁹ It is clear that $K_b > K_a$, indicating that the binding capacity between DSAC₂N and C-Apt-CAP complex is stronger than that between DSAC₂N and

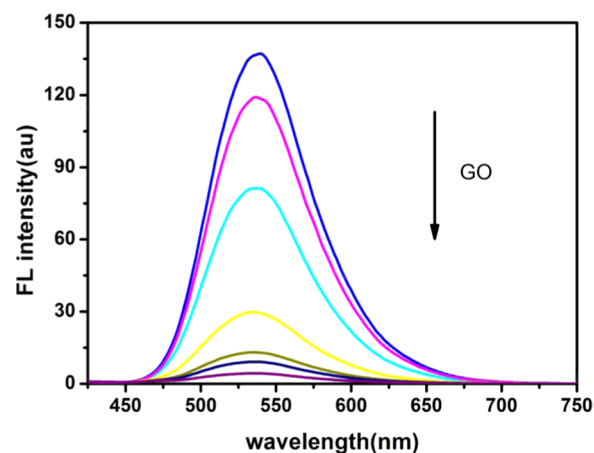


Figure 2. Fluorescence spectrum of DSAC₂N/C-Apt under different concentrations of GO. Experimental conditions: 10 μ M DSAC₂N, 150 nM C-Apt, 3–25 μ g/mL GO, 20 mM Tris-HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm.

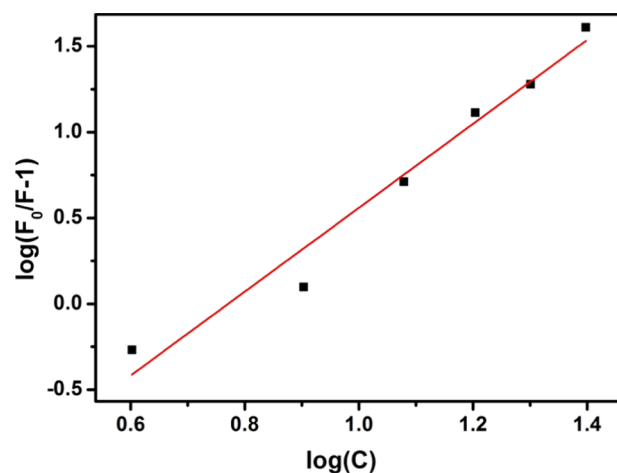


Figure 3. Double logarithm regression curve of DSAC₂N and GO. Experimental conditions: 150 nM C-Apt, 10 μ M DSAC₂N, 3–25 μ g/mL GO, 20 mM Tris-HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm.

GO, ensuring that DSAC₂N can aggregate on the C-Apt-CAP complex and light the fluorescence signal of DSAC₂N sufficiently.

2.4. Sensitivity of the Fluorescent Aptasensor. The fluorescent spectra of DSAC₂N in the presence of different CAP concentrations were recorded, so that the sensitivity of the fluorescent aptasensor was investigated. As illustrated in Figure 5a, with increase in the CAP concentration, the fluorescent intensity of DSAC₂N is gradually enhanced, indicating that more and more C-Apt-CAP complexes were formed in the system, and the aggregation degree of DSAC₂N gradually increased, resulting in gradual enhancement in the fluorescence signal. Figure 5b shows the relationship between the fluorescent intensity of DSAC₂N and the concentration of CAP, and the inset in Figure 5b exhibits the linear part of this relationship at the low concentration of CAP. It can be seen that when the concentration of CAP reaches 10 ng/mL, the fluorescence intensity of the solution no longer increases greatly and gradually tends to be stable, indicating the interaction between C-Apt and CAP gradually tends to balance and saturate; accordingly, the combination of DSAC₂N and C-

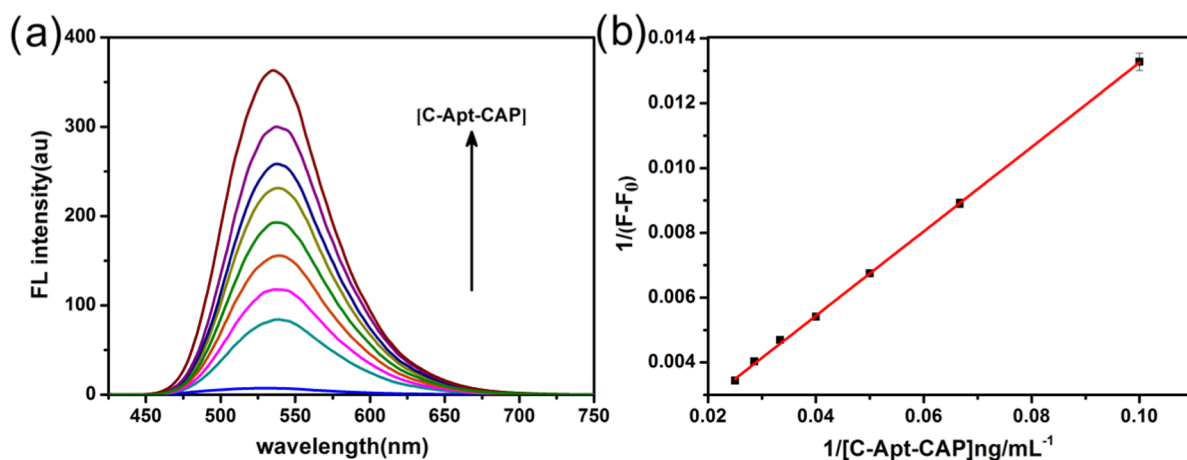


Figure 4. (a) Fluorescence spectra of DSAC₂N in the presence of increasing amounts of C-Apt–CAP complex. (b) Benesi–Hildebrand plot of DSAC₂N and C-Apt–CAP complex. Experimental conditions: 10 μ M DSAC₂N, 0–60 ng/mL C-Apt–CAP, 20 mM Tris–HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm.

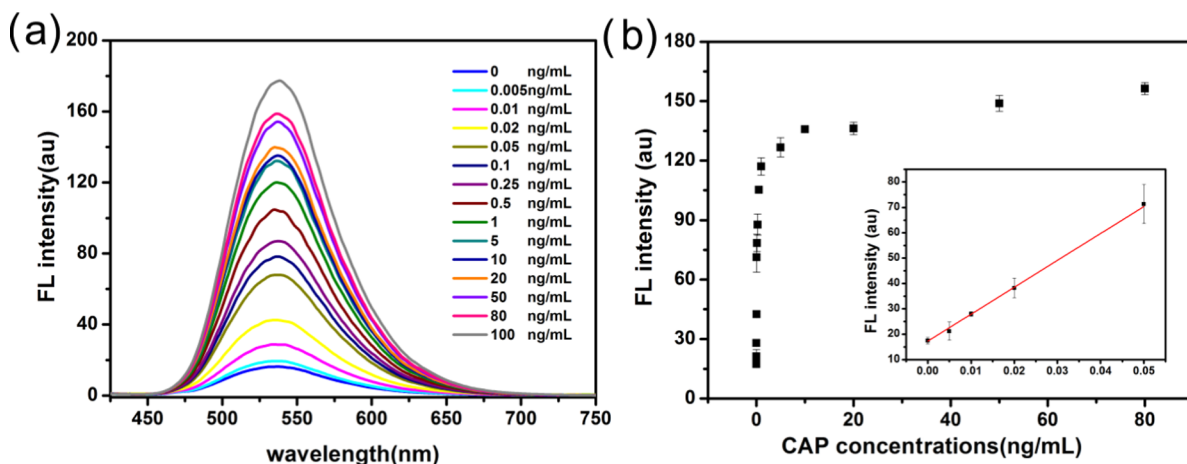


Figure 5. (a) Fluorescence emission spectra of DSAC₂N in the presence of different concentrations of CAP. (b) Plot of fluorescence intensity at 535 nm vs CAP concentration. Inset: linear part of the plot in (b). Experimental conditions: 10 μ M DSAC₂N, 9 μ g/mL GO, 150 nM C-Apt, 0–100 ng/mL CAP, 20 mM Tris–HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm.

Apt–CAP is saturated gradually. The limit of detection (LOD) was calculated to be 1.26 pg/mL ($\text{LOD} = 3\sigma/\kappa$, where σ is the standard deviation of the three blank measurements (without CAP) and κ is the slope of the fluorescence intensity of DSAC₂N relative to the CAP concentration).

2.5. Selectivity of the Fluorescent Aptasensor. The selectivity of the sensing platform to CAP was investigated. The controlled experiments were carried out under the same experimental conditions by selecting several common antibiotics such as chlortetracycline (CTC), thiamphenicol (TAP), florfenicol (FF), tetracycline (TC), ampicillin (APC), kanamycin (Kana), bovine serum albumin (BSA), and oxytetracycline (OTC) as the interfering molecules (Figure 6). We can find that only in the presence of CAP, the fluorescence intensity of DSAC₂N has a great enhancement. Nevertheless, the fluorescence intensity of DSAC₂N is very weak when adding the above interfering molecules instead of CAP, indicating that these interfering molecules cannot form a complex with C-Apt and accordingly cannot bring obvious effect to the raising of the fluorescence intensity of DSAC₂N. It strongly confirms that this sensing platform has good selectivity to CAP.

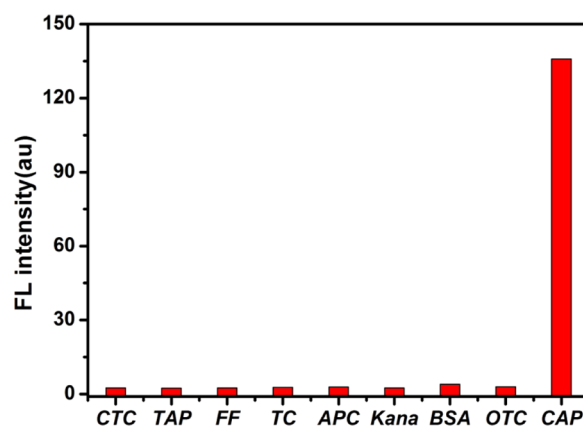


Figure 6. Fluorescent spectra of DSAC₂N in the presence of different interfering molecules. Experimental conditions: 10 μ M DSAC₂N, 9 μ g/mL GO, 150 nM C-Apt, 10 ng/mL CAP, interfering molecules, 20 mM Tris–HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm.

We also listed some literature reports about fluorescent biosensors for detecting CAP in Table 1. As to the selectivity, we investigated more kinds of interfering molecules than the

Table 1. Comparison of Fluorescent Biosensors for CAP Detection in Selectivity, Sensitivity, Biosensor Structure, and Fluorescent Operation Mold

no.	selectivity	sensitivity (LOD) (pg/mL)	structure and operation	reference
1	have a selectivity to CAP, no selectivity to thiamphenicol (TAP), florfenicol (FF), chlortetracycline (CTC), tetracycline (TC), ampicillin (APC), kanamycin (Kana), bovine serum albumin (BSA) and oxytetracycline (OTC)	1.26	based on aptamer, an AIE probe and GO (fluorescent turn-on probe)	this work
2	have a selectivity to CAP, no selectivity to TAP and FF	100	based on aptamer and realtime fluorescent quantitative polymerase chain reaction	Talanta, 2017, 165, 671
3	have a selectivity to CAP, no selectivity to TAP, nitrofurantoin and metronidazole	31.7	based on aptamer, CdTe quantum dots and GO (fluorescent turn-on probe)	Mat. Sci. Eng. C, 2015, 48, 611
4	have a selectivity to CAP, no obvious selectivity to TAP, FF, Kana and streptomycin sulfate	10	based on aptamer, conjugated magnetic nanoparticles and complementary DNA modified with upconversion nanoparticles (fluorescent turn-off probe)	Food Control, 2015, 50, 597
5	have a selectivity to CAP, no selectivity to TAP FF, Kana, OTC, Streptomyces erythreus (SE)	2	based on aptamer, complementary strand DNA, double-stranded DNA antibody and CdSe quantum dot probes (fluorescent turn-on probe)	Anal. Methods, 2016, 8, 3006
6	have a selectivity to CAP, no selectivity to CTC, Kana, OTC, streptomyces erythreus (SE) and gentamicin sulfate	0.32	based on magnetic aptamer, single-stranded DNA binding protein and DIL coimmobilized liposome vesicle probe (fluorescent turn-on probe)	Biosens. Bioelectron., 2016, 81, 454

cited articles, and our fluorescent biosensor exhibited a good selectivity to CAP against eight kinds of interfering molecules. Moreover, the LOD of our fluorescent biosensor is much lower than that of the cited articles except for the last one. In addition, the fluorescent biosensor in this work is much simpler, of lower cost, and more environment-friendly than those in the cited articles because they either need modified DNA or aptamer (nos 4 and 6) or need materials containing heavy metal (nos 3 and 5).

2.6. Detection of CAP in Milk Samples. To evaluate the feasibility of the fluorescent aptasensor in practical applications, we detect the CAP in milk by adding different concentrations of CAP solutions into the diluted milk samples (5%) under the same conditions. As shown in Figure 7a, when the milk sample was used as the matrix, the fluorescence of DSAC₂N was much stronger and the fluorescence peak blue-shifted about 20 nm than that in the buffer solution. It indicates that DSAC₂N molecules interact with other substances in the milk sample at the same time, which changes the molecular energy levels of DSAC₂N, so the emission peak of DSAC₂N in the buffer solution has a blue-shift compared with that in the milk. Figure 7b shows the fluorescence response of DSAC₂N at different CAP concentrations. It is clear that with the increasing concentration of CAP, the fluorescence intensity of the system increased steadily. The detection limit was calculated to be 0.36 ng/mL. These results confirm the feasibility and reliability of our sensing platform in monitoring CAP in real samples.

3. CONCLUSIONS

A facile, sensitive, and label-free fluorescent aptasensor was developed by using an AIE molecule DSAC₂N as the fluorescence probe, the low oxidation degree GO as the fluorescence quenching agent and specific nucleic acid aptamer C-Apt as the recognition part, realizing the ultrasensitive and specific detection of CAP. In absence of CAP, C-Apt and DSAC₂N can be adsorbed on GO, and the fluorescence of DSAC₂N is quenched by GO due to the FRET interaction between DSAC₂N and GO, and the solution exhibits no fluorescence. After adding CAP, a C-Apt–CAP complex is formed by the virtue of the specific binding of C-Apt and CAP, resulting in the leaving of C-Apt from the surface of GO. As the binding force between DSAC₂N and C-Apt–CAP is stronger than that between DSAC₂N and GO, DSAC₂N separates from the GO and aggregated on the surface of the C-Apt–CAP complex, and accordingly the fluorescence of the solution turns on. The results indicate that C-Apt cannot specifically bind with other antibiotics, so the fluorescent biosensor platform has a good selectivity to CAP. Moreover, we also applied this fluorescent aptasensor to the detection of CAP in milk samples. The sensing strategy can be applied to other target molecules detection thanks to its advantages of simple operation, high sensitivity, strong specificity, and excellent universality.

4. EXPERIMENTAL SECTION

4.1. Materials. All the reagents and starting materials were commercially available and were used without further purification. The detailed synthesis and characterization of DSAC₂N has been reported in our previous work.²⁷ GO was also obtained from our previous work.²⁷ Chloramphenicol (CAP), tetracycline (TC), thiamphenicol (TAP), and ethanol

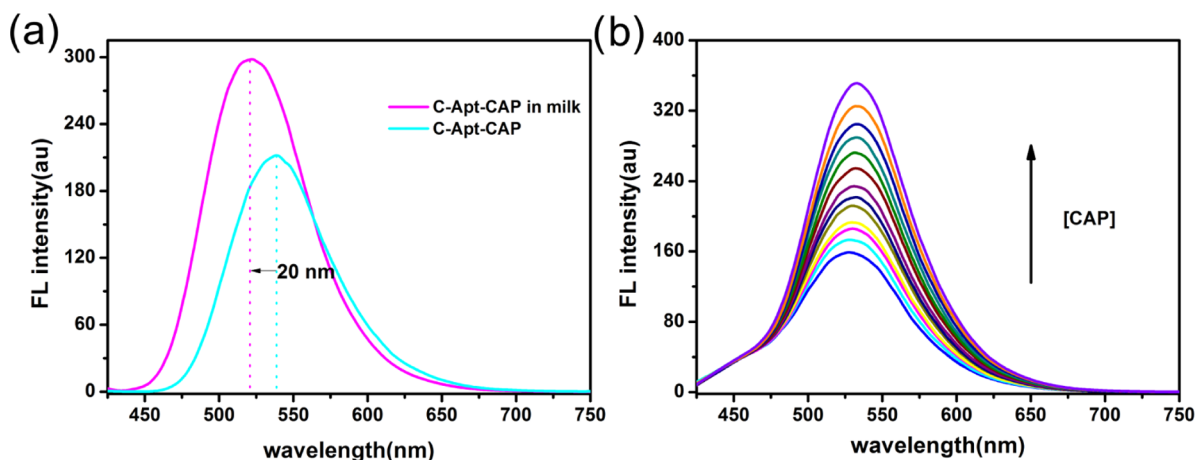


Figure 7. (a) Fluorescence spectra of DSAC₂N (10 μ M) in the presence of CAP in buffer solution and in 5% milk, respectively. Experimental conditions: 10 μ M DSAC₂N, 9 μ g/mL GO, 150 nM C-Apt, 10 ng/mL CAP, 20 mM Tris–HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm. (b) Fluorescence spectra of DSAC₂N in the 5% milk with different concentrations of CAP solutions. Experimental conditions: 10 μ M DSAC₂N, 9 μ g/mL GO, 150 nM C-Apt, 0–20 ng/mL CAP, 20 mM Tris–HCl buffer solution (pH = 7.4), and λ_{ex} = 405 nm.

were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Oxytetracycline (OTC), chlortetracycline (CTC), and kanamycin (Kana) were obtained from Sigma-Aldrich (St. Louis, MO). Florfenicol (FF) was bought from Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China). Ampicillin (APC) was obtained from Beijing J&K Scientific Co., Ltd. (China). Tris ($\text{C}_4\text{H}_{11}\text{NO}_3$) and the specific aptamer C-Apt were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The production process of C-Apt was reported in the literature.³⁰ C-Apt has a sequence of 5'-ACT TCA GTG AGT TGT CCC ACG GTC GGC GAG TCG GTG GTAG-3' and was purified by using HPLC. The crystal of C-Apt was dissolved in deionized water and the concentration of the C-Apt solution was measured by UV–vis absorption spectroscopy. The C-Apt stock solution was stored at 4 $^{\circ}\text{C}$ before use. All the other chemicals were purchased as analytical grade from either Beijing Chemical Reagent Co. (China) or Sinopharm Chemical Reagent Co., Ltd. The stock solution of CAP (5 μ g/mL) was prepared by dissolving CAP in ethanol and stored at 4 $^{\circ}\text{C}$. Buffer solution used in this work is Tris–HCl buffer solution (10 mM, pH = 7.4). Deionized water (18.2 M Ω cm resistivity) from a Milli-Q water system was used throughout the whole experiments.

4.2. Experimental Instrument. Fluorescence spectra were recorded out on a Shimadzu RF-5301PC spectrophotometer. All the spectra were taken at ambient temperature. UV–vis absorption spectra were recorded on a Lambda-800 spectrophotometer. The measurement of pH value was carried out with an FE20 pH meter (Mettler-Toledo, Switzerland).

4.3. Detection of CAP. C-Apt (150 nM) and GO (9 μ g/mL) were dispersed in the buffer solution of Tris–HCl (20 mM, pH = 7.4) at room temperature. After the solution was mixed, CAP (10 ng/mL) was added to the mixture. After 1 h of incubation at 37 $^{\circ}\text{C}$, the solution was allowed to slowly cool to room temperature. Eventually, DSAC₂N (10 μ M) was added to the solution (the total volume of deionized water was 1000 μ L) to measure the fluorescence spectra.

4.4. Fluorescence Quenching. C-Apt (150 nM) and DSAC₂N (10 μ M) were dispersed in the buffer solution of Tris–HCl (20 mM, pH = 7.4) at room temperature. After the solution was mixed, different concentrations of GO (3, 6, 9, 12,

15, 20, and 25 μ g/mL) were added to the mixture (total volume = 1000 μ L) to measure the fluorescence spectra.

4.5. Fluorescence Titration. C-Apt and CAP complex solutions (mixed at a molar ratio of 1:1 and incubated at 37 $^{\circ}\text{C}$ for 1 h in the buffer solution of Tris–HCl (20 mM, pH = 7.4)) with different concentrations (0, 10, 15, 20, 25, 30, 35, 40, and 60 ng/mL) were added to the DSAC₂N (10 μ M) solution (total volume = 1000 μ L) to measure the fluorescence spectra.

4.6. Sensitivity Experiment. Different concentrations of CAP (0, 0.005, 0.01, 0.02, 0.05, 0.1, 0.25, 0.5, 1, 5, 10, 20, 50, 80, and 100 ng/mL) were added to the buffer solution of Tris–HCl (20 mM, pH = 7.4) containing C-Apt (150 nM) and GO (9 μ g/mL), incubated at 37 $^{\circ}\text{C}$ for 1 h, and the solution was slowly cooled to room temperature. Then, DSAC₂N (10 μ M) was added to the mixtures (total volume = 1000 μ L) to measure the fluorescence spectra.

4.7. Selective Analysis. C-Apt (150 nM) and GO (9 μ g/mL) were dispersed in the buffer solution of Tris–HCl (20 mM, pH = 7.4) at room temperature. After the solution was mixed, different antibiotics of chloramphenicol (CAP), chlortetracycline (CTC), thiamphenicol (TAP), florfenicol (FF), tetracycline (TC), ampicillin (APC), kanamycin (Kana), bovine serum albumin (BSA), and oxytetracycline (OTC) (10 nM) were added to the solution, incubated at 37 $^{\circ}\text{C}$ for 1 h, and the solution was slowly cooled to room temperature. Then, DSAC₂N (10 μ M) was added to the mixtures (total volume = 1000 μ L) to measure the fluorescence spectra.

4.8. Detection of CAP in Milk Samples. DSAC₂N (10 μ M), GO (9 μ g/mL), and C-Apt (150 nM) were dispersed in the buffer solution of Tris–HCl (20 mM, pH = 7.4) at room temperature and stirred to a mixture solution. Ten milliliters of milk sample was centrifugated at 7000 rpm for 10 min at 10 $^{\circ}\text{C}$. The fat layer was removed and the supernatant was diluted 20 times with ultrapure water. Then, the dilute solution was filtered with a disposable filter head (0.22 μ m). The CAP solution of different concentrations (0, 0.01, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10, and 20 ng/mL) was added to the mixture solution, incubated at 37 $^{\circ}\text{C}$ for 1 h, and the solution was slowly cooled to room temperature to record the fluorescence spectra.

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Notes

The authors declare no competing financial interest.

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