A Fluorescence Resonance Energy Transfer-Based Molecular Probe for Cisplatin Detection

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***Abstract*—Cisplatin, one of the most widely used clinical medications in the field of cancer chemotherapy, which is an indispensable drug type in traditional chemotherapy. Due to the significant toxicity and side effects of typical chemotherapy medications, precise drug concentration monitoring becomes the key. However, because of individual variances, various individuals' metabolic capacities to medications vary, resulting in differences in actual blood drug concentration in the body under the same dosage. Therefore, it is imperative to create a new low-cost and rapid detection method for the concentration of serum active cisplatin, which is crucial to the chemotherapy effect and quality of life for cancer patients. In this work, based on the chemically cross-linked reaction between active cisplatin and DNA bases, as well as the high sensitivity of Fluorescence Resonance Energy Transfer (FRET), we proposed a new FRET-based method for detecting serum active cisplatin concentration. We constructed a fluorescent molecular probe with a dumbbell shape consisting of graphene quantum dots (GQDs) modified with carboxyl, gold nanoparticles (AuNPs), and a single-stranded DNA (ssDNA) sequence. The results reveal that the molecular probe has a strong linear correlation across the active cisplatin concentration range of 52-832 μM. This method can accomplish low-cost, rapid and simple detection of active cisplatin concentration, and further advancements in this work can give a decision-making basis for tailored and accurate cancer treatment.**

***Keywords*—Active cisplatin, FRET, Molecular probe, Concentration detection**

I. Introduction

Cisplatin is one of the most commonly utilized chemotherapeutic medications that produces a cross-link by binding to specific bases in the DNA of rapidly dividing cancer cells. This cross-link can induce damage and disruption of DNA replication and transcription by altering the typical double helix shape within DNA so that cisplatin can treat cancer by effectively inhibiting the division and proliferation of cancer cells. As a result, people use cisplatin clinically as a first-line anticancer agent for the treatment of testicular cancer, ovarian cancer and other malignant tumors [1]. However, individual disparities in metabolic capabilities amongst patients are noticeable. Cisplatin at high concentration will create gastrointestinal side effects, whereas cisplatin at low concentration will affect the chemotherapeutic effect. Simultaneously, cisplatin has substantial side effects including ototoxicity, bone marrow suppression and neurotoxicity, with large individual variability in the occurrence of these side effects during different chemotherapy cycles. Several approaches for detecting cisplatin have been developed, such as colorimetric [2], inductively coupled plasma mass spectrometry (ICP-MS) [3], high performance liquid chromatography (HPLC) [4] and improved schemes based on above three methods [5] [6]. However, these approaches are high-interference, expensive with advanced analytical instruments, and require extensive sample pre-treatment, which cannot meet the demand for clinical sample detection. Hence, it is highly desirable to develop a real-time, rapid and novel approach for detecting cisplatin in patients' serum during various chemotherapy cycles, allowing clinicians to develop precise and individualized dosing regimens.

In recent years, FRET-based approaches for detecting heavy metal ions, small molecules and organic macromolecules have aroused enormous interest owing to their simplicity, high sensitivity, selectivity and low cost [7]–[9]. Herein, we present a novel FRET molecular probe for the first time, using GQDs, AuNPs and ssDNA connected to the middle of them, for the detection of cisplatin. The detection mechanism is based on cisplatin-induced fluorescence quenching caused by the presence of GQDs and AuNPs in close proximity. The proposed FRET molecular probe in our work basically achieves a rapid and simple quantitative analysis of cisplatin.

In this work, the ssDNA strand was employed as the medium to join AuNPs and GQDs in the FRET molecular probe. Based on the intense fluorescence of GQDs, fluorescence quenching between AuNPs and GQDs is used to quantify the concentration of cisplatin. Fig. 1(a) shows the design principle of cisplatin detection based on the FRET molecular probe. Here, the freezing method was used to modify the AuNPs with the ssDNA strand. [10] [11]. Then, the GQDs were linked to ssDNA-AuNPs by 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC). After thoroughly mixing the solution, we can obtain the FRET molecular probe. Since the length of the ssDNA strand was around 20 nm, the GQDs and AuNPs at the end of the FRET molecular probe did not generate the FRET effect (We call this state "FRET off"). Following that, as presented in Fig. 1(b), the addition of cisplatin will form mono adducts, diadducts and cross-linked adducts on the purine bases of ssDNA, causing a considerable bending of the ssDNA into rings and therefore shortening the distance between GQDs and AuNPs [12]. When the distance between AuNPs and GQDs was smaller than 10

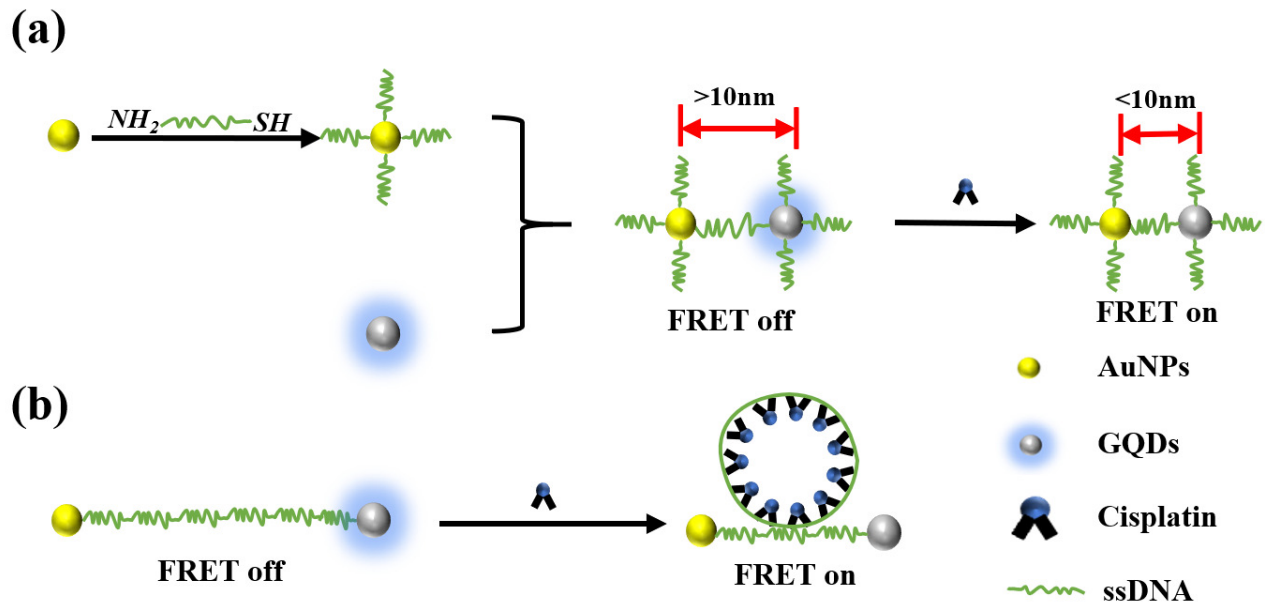

Fig. 1. (a) Design principle of FRET molecular probe for cisplatin detection; (b) Schematic illustration of cisplatin interaction with ssDNA leads to FRET effect between GQDs and AuNPs.

nm, a relatively significant FRET effect was formed, resulting in fluorescence quenching emitted by GQDs (We call this state "FRET on"). Based on the relationship between the intensity of fluorescence emission of the FRET molecular probe and the concentration of introduced cisplatin, a simple and efficient detection approach of cisplatin can be achieved.

II. Materials and Methods

A. Experimental materials

In our research, sodium citrate solution, Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), $FeCl_3$, $CuCl_2$ and etoposide were all bought from Sigma-Aldrich (St. Louis, MO, USA). Gemcitabine and paclitaxel were bought from Aladdin (Shanghai, China). EDC was bought from Hebei Bailingway Superfine Material Co. (Langfang, China). $AgNO_3$ was bought from Acros Organics (Belgium). Carboxylated GQDs were bought from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). The Cisplatin injection was bought from Jiangsu Haosen Pharmaceutical Co., Ltd. (Lianyungang, China). The ssDNA strand (5'-/HS-SH-C6/TTTTTTTTTGGTTGGTGGGTTTGGGTTTGTGTTTTG GGTTTGTGGTTGGTTGGGTTTGGTTTTTTTTT/NH_2-C7/-3') and TCEP were both bought from Shenggong Bioengineering Ltd. Company (Shanghai, China). The phosphate buffer solution (PBS) was bought from Solebro Technology Co. (Beijing, China), which consists of 0.137 M NaCl, 2 mM NaH_2PO_4 and 8 mM Na_2HPO_4. All aqueous solutions in this research were prepared by deionized water. We used a Talos F200S transmission electron microscopy (TEM) to characterize the diameter and morphology of AuNPs, GQDs and FRET molecular probe, a UV-5200PC spectrophotometer to observe the absorbing range of AuNPs and a Fluo Time 300 automatic fluorescence lifetime spectrometer to analyze the emission spectrum of GQDs and FRET molecular probe.

B. Synthesis of AuNPs and ssDNA-AuNPs

To synthesize the AuNPs, we mixed 95.88 mL of deionized water and 4.12 mL of 24.28 mM $HAuCl_4$ and then heated it to boiling. After refluxing, we injected 10 mL of 38.8 mM trisodium citrate immediately, thus changing the appearance of the mixture from almost colorless to burgundy. Next, we stirred and refluxed the boiling solution rapidly for about 20 minutes and then stopped heating. Finally, after 30 minutes of stirring at room temperature, we collected and stored the solution in the refrigerator at $4\,^{\circ}\mathrm{C}$ [13].

To couple the ssDNA to the surface of AuNPs, we measured and mixed 6 μL of 100 μM ssDNA and 4 μL of 10 mM TCEP in a centrifuge tube. Then, we stood the mixed solution for 1 hour at room temperature away from light to fully break the disulfide bonds present in the ssDNA. Next, we mixed 100 μL of AuNPs and 10 μL of the above solution and chilled it at $-20\,^{\circ}\mathrm{C}$ for 2 hours [10]. Finally, we centrifuged the thawed solution three times at 15,000 rpm for 20 minutes and then acquired the stable ssDNA-AuNPs complexes at the bottom of the centrifuge tube.

C. Synthesis of FRET molecular probe

To synthesize the FRET molecular probe, we sonicated 100 μL of 1 mg/mL carboxylation-modified GQDs for 10 minutes and then added 100 μL of 27 mM EDC. Next, we shook the entire solution for 2 minutes and then sonicated it for roughly 30 s [7]. Finally, we obtained the FRET molecular probe by adding the above solution to the centrifuged ssDNA-AuNPs in Section B and incubating the whole solution for 2 hours at room temperature away from light.

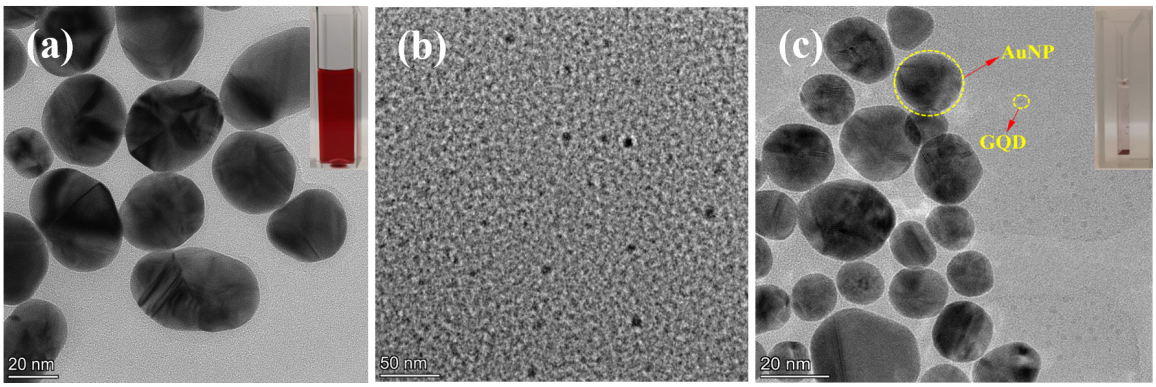

Fig. 2. The size and morphology images of (a) AuNPs with average diameter of 17.1 nm. Scale bar: 20 nm; (b) GQDs with average diameter of 5 nm. Scale bar: 50 nm; (c) FRET molecular probe. Scale bar: 20 nm.

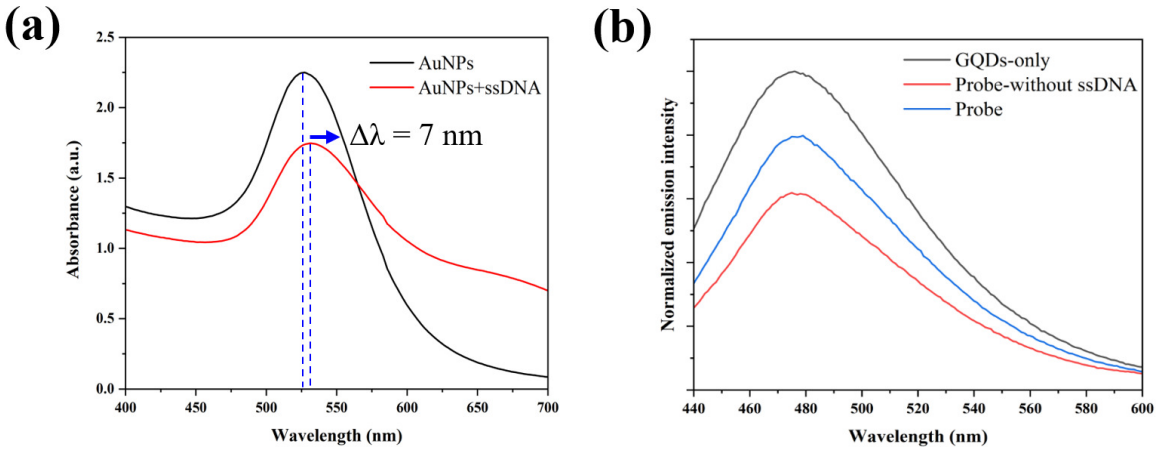

Fig. 3. (a) Absorption spectrum of AuNPs before and after ssDNA coupling; (b) Fluorescence emission spectrum of GQDs, FRET molecular probe and simple hybrid solutions of AuNPs and GQDs in the absence of ssDNA.

D. Pretreatment of cisplatin

To fully precipitate the chlorine element in cisplatin and allow it to form adducts with ssDNA, we measured 500 μL of 2 mM cisplatin and added it into 702 μL of 16 mM silver nitrate, then protected away from light for 10 minutes. Next, we centrifuged the product twice at 12,000 rpm for 10 minutes and obtained the supernatant which was dechlorinated cisplatin solution [12]. Finally, we diluted the collected supernatant with deionized water at a certain ratio to obtain different concentrations of cisplatin.

III. Results and Discussion

This part focuses on the characterization of the FRET molecular probe and the evaluation of its detection performance. Section A briefly describes the characterization of each component of the molecular probe. Section B constructs a quantitative model of the molecular probe for cisplatin detection. Section C evaluates the specificity of the molecular probe for cisplatin detection.

A. Morphological and spectral characterization

The morphology of the individual components of the FRET molecular probe was described by TEM experiments. The results showed that the AuNPs and GQDs have average diameters of 17.1 nm (Fig. 2(a)) and 5 nm (Fig. 2(b)) as well, and both are well dispersed in the solution. When a strand of ssDNA is attached between AuNPs and GQDs to construct a FRET molecular probe, the smallest distance between GQDs and AuNPs will be larger than the critical distance (10 nm) for FRET to occur, as illustrated in Fig. 2(c).

Fig. 3(a) depicts the absorption spectrum of AuNPs and ssDNA-AuNPs. The absorbance peak of AuNPs (black curve) is around 525 nm. When AuNPs was modified by a strand of ssDNA, the peak of the absorption spectrum obtained was red-shifted by roughly 7 nm, and the absorbance was reduced (red curve). Since the wavelength of the absorbance peak of AuNPs is shape-dependent, the absorbance peak is shifted as the shape changes. Therefore, the red shift in the absorption spectrum of ssDNA-AuNPs indicates that the AuNPs are in touch with additional nanoparticles that must be ssDNA on the surface of AuNPs. After coupling with ssDNA, the surface plasmon resonance (SPR) of AuNPs will become stronger, causing the absorbance reduced and the absorbance peak to red-shift. Fig. 3(b) shows the normalized fluorescence emission spectrum of GQDs, FRET molecular probe, and simple hybrid solutions of

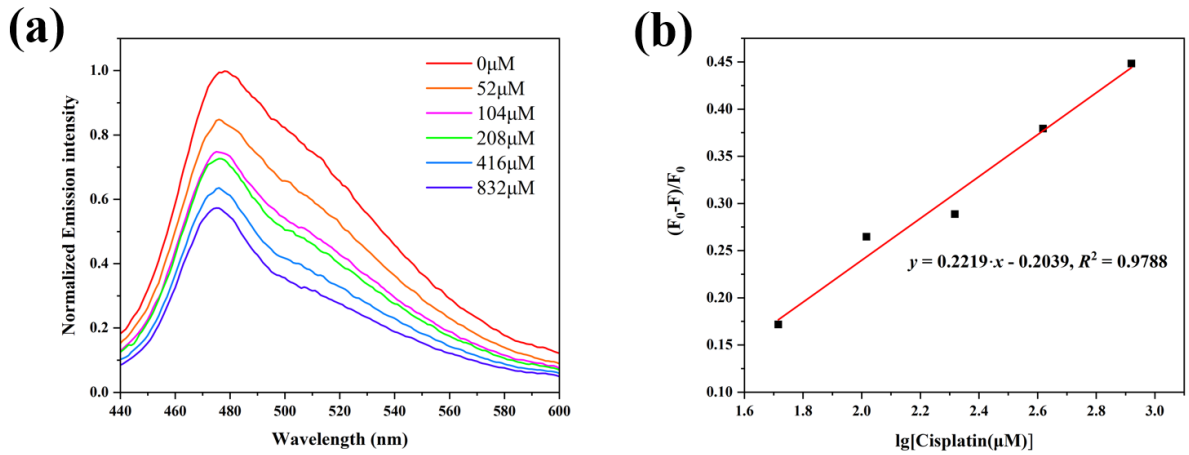

Fig. 4. (a) Fluorescence emission spectrum of FRET molecular probe when adding different concentrations of dechlorinated cisplatin (from top to bottom: 0, 52, 104, 208, 416 and 832 μM); (b) Plot of the relative reduction of the fluorescence central emission intensity $(\mathrm{F}_0 - \mathrm{F})/\mathrm{F}_0$ versus different logarithm concentrations of dechlorinated cisplatin from 52-832 μM.

AuNPs and GQDs in the absence of ssDNA. It can be seen that when exposed to high ultraviolet light with a wavelength of 405nm, the intensity of the central mission band was the highest when only GQDs were presented in the solution (black curve). The intensity of the central mission band of the FRET molecular probe has a partial decline compared to that when just GQDs were presented (blue curve). However, when ssDNA was absent, the intensity of the central mission band of simple hybrid solutions of AuNPs and GQDs was the lowest, about 60% of that when just GQDs were present (red curve). These observations highlight the critical function of ssDNA in the construction of FRET molecular probe: By rigidly connecting AuNPs and GQDs, the distance between AuNPs and GQDs is forcibly stretched so that the FRET effect cannot occur between most of them. When ssDNA is absent, both AuNPs and GQDs are in a free state in the solution, and the FRET effect between them considerably quenches the fluorescence emitted by GQDs, thus making the intensity of the central mission band of the whole solution lowest.

B. Quantitative model for cisplatin detection

To obtain the quantitative model for cisplatin detection, we researched the effect of different cisplatin concentrations on fluorescence quenching efficiency by adding a series of dechlorinated cisplatin in the concentration range of 52-832 μM to the FRET molecular probes with the volumetric ratio of 1:10. After that, we incubated all the solutions in a water bath at 37 °C for about 2 hours to sufficiently shorten the length of ssDNA and measured their fluorescence intensity after incubation. Fig. 4(a) illustrates the normalized fluorescence emission spectrum of the FRET molecular probe with different concentrations of dechlorinated cisplatin from 52-832 μM. It can be seen that with the increasing concentration of dechlorinated cisplatin, the fluorescence intensity of the FRET molecular probe was gradually decreasing. When no dechlorinated cisplatin is introduced, the central mission band has a normalized intensity of 1 at 479 nm. However, as we gradually increase the concentration of the introduced dechlorinated cisplatin, the distance between AuNPs and GQDs gradually decreases as the length of the ssDNA coupled between them continues to shorten, resulting in more FRET effect between AuNPs and GQDs. As a result, when the increased concentration of dechlorinated cisplatin reached 832 μM, the intensity of the central mission band of the FRET molecular probe reduced to 57.4% of what it was without the addition of dechlorinated cisplatin. The quantitative model of the relationship between the logarithm concentration of the dechlorinated cisplatin and the fluorescence central emission intensity of the FRET molecular probe is established in Fig. 4(b). The relative reduction of the fluorescence central emission intensity of the FRET molecular probe after the introduction of dechlorinated cisplatin with the expression $(\mathrm{F}_0 - \mathrm{F})/\mathrm{F}_0$ demonstrates a strong linear relationship for the dechlorinated cisplatin, which can be expressed by $y = 0.2219x - 0.2039$ with $R^2 = 0.9788$. As a result, the FRET molecular probe has a good working performance when the range of the concentration of introduced dechlorinated cisplatin is 52-832 μM.

C. Specificity evaluation

Specificity is a measure of the ability of the FRET molecular probe to detect the target when other interferences are introduced. In this section, we evaluated the specificity of the FRET molecular probe for the detection of cisplatin. Here, we selected five common metal ions in human serum and other chemotherapeutic agents combined with cisplatin: Fe^{3+}, Cu^{2+}, gemcitabine, etoposide and paclitaxel. Then, we introduced them separately to the FRET molecular probe at a concentration of 832 μM for detection. After that, we calculated the detected concentrations of these interferences based on the value of the expression $(\mathrm{F}_0 - \mathrm{F})/\mathrm{F}_0$ of the quantitative model mentioned in Section B and compared them with the detected concentration obtained by adding 832 μM dechlorinated cisplatin based on the quantitative model, as shown in Fig. 5. It can be obtained from the quantitative

model described in Section B, when five interferences with concentrations of 832 μM were introduced to the FRET molecular probe, the detected concentrations obtained were all near a few tens of micromolar range. The detected concentration for dechlorinated cisplatin, on the other hand, was close to 700 μM, indicating that the FRET molecular probe did not respond appreciably to the other five interferences compared with dechlorinated cisplatin. As a result, the FRET molecular probe has the specificity for the detection of cisplatin.

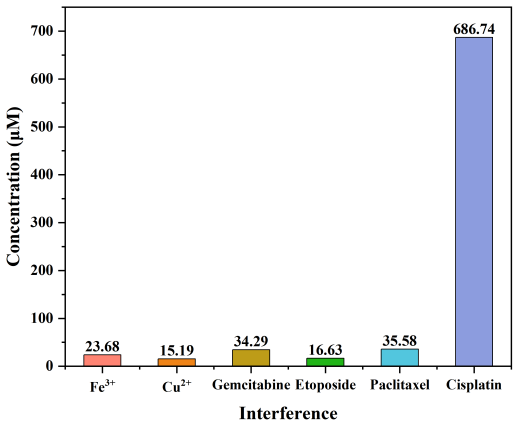

Fig. 5. Specificity of the FRET molecular probe in presence of Fe^{3+}, Cu^{2+}, gemcitabine, etoposide, paclitaxel and cisplatin (832 μM).

IV. Conclusion and Prospects

In this work, a FRET-based fluorescence molecular probe for detecting active cisplatin has been demonstrated. The bending of ssDNA caused by cisplatin significantly shortens the distance between GQDs and AuNPs, resulting in a fluorescence quenching. The proposed quantitative model has a cisplatin detection range of 52-832 μM, and the additional experiment validated the specificity of the molecular probe. In the future, by continuously optimizing the structure of the FRET molecular probe and its corresponding detection conditions, the detection range will be extended to low concentrations for better application in the detection of serum active cisplatin in patient samples, which will provide a decision-making basis for individualized and precise treatment of cancer patients.

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