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RESEARCH ARTICLE

Quantum dots-based fluorescence resonance energy transfer biosensor for monitoring cell apoptosis

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Abstract

The development of advanced methods for accurately monitoring cell apoptosis has extensive significance in the diagnostic and pharmaceutical fields. In this study, we developed a rapid, sensitive and selective approach for the detection of cell apoptosis by combining the site-specific recognition and cleavage of the DEVD–peptide with quantum dots (QDs)-based fluorescence resonance energy transfer (FRET). Firstly, biotin-peptide was conjugated on the surface of AuNPs to form AuNPs-pep through the formation of an Au-S bond. Then, AuNPs–pep–QDs nanoprobe was obtained through the connection between AuNPs–pep and QDs. FRET is on and the fluorescence of QDs is quenched at this point. The evidence of UV–vis spectra, transmission electron microscopy (TEM), and Fourier transform infrared (FT-IR) spectroscopy revealed that the connection was successful. Upon the addition of apoptosis cell lysis solution, peptide was cleaved by caspase-3, and AuNPs was dissociated from the QDs. At this time, FRET is off, and thus the QDs fluorescence was recovered. The experimental conditions were optimized in terms of ratio of peptide to AuNPs, buffer solution, and the temperature of conjugation and enzyme reaction. The biosensor was successfully applied to distinguishing apoptosis cells and normal cells within 2 h. This study demonstrated that the biosensor could be utilized to evaluate anticancer drugs.

KEYWORDS

apoptosis, biosensor, fluorescence resonance energy transfer, gold nanoparticles, quantum dots

1 | INTRODUCTION

Apoptosis, also known as programmed cell death (PCD), is a specialized form of cell death that exists in multicellular organisms. It is also an essential biological process in normal tissue homeostasis and diseased tissue.[1] Deregulation of the apoptosis pathway can bring about a variety of debilitating diseases including cancer, neurodegenerative diseases (e.g. Alzheimer's disease, Parkinson's disease), autoimmune diseases (e.g. lupus erythematosus, rheumatoid arthritis), atherosclerosis and myocardial infarction.[2–4] Therefore, it is desirable to develop specific and sensitive methods for diagnosing apoptosis, and thus much effort has been taken to this end.[5–7] To date, several methods have become available to monitor apoptosis, such as morphological detection,[8] flow cytometry,[9] detection of DNA degradation[10] or intracellular calcium concentration[11] and enzymatic analysis.[12] Among these, enzymatic analysis, based on caspase family cleavage of functional and structural intracellular proteins, is one of the common ways to detect apoptosis.[13–15] The caspase family is considered as a central, indispensable executioner of apoptosis. It is responsible for major parts of morphological changes.[16] in the process of apoptosis. Among these, caspase-3 is especially important in this family, because it takes part in both intrinsic and extrinsic apoptotic pathways. It can typically recognize the 4-amino acid peptide sequence Asp-Glu-Val-Asp (DEVD) and cleaves its substrate at the C-terminal Asp residue.[17]

With the development of fluorescence-based techniques, much attention has focused on fluorescence resonance energy transfer (FRET)-based biosensors.[7,18] In the FRET-based system, quantum dots (QDs) are becoming an effective energy donor due to their intrinsic optical properties, including high quantum yield, size-dependent photoluminescence with broad excitation spectra and sharp emission spectra, high photostability and excellent chemical stability.[19–22] Over the past decade, numerous materials have been used as acceptors, principally organic dyes or fluorescent proteins, but most materials were limited due to photobleaching problems, complex preparation, and high-cost.[23–25] At this time, mounting evidence suggests that

Abbreviations: FCS, fetal calf serum; FRET, fluorescence resonance energy transfer; PCD, programmed cell death; QD, quantum dots; QE, quenching efficiency; TEM, transmission electron microscopy.

gold nanoparticles (AuNPs) are a good energy acceptor in view of their strong absorbance in the visible range, exceptionally for biocompatibility and photobleaching resistance.[26–28] Thus, QDs and AuNPs are a useful alternative with which to construct FRET biosensors.

Despite efforts to use energy transfer biosensors, there have been no reports in the literature on the use of QDs-based FRET biosensors for monitoring apoptosis. In this study, using the combination of the large extinction coefficient of AuNPs with the strong fluorescence of QDs, we developed a sensitive and selective FRET biosensor for monitoring apoptosis within 2 h. The constructed platform was proven to be facile and cost effective as well as highly sensitive, selective and reliable, making it a promising candidate for trace inhibitor screening in the future.

2 | EXPERIMENTAL

2.1 | Reagents

Biotin–peptide (GDGDEVDGC) and QDs 605–streptavidin were purchased from Sangon Biotechnology Co., Ltd (>98%, Shanghai, China) and Jiayuan Quantum Dots Co., Ltd (Wuhan, China), respectively. Chloroauric acid ($HAuCl_4{\cdot}4H_2O$), trisodium citrate and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from the Shanghai Reagent Co., Ltd. (Shanghai, China). Apoptosis inducers, cell lysis solution and phenylmethanesulfonyl fluoride (PMSF) were obtained from the Beyotime Institute of Biotechnology. Other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with double-distilled water.

2.2 | Apparatus

UV–vis absorption spectra were recorded on a UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan). The fluorescence measurements were carried out at room temperature on an Edinburgh FLS920P fluorescence spectrometer (Edinburgh Instruments Ltd, UK). All samples were excited at 300 nm and the emission spectra measured from 500 to 650 nm. Fourier transform infrared (FT-IR) spectroscopy was recorded on a Vector 22 FT-IR spectrometer (Bruker). Samples were thoroughly ground with exhaustively dried KBr. Transmission electron micrographs (TEM) images were acquired using a JEOL JEM-2100 (JEOL, Japan) with a transmission electron microscope operating at an acceleration voltage of 200 kV.

2.3 | Synthesis of AuNPs–pep

AuNPs were prepared according to the literature procedures.[29] Typically, 2 ml 50 mM $HAuCl_4$ was added to 98 ml water. After stirring for 1 min, the mixture was heated until boiling. Then, 2.0 ml of 38.8 mM sodium citrate was added. The resulting colloidal solution was stirring for 5 min, and finally stored in a dark bottle at 4°C.

The AuNPs–pep was prepared via the formation of Au-S bond between cysteine-terminated peptide and AuNPs. Typically, biotin–peptide (20 μl, 50 μmol/L) was activated by TCEP (10 μl, 10 mM), and then 1 ml of the above mentioned AuNPs was added to the mixture. The resulting solution was incubated at 4°C for more than 18 h. After that, the obtained AuNPs–pep were purified by centrifugation at 12 000 rpm for 10 min, washed twice with double-distilled water and then dispersed in appropriate buffer solution.

2.4 | Preparation of QDs–pep–AuNPs probe

QDs–pep–AuNPs probe was prepared by adding 0.5 μl of 1 μM QDs–streptavidin and varying volumes (0–80 μl) of the obtained AuNPs–pep to a buffer solution (10 mM Tris–HCl 7.4) with a final volume of 500 μl. The mixture was incubated at 37°C for 1 h to allow specific association between streptavidin and biotin.

2.5 | Cell culture and induction of apoptosis

HL-60 cells were cultured in a flask with the RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg/ml) and streptomycin (100 μg/ml) in an incubator (5% CO_2, 37°C) for 2 days at a concentration of 10^6 cells/ml. Then, cells were treated with reagents from an Apoptosis Inducers Kit (10 μl/ml) to induce apoptosis for a certain time varying from 0.5 to 2 h. Subsequently, cells were harvested, washed twice with phosphate buffer solution (PBS, 50 mM, pH 7.4) and lysed in lysis buffer (cell lysis solution and PMSF with 100:1) with repeated freeze–thaw lysis cycles. The obtained solution was centrifuged at 12 000 rpm for 5 min and the supernatant was used for apoptosis assays immediately.

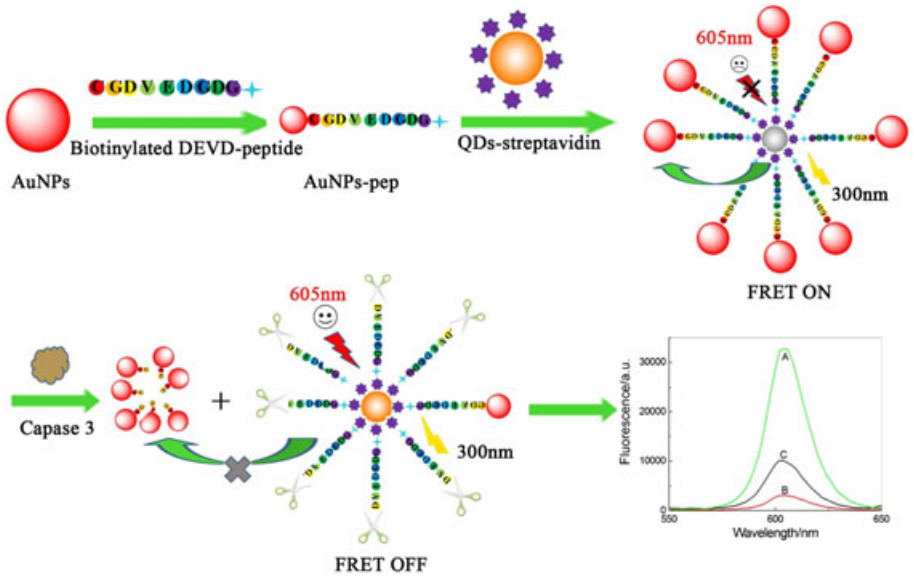
2.6 | Apoptosis assays

Apoptosis assays were initiated by adding cell lysis solution to the above QDs–pep–AuNPs probe. Subsequently, the obtained mixture were incubated at 37°C for 60–120 min by shaking vigorously, and then measured the fluorescence spectra to monitor cell apoptosis quickly and easily.

3 | RESULTS AND DISCUSSION

3.1 | Principle of the biosensor

As shown in Scheme 1, the biotin–peptide containing four amino acids (DEVD) was designed and used as a bridge to make the QDs-based FRET biosensor. Firstly, the biotinylated DEVD–peptide was modified on the surface of AuNPs via the formation of a Au–S bond between the terminal amino acid-cysteine of peptide and AuNPs. Subsequently, the AuNPs–peptide was linked with QDs by streptavidin–biotin-specific binding. When the process was accomplished, FRET between QDs and AuNPs was on and the fluorescence of QDs was quenched. It is known that caspase 3, which cleaves the peptide at the DEVD site, is produced in apoptosis cell lysis solution. Thus, in the presence of apoptosis cell lysis solution, the above QDs–AuNPs probe was cleaved and AuNPs detached from the QDs. Then, FRET was turned off and the QDs fluorescence was recovered. Thus, apoptosis was monitored easily by measuring the fluorescence change of the QDs-based FRET probe.



SCHEME 1 The principle of QDs-based FRET biosensor for monitoring cell apoptosis

3.2 | Characterization of AuNPs and QDs

In this study, QDs and AuNPs were selected as the donor and acceptor pair for the FRET biosensor. Thus, the properties of QDs and AuNPs were characterized by HRTEM, UV–vis absorbance, and fluorescence spectra, as shown in Figure 1. In Figure 1(a), we can see that the as-prepared AuNPs are well dispersed spherical objects with average diameter of 15 nm (based on statistical analysis of more than 100 nanoparticles). Furthermore, AuNPs exhibit a maximum absorption peak at 519.5 nm in the range of 400–650 nm while QDs have a narrow fluorescence emission with the maximum peak at 605 nm (Figure 1b). Distinctly, the fluorescence emission of QDs has a considerable spectral overlap with surface plasmon absorption of AuNPs, which laid a good foundation for efficient energy transfer.[30]

3.3 | Characterization of AuNPs-pep

Biotinylated DEVD–peptide was modified on the surface of AuNPs through formation of strong Au-S bonds, and the modified AuNPs was purified with centrifugation, characterized with UV–vis, and FT-IR, as shown in Figure 2. Figure 2(a) is the UV–vis absorbance spectrum of AuNPs and AuNPs–pep. It can be seen that the maximum absorption wavelength of AuNPs redshifts from 519.5 to 525.5 nm after

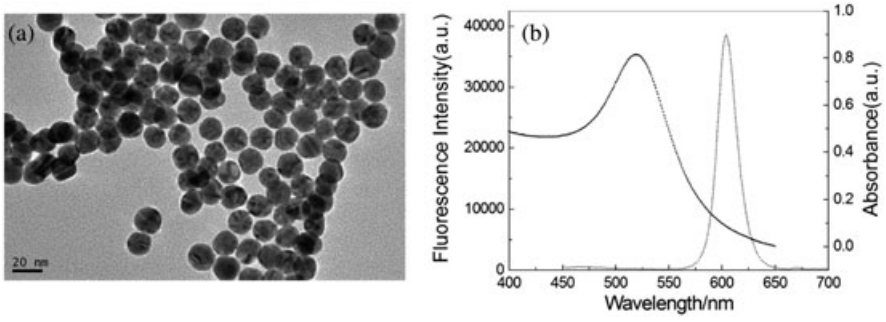


FIGURE 1 (a) TEM images of AuNPs. (b) UV–Vis absorption spectra of AuNPs (dot line) and fluorescence spectra of QDs (straight line)

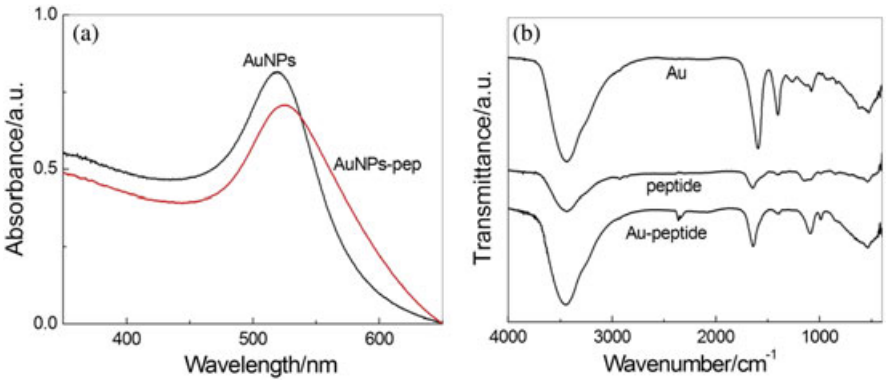


FIGURE 2 (a) UV–Vis absorption spectra of AuNPs and AuNPs–pep. (b) FT-IR spectra of AuNPs, peptide, and AuNPs–pep

conjugated with peptide. This fact is attributed to the increase of average size for AuNPs. Meanwhile, the absorbance intensity was reduced. It may be as a consequence of the increase of AuNPs' size and centrifugation operation. In addition, FT-IR spectra of AuNPs–pep and peptide exhibit the amino absorption features of peptide at 1640, 1088 and 990 cm^{-1}, and the shapes of these are similar (Figure 2b), indicating that the peptide was modified on the surface of AuNPs successfully.

3.4 | FRET between QDs and AuNPs

As described in Scheme 1, the apoptosis assay is based on the FRET principle. To further verify the feasibility of fluorescence energy transfer between QDs and AuNPs–pep, photoluminescence (PL) changes of the QDs–AuNPs probe in the absence or presence of apoptosis cell lysates were monitored. The QDs-streptavidin emitted a strong red fluorescence at 605 nm, as shown in Figure 3(a). Then, the AuNPs–pep was bounded to QDs-streptavidin to form QDs–pep–AuNPs probes through specific association between streptavidin and biotin. At this time, the PL of QDs was quenched (Figure 3b). Subsequently, with the addition of apoptosis cell lysates, AuNPs was detached from the surface of the QDs, and thus PL was recovered (Figure 3c). This change is because that lysates contain a kind of protease - caspase 3 - that cleaves the peptide substrate with tetrapeptide DEVD. Thus, we can detect apoptosis using the proposed QDs-based FRET biosensor.

To achieve the best sensing performance, two important parameters were optimized. Firstly, the background on the fluorescence signal of the AuNPs–pep–QDs, which is achieved by quenching PL of QDs by AuNPs–pep, was investigated. Quenching efficiency (QE) plays a crucial role in this case and was calculated using the following equation: $QE = 1 - F_a/F_d$, where F_d and F_a are the fluorescence intensities of QDs in the presence or absence of AuNPs–pep, respectively.[31] The ratio of acceptor to donor is a very important factor for QE. Thus, the ratio of acceptor to donor was optimized. For this purpose, different volumes of AuNPs–pep were added to the QDs while the molarity of QDs was kept at one set value. As depicted in the inset of Figure 3(a),

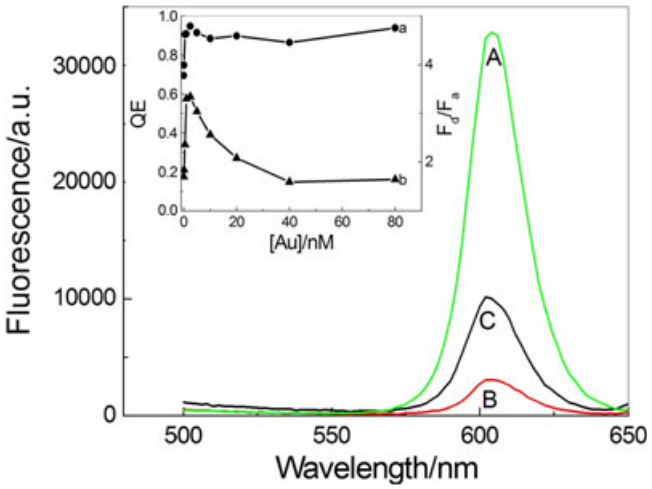


FIGURE 3 PL changes in QDs–AuNPs by apoptosis cell lysates: (a) QDs; (b) QDs–pep–AuNPs; (c) addition of apoptosis cell lysates. Inset: changes in QE (a) and F_d/F_a (b) with varying volumes (0.02, 0.1, 0.5, 1, 2.5, 5, 10, 20, 40, 80 µl) of AuNPs–pep and QDs concentration of 1 nM

QE increases with increase in volume of AuNPs–pep and starts to level off after 2.5 µl. After peptide is cleaved by apoptosis cell lysates, the fluorescence of the AuNPs–pep–QDs probe should be recovered, based on the difference in PL values for the presence or absence of apoptosis cell lysates. This change is defined as F_d/F_a, in which F_d and F_a is the fluorescence intensity of QDs in the presence or absence of apoptosis cell lysates, respectively. From the inset in Figure 3(b), it can be seen clearly that F_d/F_a increases with increase in the volume of AuNPs–pep. When the volume reached 2.5 µl, F_d/F_a reached maximum. After that, when the volume increased, F_d/F_a decreased clearly. Considering the above factors comprehensively, we selected 2.5 µl as the optimum AuNPs–pep volume.

3.5 | Optimization of the experimental conditions

To obtain the best sensing performance, we also optimized several experiment parameters such as the ratio of peptide to AuNPs, buffer solution, and the temperature for conjugation and enzyme reaction. To improve the sensitivity, the ratio of peptide to AuNPs was optimized first. The experiments showed that QE increased with increase in the ratio of peptide to AuNPs. When the ratio was up to 10, QE achieved maximum value. Therefore, 10 was selected as the best ratio of peptide to AuNPs. In addition, buffer solution and pH are very important parameters for the fluorescence of QDs and for enzyme activity. Thus, the effect of buffer solution and pH on the response of apoptosis of 0.01 M PBS (7.4), Tris–HCl (7.4), Dulbecco's PBS and HBO_3-$Na_4B_2O_7$ solution was investigated. The results exhibited that HBO_3-$Na_4B_2O_7$ is the optimum buffer solution. Lastly, temperature is an important parameter for the QDs and AuNPs–pep conjugation and enzyme catalysis. We conducted the experiment at 25°C and 37°C, and then compared the results. The data showed that 37°C is more appropriate than 25°C. Furthermore, in order to keep the best enzyme activity, 37°C was selected as the enzyme reaction temperature.

3.6 | Apoptosis assay using QDs–pep–AuNPs FRET probes

To check our prepared QDs–pep–AuNPs FRET probes, the fluorescence spectra of QDs–pep–AuNPs FRET probes were monitored and the F_d/F_a value in the presence of apoptosis cell lysis solution at different time points was calculated. Herein, F_d and F_a is the fluorescence intensity of QDs–pep–AuNPs FRET probes in the presence or absence of cell lysates, respectively. As shown in Figure 4, PL intensity of QDs decreased after they bound to the surface of AuNPs due to the effect of FRET between QDs and AuNPs. After apoptosis cell lysis solution was added and incubated for different time intervals (0.5–2 h), PL intensity of QDs–pep–AuNPs FRET probes and F_d/F_a increased with incubation time. The value of F_d/F_a was defined as 1 at the start of the experiment. After the probes were incubated with apoptosis cell lysis solution for 0.5, 1, 1.5, or 2 h, the F_d/F_a value increased from 1.44 to 3.425. However, F_d/F_a was kept at 1.2, if normal cell lysis solutions were employed. This phenomenon is due to recognition and cleavage of the tetrapeptide DEVD by caspase-3 in the apoptosis cell lysis solution. Caspase-3 can typically recognize the 4-amino acid peptide sequence Asp-Glu-Val-Asp (DEVD) and cleaves their substrate at

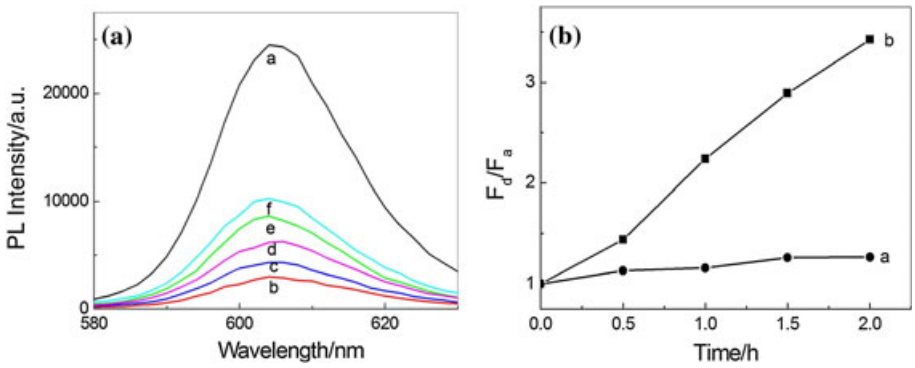


FIGURE 4 (a) PL changes of QDs–pep–AuNPs FRET probes due to apoptosis cell lysates and increasing reaction times. (a) QDs only, (b) QDs-pep-AuNPs, (c-f) addition of apoptosis cell lysates and incubation at 37°C for 0.5, 1, 1.5, or 2 h. (B) relationship between F_d/F_a and QDs–pep–AuNPs probe incubation time for normal cells (a) and apoptosis cell (b) lysates at 37°C

the C-terminal Asp residue. So as more caspase-3 is added, more peptide is cleaved. Consequently, more QDs detach from the AuNP–pep and the intensity of QDs–pep–AuNPs FRET probes increases, which results in the F_d/F_a value increasing further. In our study, after repeated freeze–thaw lysis cycles, the apoptosis cell lysis solution had an increased caspase-3 content, while the abnormal cell lysis solution has little caspase. Consequently, the F_d/F_a of the system with apoptosis cell is higher than that of the abnormal cell. Thus, we can come to the conclusion that our designed QDs–pep–AuNPs FRET probes can recognize and detect apoptotic cells.

3.7 | Control experiment

BSA and type IV collagenase was used to conduct control experiments by comparing the fluorescence response of QDs–pep–AuNPs FRET probes after the addition of pure apoptosis cell lysis solution and measuring difference in response The response changes were 6.5 and 7.2% respectively which indicated that the specificity was acceptable. Conversely, when peptide (GDGDEVDGC) was changed to peptide (PLGLCG) the cell lysis solution containing caspase-3 could not cleave the peptide substrate of peptide PLGLCG. These factors guarantee that the biosensor is excellent for application.

4 | CONCLUSIONS

In summary, a novel biosensor monitoring cell apoptosis was constructed by coupling FRET between QDs and AuNPs with site-specific recognition and cleavage of the DEVD–peptide bond. The excellent quenching ability of AuNPs allows the PL intensity of the QDs to turn off, and then turn on by the addition of apoptotic cell lysis solution. Under optimized analytical conditions, this method was able to distinguish between apoptotic cells and normal cells within 2 h. Furthermore, this system exhibited excellent selectivity, remarkable repeatability and a fast response.

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