Applications of fluorescent biosensors based on quantum dots

Zehan Ren[1, †] Jiyue Zhang[2, *, †]

[1]Shanghai High School, Shanghai, 200237, China

[2]School of Chemical and Environmental Engineering, Anhui Polytechnic University, 236000, Wuhu, Anhui

*Corresponding author. Email: 3190409123@stu.ahpu.edu.cn

[†]These authors contributed equally

Abstract. In modern analytical sciences, fluorescent biosensors are widely used because of their have high sensitivity, high selectivity and stability. Quantum dots (QDs) are a kind of new semiconductor materials which are popular in recent years, which have been widely used in bioimaging, sensing, drug delivery and photocatalysis. QDs have unique and excellent photoelectric characteristics, and are mainly composed of II-VI period element. Due to its good biocompatibility, high stability, excellent water solubility and easy surface modification, the prepared QDs come from a wide range of sources and are considered to be good fluorescent probes for the development of a diverse of fluorescent biosensors. This research describes the progress of fluorescent biosensors based on QDs in recent years, focusing on the three mechanisms and applications in detection, such as the detection of glucose, uric acid and cell apoptosis. And this research also looks forward to the future development trend of QDs-based fluorescent biosensors.

Keywords: quantum dots, guanine, uric acid, cell apoptosis, fluorescent biosensors.

1. Introduction

Biological studies require looking into the mechanisms of intracellular reactions. With the development of modern physiological sciences, it has been better understood that certain reactions may serve as signs of phenomena of interest. For example, high concentration of special proteins can suggest the progression of cancer. However, deep insights into the microstructure of cells and compounds and the reactions that take place among them remain limited. Scientists consider it difficult to trace these reactions, mostly due to the fact that the majority of the reactions cannot be easily represented outside the organism, making it almost impossible to adopt the methods that are widely used for the detection of reactions *in vitro*. This remained a challenge for a long time, until new techniques were finally developed in the years that followed.

Fluorescent biosensors are considered one of the most widely adopted biosensing techniques, which proved to be very efficient when probing small molecule reactions. The technique is based on the fact that certain molecules or nanomaterials send out optical signals when they are exposed to light rays. First discovered by Spanish doctor and botanist Nicolas Monardes in 1575, who observed a strange blue color in lignum nephriticum, the phenomenon remained an enigma until 1852, when Irish physicist George Stokes proved by experiment that the color results from the substance emitting light whose wavelength is not equivalent to that of the light absorbed, later referred to as "fluorescence". It has been shown that the electron which absorbs light is lifted from the ground state to the excited state, carrying much more energy than before. The excited state, however, is highly unstable, and when a minor disturbance occurs, the electron releases the energy and returns to the ground state. According to numerous researches that have been carried out through the decades, we learn that in most cases, the energy is released in the form of heat. Certain electrons, on the other hand, release the energy by emitting light, the wavelength of it depending on the structure of the molecule, resulting in different colors that can be used for qualitative sensing. The fluorescence intensity can also be used to carry out quantitative analysis by applying theories developed later (which will be mentioned in detail in the main part of this paper). Due to the fact that fluorescence is

common among biomolecules, and that different molecules show different colors, the sensing technique is highly specific and can be adopted in a wide range of reaction systems.

From biomedical to environmental applications and food analysis using different biosensing methods are closely related to human daily life. There is an increasing demand for quick response and low-cost simple analysis of equipment materials for application in the market. In medicine, many human diseases cannot be detected in the early stage, so it is particularly important to detect whether the content of some special substances in the human body changes, which is of great help to the prevention and treatment of disease progress and means that we need to visualize it through specific technology to directly or indirectly understand. At the same time, some drug residues will also have a bad effect on the human body. Due to the high sensitivity of fluorescent biosensors, some trace concentrations can be detected through the significant changes in fluorescence biosensors, reaching nanomolar or picomolar concentrations of biomolecules [1], which are of paramount importance in biomedical applications.

Many studies have shown that different materials are designed for efficient and sensitive fluorescent biosensors. Traditional sensors have been used for these detections, but they have many limitations. The fluorescent biosensors have a vastly improved detection range of substances due to its unique physical properties. Compared with traditional biosensors, it can detect lower concentrations and can directly see the changes through changes in the peak value of fluorescence spectra. In terms of materials, with the development of science and technology, people use smaller and smaller sizes of materials, which is not only conducive to reducing equipment, but also to reflect its sensitivity. Nanomaterials are widely used in the development of sensitive and efficient fluorescence sensors due to most of them have unique photoelectric characteristics such as photoelectric stability, adjustable size emission spectrum, long fluorescence lifetime, and multifunctional indication modification. They can be used for some basic substances of human body such as DNA [2], RNA [3] and protein [4], and can also detect the content of some drugs such as cocaine [5] and DOX [6]. At present, many fluorescent biosensors also have certain limitations, such as easy to be interfered by detected substances and some environmental factors. At the same time, most of the time, these sensors need chemical synthesis, so sometimes there is a certain toxicity. It is still worth exploring innovation in this area to develop sensors that are less toxic or even non-toxic while improving their stability and accuracy in complex environments. This research describes the recent advances and applications of fluorescent biosensors, including the basic principles of detection of various substances in the human body, and discusses the development direction in this field.

2. Mechanism of fluorescence biosensors

2.1. Interpretation of the Phenomenon

As we know by experiment, when a molecule is exposed to light, the energy it absorbs is often sent out in the form of heat due to collision. Some molecules, however, only lose part of the energy via collision, sending out the rest by photon emission. The photon carries less energy than the incident light, and therefore has a longer wavelength, resulting in certain optical signals. This process is commonly referred to as fluorescence.

Certain molecules bear a structure called “fluorophore”, which can send out fluorescent signals and serve as a specific biomarker. When the molecule is hit by a beam of light, the structure absorbs the photon in about 10^{-15} seconds, obtaining the energy contained. Its electrons are then raised to a state with higher energy levels, namely, an excited state. For each state (S_0, S_1, S_2, etc), there are different vibrational levels. It is observed that for most molecules of interest (often proteins or other organic compounds), the electrons tend to take steps to higher vibrational levels of exited states, resulting in the absorption spectrum, which is crucial to fluorescence sensing and later stages of analysis. These states (or levels) are highly unstable, meaning that the electros will drop back to ground state, due to 2 main processes referred to as internal conversion and vibrational relaxation. The first, internal conversion, is a process when the electron transits from a lower vibrational level of

a higher state to a higher vibrational level of a lower state, which carries the same energy as the previous one. This occurs when the electron is lifted to electronic states above the first excited state (S_1). The latter, vibrational relaxation, means that the electron drops to lower vibrational levels of the same electronic state, mostly due to cross-molecule collision that sends out the energy in the form of heat. The result is that practically all electrons are in the lowest vibrational level of the first excited state (S_1V_0) within no more than 10^{-12} seconds, making the molecule reach the first excited singlet state.

The transition to the ground state, on the other hand, is not governed by any of the two processes mentioned above. Due to the relatively longer time scale (10^{-9} to 10^{-6} seconds), fluorescence becomes one of the important processes that contribute to the energy loss. This means the molecule sends out the energy by emitting a photon. However, the excited electron has already lost a large part of the energy it absorbs from the incident photon, leading to the fact that the photon it emits carries a lesser amount of energy, therefore having a greater wavelength. In certain situations, the electron absorbs two or even more photons at the same time, resulting in shorter wavelength than that of the photons absorbs.

Not all molecules can send out fluorescent signals. A major requirement is that the molecule should be well-structured to absorb incident light. The molecule should also have high fluorescence efficiency (or fluorescence quantum yield), which is defined as the ratio of the number of photons emitted to the number of photons absorbed. This also depends on the structure of the molecule, and on environmental factors as well, such as temperature and pH.

2.2. Basic Principles of Fluorescence

In fluorescence, the intensity F is given by the following formula

$F=\phi I0\ (1-10-\varepsilon cl)$

Where ϕ is the quantum yield, I_0 is the power of incident light, ε is the molar absorptivity, c is the concentration, and l is the pathlength. For large enough εcl, the product $10^{-\varepsilon cl}$ tends to 0, making the fluorescence intensity depend solely on the quantum yield and the incident light power. If εcl is relatively small, the formula above can be written as

$F=2.303\ \phi I0\ \varepsilon cl$

Demonstrating that for small εcl and a fixed pathlength l, the intensity F is proportional to the concentration c, and the power of the incident light I_0.

We plot the intensity-concentration graph during analysis. From the interpretation above, we can show that the linear region appears in lower concentrations. For high concentrations, the linearity is broken due to cross-molecule processes such as collision.

2.3. Fluorescence Quenching

Fluorescence quenching is a common phenomenon that plays an important role in fluorescence sensing, especially when it comes to the dynamic properties of complex structures. For instance, a lot of scientists have found ways to analyze micelles by using fluorescence quenching. Fluorescence quenching can also be used for other purposes, such as chlorophyll detection, tissue observation and the analysis of different compounds, from nitrated explosives to cancer biomarkers.

As is mentioned above, fluorescence occurs when all the electrons reach the lowest vibrational level of the first excited state (S_1V_0). However, these electrons can lose energy further, making them not able to emit fluorescent photons. This results in lower fluorescence intensity or the complete vanish of the optical signal.

Various processes can lead to fluorescence quenching. Among those are excited-state energy transfer (EET), electron transfer (ET), cross relaxation (CR) and many others. Here, we introduce the mechanism of concentration quenching first.

Normally, in the solution, fluorescent molecules maintain a relatively long distance between them. When the concentration is too high, however, the distance becomes so short that molecules begin to affect the behaviour of others. Therefore, a process called cascade energy transfer occurs, until a large

amount of energy is passed to the quenching centre, making the optical signal to weaken or almost disappear. Because no other chemical compound is introduced into the system, this process is also referred to as self-quenching.

Another way of fluorescence quenching is to add specific quenchers to the solution. The process of quenching is complex: the energy may be lost due to colliding with the quencher; the molecule and the quencher may form a compound which is not fluorescent; and energy transfer can also occur, which will be the topic of our next section.

2.4. Fluorescence Resonance Energy Transfer

Different fluorescent molecules have different absorbance and emission spectrums. When the emission spectrum of one molecule overlaps with the absorbance spectrum of another molecule, then the latter molecule is likely to be excited if the former one is excited by the incident light. This will lead to the energy transfer from one fluorescent molecule to another. The former molecule, known as the donor, loses the energy, and the latter, known as the accepter, receives the energy. Thus, the latter molecule (though not exposed to any light beam which is in its absorbance spectrum) will be able as well to send out fluorescent signals. The process is non-radiative for it does not require the emission of any photon. This phenomenon is often referred to as fluorescence resonance energy transfer (FRET).

To achieve this phenomenon, we need to make sure the molecules are close enough to each other. We must also search with great care for the donor-accepter pairing. The most widely used donor-accepter pairs lies in the family of green fluorescent protein (GFP) and its variants, which have been studied extensively in the last century [7][8]. They have been used for detecting the concentration of certain ions, such as Ca^{2+}.

It is convenient to probe intra-cellular reactions due to the simple mechanism of the technique. We bind certain fluorescent proteins to the chemical substances of interest to form a pair of donor and accepter. Then we use a sensor to monitor the fluorescence spectrum change of the system. When the two substances react, they come together and the distance between the fluorophores becomes smaller. The closer they are, the greater FRET will be, and a bigger loss in the fluorescence intensity of the donor will be observed. The concentration of substance of interest can thus be known.

3. Applications of fluorescent biosensors

Gu et al. [9] developed a QDS-based FRET biosensor for monitoring cell apoptosis. The mechanism has been shown in Figure 1. Since the Fd/Fa (Fd and Fa are the fluorescence intensity of QDS-PEP-AuNPs probe in the presence or absence of cell lysates) value in the system with apoptotic cells was higher than that of abnormal cells, and the normal cell lysate was about 1.2, cell apoptosis could be detected by detecting changes in probe fluorescence intensity. In this study, -PEP was synthesized by hydrothermal method and prepared QDS-PEP-AuNPs probe on this basis. After the addition of apoptosis lysate, the QDS-AuNPs probe was cleaved, and the peptide and AuNPs terminal amino acids formed Au-S to modify the DEVD-peptide. Then the AUNPs-peptide bound to the quantum dots, the fluorescence of the quantum dots was quenched, and AuNPs were separated from the quantum dots.

The results showed that fluorescence spectra of the QDS-PEP-AuNPs FRET probe were monitored and Fd/Fa values were calculated for the presence of lysis solution of apoptotic cells at different time points. Under optimized analytical conditions, the method could distinguish apoptotic cells from normal cells within 2 h, with good selectivity, repeatability, and high sensitivity.

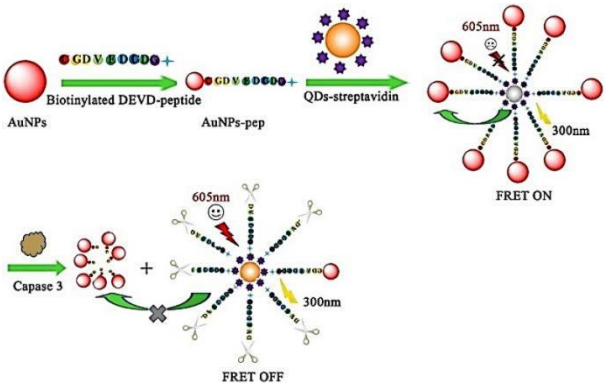

Figure 1 The mechanism of QDs‐based fluorescent biosensor for cell apoptosis analysis [9].

Wang et al. [10] used hydrothermal method to synthesize sulfur and nitrogen co-doped carbon quantum dots as fluorescent probes to detect uric acid. The mechanism has been shown in Figure 2. In this experiment, in order to make the composite carbon quantum dots have good fluorescence performance and repeatability, the factors such as hydrothermal temperature, time, and mass ratio were optimized, and the ultraviolet and infrared characterization was carried out. After that, take certain prepared CQD and add uric acid cultured with uricase in different concentrations. H_2O_2 is the main reaction product catalyzed by uricase. The Fenton reaction of H_2O_2-Fe^{2+}can produce hydroxyl radical, which can obviously quench the fluorescence intensity of S and N co-doped CQD. On this basis, Fenton reaction conditions were optimized, Fe^{2+}, pH, and uricase influence on the reaction were analyzed and studied, and uric acid was quantitatively detected under the best experimental conditions. The results showed that, by analyzing the relationship between fluorescence quenching efficiency and uric acid concentration, there was a good linear relationship between UA concentration of 0.08~10 μM and 10~50μM, and the detection limit is as low as 0.07 μM. The composite carbon quantum dots can be successfully applied to the detection of uric acid. It is a simple and environmentally friendly nano-fluorescent biosensor, which has the advantages of high selectivity, fast response speed and the corresponding range of light, and has a great application prospect in biological imaging and biological detection.

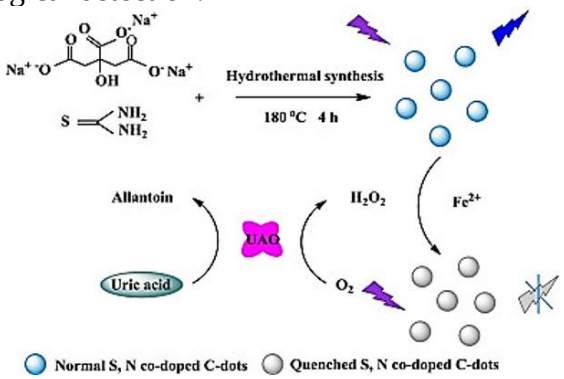

Figure 2 The proposed uric acid-sensing principle [10].

Xu et al. [11] developed a dual-emission CQDS-ZnCdTe quantum dots synthesized with S-doped carbon dots as ligands and established a guanine ratio fluorescence biosensor. The mechanism has been shown in Figure 3. Guanine exists at the same time between RNA and DNA, which is easily oxidized. Oxidation of DNA usually occurs on the bases. Accumulation of the oxidized bases can easily lead to aging, and modification and expansion of the bases can cause cancer and other lesions. Therefore, the measuring guanine content is particularly important. In this study, S-doped CQDS was synthesized by microwave method with citric acid and L-cysteine as raw materials, and ZnCdTe-QDs were synthesized with CDs as capping ligands. Guanine can be used as the electron donor of this composite quantum dot, which can easily form complexes with Cd and Zn atoms. In this system, fluorescence quenching is induced between three molecules of guanine quantum electric complex.

The fluorescence response of the composite quantum dots to some competing substances such as metal ions and proteins was also studied.

The results show that CDs-ZnCdTe QDs have high sensitivity and selectivity for the detection of guanine. The fluorescence emission of quantum dots is quenched by guanine. It has a good linear with correlation between guanine concentration and *I*605/*I*425 at the range of 0.25−40.0 mol/L with a detection limit of 76.0 nmol/L. The double emission ratio fluorescent probe was established by mixing two different nanoprobes. Compared with the traditional fluorescent probe, it has stronger stability, optical performance, and higher sensitivity, and has great application potential in the detection of guanine samples.

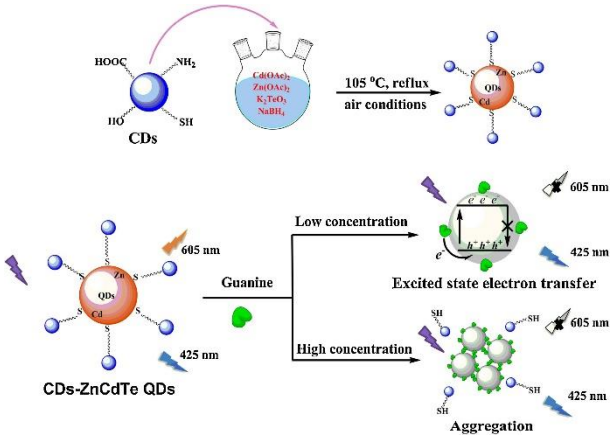

Figure 3 The proposed mechanism of fluorescence sensing for the detection of guanine [11].

Liang et al. [12] developed a ratio fluorescence biosensor for detecting double-stranded DNA (dsDNA). In this study, Water-soluble 3-MPA-capped CdTe QDs and fluorescent CDs were synthesized by hydrothermal method. The mechanism has been shown in Figure 4. In CdTe-CDs solution, mitoxantrone (MTX) with different concentrations is added MTX, as a synthetic anthraquinone derivative, can act as an electron acceptor, which can be adsorbed on the surface of quantum dots and lead to the photoluminescence quenching of quantum dots through electron transfer. Therefore, MTX can quench the fluorescence of CdTe quantum dots by an electron transfer mechanism. At the same time, CDs is not sensitive to MTX and DNA, and the change of fluorescence intensity is not obvious. Adding dsDNA (0−175 nM) to the system restores the fluorescence intensity of CdTe-CDs at 599 nm due to the strong and specific binding between dsDNA and MTX.

The quenching effect of 1000 nM MTX is 85%, and the quenching constant is 1.167×10^3 mol/L by linear fitting analysis of the different concentration of MTX. In the range of 0−50 nM, the fluorescence intensity ratio of CdTe and CDs showed a linear response to dsDNA concentration, with a detection limit of 1.0 nM. In addition, the fluorescent biosensor has good reproducibility, with a relative standard deviation ranging from 1.2% to 3.9%, and a good recovery range from 98.37% to 101.0%. This fluorescent biosensor is simple in design and low in cost, which can easily and quickly detect dsDNA. At the same time, two emission seals can reduce the influence of detection conditions, making the detection more sensitive and reliable.

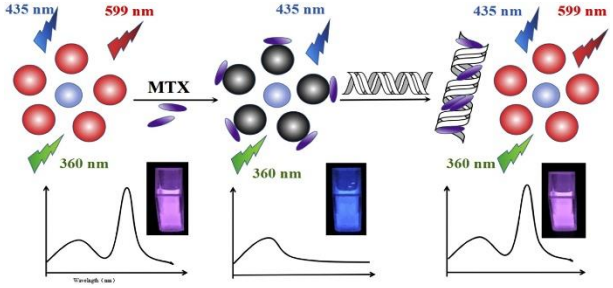

Figure 4 Schematic diagram of the fluorescent biosensor [12].

Pourghobadi et al. [13] developed a CdTe-QDs fluorescent biosensor based on thioglycolic acid (TAG) encapsulation to detect DA in human plasma samples. The mechanism has been shown in

Figure 5. The amount of dopamine in human body has a very important influence on some diseases. Low dopamine can affect the nervous system and lead to some mental diseases such as Parkinson's disease. In this study, CdTe-CQDs were synthesized with $NaBH_4$ as reducing agent, TGA as stabilizing agent, Te, and $CdSO_4$ as raw materials. The fluorescence intensity was measured by mixing the diluted dopamine solution with TGA-capped CdTe QDs at different concentrations.

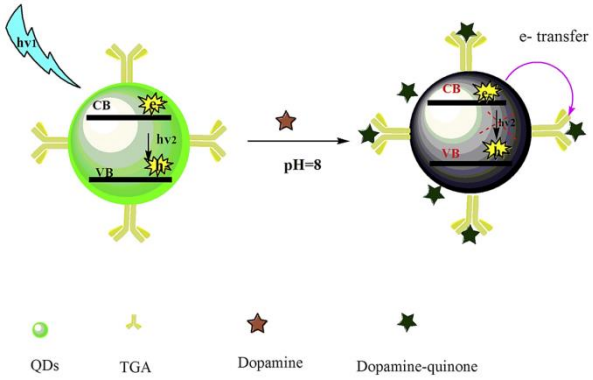

Figure 5 The fluorescence quenching mechanism for the developed fluorescent biosensor [13].

The results showed that there was a good linear relationship between dopamine concentration fluorescent intensity in the range of 0.5~10 μM, and the detection limit was 0.35 μM. Under optimal conditions, the relative standard values were 1.45% and 1.82%, respectively. Compared with the traditional carbon quantum dots and graphene sensors, the composite quantum dots bioluminescence sensor has improved selectivity and sensitivity, showing rapid response and applicability in actual sample analysis.

4. Conclusions

This research outlines the research progress of fluorescent biosensors based on quantum dots in recent years. Due to the unique characteristics of quantum dots, quantum dots have unique advantages in molecular detection, which can be developed into fluorescent biosensors with high signal-to-noise ratio, high sensitivity, and high selectivity. It has broad application prospects in clinical medicine, which can monitor apoptosis and guanine. It can also be used for food pesticides and environmental monitoring. The existing technology is gradually becoming complete in the preparation, synthesis, and application of quantum dots, but there are still many places worth exploring and studying. Some quantum dots have toxicity and their safety in the human body need to be studied. Meanwhile, the reproducibility and operation stability of existing quantum dots need to be improved.

Authors' Contributions

Jiyue Zhang and Zehan Ren complete this work together.

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