

## Advances of Fluorescent Probes Inbiomedical Science

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**Abstract:** Fluorescence probes are attracting more and more attentions in biomedical research because of their nontoxicity, sensitivity and accuracy. The advantages and disadvantages of different kinds of fluorescent probes, the research status and the progress of fluorescent labeling in biomedicine are reviewed, and the research trend and application prospect of biofluorescence probe are prospected.

**Keywords:** Fluorescence Probe; Biomedical Science; Application Progress

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Fluorescent probes are a class of small substrates that interact with proteins, nucleic acids and their macromolecules, which change their fluorescence properties. In recent years, fluorescent dye<sup>[1]</sup> and quantum dot fluorescent probe<sup>[2]</sup> have been widely used in fluorescent marking materials. Fluorescent dye has good chemical stability, large molar absorption coefficient, high fluorescence quantum yield, long fluorescence lifetime and biocompatibility of quantum dot fluorescence probe.

It has high property, large stokes position shift and good photostability. However, their defects have also seriously affected the application of quantum dot fluorescent probes and fluorescent dyes., such as the strong toxicity and relatively low chemical stability of quantum dot fluorescence probes. The photobleaching of fluorescent dyes is fast. Long afterglow nanoluminescence materials have the advantages of quantum dot fluorescence probes and fluorescent dyes, such as being excited before detection and imaging, and realizing biological sensing and imaging without excitation. Background interference caused by in situ excitation can be avoided effectively and it is a new fluorescence probe which has been reviewed in recent years. Fluorescent probes have been found in biomarkers It has the advantages of high sensitivity, low dosage, accurate detection and so on. It is often used for imaging tracers in vivo and tissues, and can also be used for the detection of substances in and out of cells<sup>[3,4]</sup>, such as detecting eggs.

Active site of white matter, destruction and repair of DNA bases.

It plays an important role in the early diagnosis of diseases<sup>[5]</sup>, and has been widely used in biological and medical research. In this paper, the research progress of fluorescent probes in recent years is reviewed, with emphasis on the ap-

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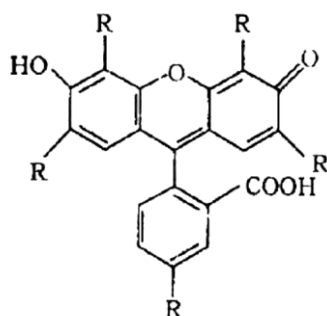
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plication of several new fluorescent probes in biomedical research.

## 1. Several Important Fluorescent Probes

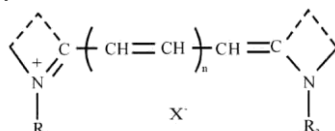
### 1.1 Fluorescent dyes

Fluorescent dyes commonly used in cell labeling include fluorescein dyes, rhodamine dyes<sup>[6,7]</sup> and cyanine dyes. Fluorescein dyes have high fluorescence quantum yield and good photostability. Their active groups are easy to be labeled with -OH, -NH<sub>2</sub>, -SH and other reactions, **Figure 1** is the main structure of fluorescein dyes.

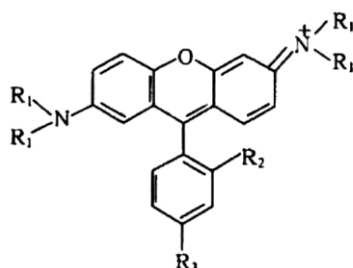


**Figure 1;** Main structural formula of fluorescein dyes.

Cyanine dyes are mainly used to label nucleic acids. In recent years, more cyanine dyes have been studied, such as thiazole (TO), oxazole orange (YO) and polymethachine series such as Cy3, Cy5, Cy7. These dyes have a wide spectrum distribution. The emission wavelength of fluorescence can reach the near infrared region (780-1200 nm), so they can avoid the ultraviolet and visible regions, improve the sensitivity and reduce the background interference. Berger *et al.* labeled sheep anti-human immunoglobulin with cyanine squareene dye containing N-hydroxysuccinimide ester active group for the first time and achieved good results. Cheng *et al.*<sup>[8]</sup> Synthesized methyl cyanide fluorescent dye Cy5, which has good biocompatibility, fluorescence emission wavelength is 780 nm, can also quickly accumulate to the location of tumor cells, using it to label mouse lung cancer cells, can clearly distinguish normal tissue and tumor tissue. **Figure 2** is the general formula of cyanine dyes.



Rhodamine dye is structurally stable and is often used as a fluorin substitute with higher fluorescence yield, lower pH sensitivity and stronger photostability. **Figure 3** is the main structural formula of Rhodamine dyeing material, where R<sub>2</sub>OR<sub>3</sub> is an active group, mainly composed of NCSL-SO<sub>2</sub>X, *et al.* It's easy to handle with the labeled ammonia. The base reaction was used to realize the labeling properties.



**Figure 3;** Main structures of Rhodamine.

Although fluorescent dyes have many advantages, they will decompose after many times of excitation and emission, and the quantum yield of fluorescence will be reduced in the physiological environment of organisms. Its Stokes shift is small, which makes the background of the sample interfere with the fluorescence greatly<sup>[11]</sup> and fluorescence. Photodyes must absorb specific energy photons in order to transit from the ground state to the excited state, and the light used must have precise wavelengths. Therefore, the shortcomings of fluorescent dyes also cause shortcomings in

applications.

## 1.2 nano quantum dots

Quantum dots are semiconductor nanoparticles with diameters ranging from 1 nm to 100 nm that can accept fluorescence generated by excited light. The application of quantum dots in biomedical field is an important prerequisite for water-solubility. Nie<sup>[12]</sup> of Indian University and others have studied the water-solubility of quantum dots in organic phase. Mercaptoethanol is attached to the ZnS shell of CdS/ZnS, and the carboxyl group is separated upstream so that the quantum dots have good hydrophilicity. It also provides conditions for the connection of quantum dots with biomolecules, and lays a foundation for the practical application of quantum dots in biofluorescence labeling.

Studies at home and abroad show that quantum dots have many incomparable advantages compared with traditional fluorescent molecular dyes, and can be better used in optical imaging. If color can be controlled, a single wavelength of light stimulates quantum dots of different sizes and emits different colors of light, the emission wavelength range from 400 nm to 2 microns, can simultaneously achieve the same cell multi-color labeling imaging; excitation spectrum is continuous and wider, emission spectrum symmetrical distribution and narrow width, can be reduced. Less spectral overlap; high quantum yield, strong photobleaching resistance, large Stokes shift and good stability<sup>[13-15]</sup>. At present, the quantum dot is a core-shell structure with ZnS as the shell and CdSe as the core. This structure has good photochemical stability and high luminescent yield<sup>[16,17]</sup>. Pang *et al.*<sup>[18]</sup> Conjugated alpha-fetoprotein monoclonal antibody with CdSe/ZnS quantum dots to detect human hepatocellular carcinoma cell line HCCLM6. The results showed that CdSe/ZnS quantum dots had targeted distribution of hepatocellular carcinoma cells, good biocompatibility and tumor location fluorescence. The signal is strong. Fig. 4 is a core-shell diagram of water-soluble CdSe / ZnS quantum dots.



**Figure 4;** Core-shell structure of CdSe/ZnS quantum dots.

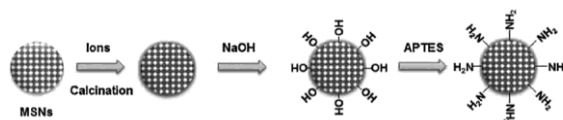
Quantum dots (QDs) as biological probes have good application prospects in the biological field. However, QDs have potential toxicity and poor photostability *in vivo*. These shortcomings seriously affect the clinical application of QDs.

## 1.3 long afterglow fluorescent probe

Long afterglow materials absorb and store energy after light can be stimulated, and can continue to emit light for a long time when the external light source is removed. Long afterglow luminescent materials have long luminescent lifetime, and with the development of preparation research, the emission spectrum has been expanded from 400 nm to 750 nm. The light penetration of this band is strong, and the absorption of biological tissues is weak, so long afterglow luminescent materials *in vivo* Imaging shows more prominent advantages. With the in-depth study of long afterglow nanomaterials, their applications in the biological field have gradually attracted much attention<sup>[20-23]</sup>.

Long afterglow nanoparticles (PLNPs) are one of the ideal imaging probes. Because of its long luminescent lifetime, it can be stimulated *in vitro*, and then be able to "excitation-free" mode of bioimaging. PLNPs have advantages that other traditional fluorescent probes do not have, such as avoiding background noise caused by excitation light and potential damage to organisms caused by excitation light, and can obtain high sensitivity and high signal-to-noise ratio imaging effect; because near-infrared light penetrates deep into biological tissues, so near-infrared long afterglow probes can be used. One step is to achieve deep tissue imaging. However, the synthesis of long afterglow luminescent materials is complex, and it needs high temperature calcination to crystallize, and even some materials are calcined at temperatures higher than 1000%. However, the materials calcined at high temperature inevitably agglomerate seriously and have poor dispersibility. After calcined at high temperature, the long afterglow materials of silicate have larger particle size and reach micron level, and almost completely precipitate in water, which is not conducive to the application

of luminescent materials in the biological field. There are almost no functional groups on the surface of long afterglow materials synthesized by traditional methods. Therefore, it is very important to modify the surface of long afterglow materials to improve their biocompatibility and water-solubility. In this research group, Sr<sub>2</sub>MgSi<sub>2</sub>O<sub>7</sub>:Eu<sup>2+</sup>, Dy<sup>3+</sup> + nano luminescent materials were prepared by sol-gel method and hydrothermal co precipitation method by adding surfactants and metal complexing agents. The water-soluble and biocompatible materials were prepared by surface hydroxylation, amination and PEG wrapping. The Sr<sub>2</sub>MgSi<sub>2</sub>O<sub>7</sub>:Eu<sup>2+</sup> and Dy<sup>3+</sup> long afterglow Nanofluorescent probes with good properties have laid a foundation for their application in biofluorescence labeling. Fig. 5 is the surface modification process of long afterglow nanoparticles.



**Figure 5;** Surface modification process of long afterglow nanoparticles.

## 2. Application of fluorescent probes in biomedicine

### 2.1 Application of fluorescent probes in tissue imaging of tissues and in vivo

Nie Shu-ming *et al.*<sup>[24]</sup> for the first time used quantum dots for in-vivo localization imaging. Quantum dots are coated with ABC triblock polymer nanoparticles and PEG, The antibody can bind to specific antigen on prostate cancer cells. Hoshion *et al.*<sup>[25]</sup> use quantum dot fluorescence probe to perform fine cell imaging of murine lymphoma. Quantum dots can enter lymphoma cells by phagocytosis. The results showed that the QDs did not affect the normal activity and function of the cells, and the survival time was long and stable.

In 2007, Chermont *et al.*<sup>[26]</sup> prepared Eu, Dy, Mn doped long afterglow nanomaterial Ca<sub>0.2</sub>Z by sol-gel method, which was applied to in vivo imaging after surface modification. The emission peak of this nanoprobe is 690nm, and the wavelength of near-infrared optical imaging. Inside, it is suitable for optical imaging. Scherman in vivo.<sup>[27]</sup> Silicate Long Afterglow was prepared by sol-gel method.

Nanomaterials, and used in animal imaging in vivo. This method is the first time to realize the image of "non-excitation" in organisms and avoid the potential damage of the traditional fluorescent labeling method to organisms.

### 2.2 Application of fluorescent probes in the detection of intracellular and extracellular substances

Fluorescent labeling probes can be used not only to label in vivo and tissues, but also to detect the movement, location and interaction of biological molecules in living cells directly and dynamically by targeting.

Lingerfelt *et al.*<sup>[28]</sup> used quantum dot biotin and immunoaffinity chromatography to detect protein toxin, and the lowest concentration was 10 g / L. Zheng Xiaoluan *et al.*<sup>[29]</sup> prepared the quantity sub-point -CK19 antibody probe, used it to label breast cancer cells, and observed by laser confocal microscope. The fine cytoplasm and membrane of the specifically labeled CK19 can be observed, and bright yellow fluorescent light can be observed. Anti-body immunofluorescence probe can be used to trace breast cancer cell lines with good biological targeting and optical properties. In 2011, Yan *et al.*<sup>[30]</sup> prepared Ca<sub>1.86</sub> Mg<sub>0.14</sub> ZnSi<sub>2</sub> O<sub>7</sub>:Eu<sup>2+</sup>, Dy<sup>3+</sup> long afterglow nanoparticles for the first time. PLNPs were coated with polyethylene imide (PEI) and combined with AFP antibody modified gold nanoparticles (Ab-AuNPs) by electrostatic interaction to apply to biological molecules alpha-fetoprotein (AFP). Application of detecting. 2.3 fluorescent probe in disease diagnosis

In recent years, fluorescent probes have also been widely used in the detection of fine bacteria, diseases, fungi and various cancerous cells, especially in the identification of tumours. The advantages of rapid detection, good reproducibility and amperometric accuracy play an important role in the early diagnosis of related diseases<sup>[31]</sup>, which has aroused the widespread concern of the authors of scientific research.

In 2012, Pan Zhenwei *et al.*<sup>[32]</sup> prepared near infrared long afterglow nanomaterials excited by visible light using

ZnGa<sub>2</sub>O<sub>4</sub> as the substrate and doped with Cr<sup>3+</sup>, the remaining glow time exceeded 100 hours; in 2013, on the basis of this study, M Aldiney, *et al.*<sup>[33,34]</sup> by using sol-gel method, the nanocrystalline materials of NG2 + 4: Cr<sup>3+</sup> were prepared by sol-gel method, and their luminescent properties were superior. Surface modification of long afterglow nanoparticles was carried out by PEG encapsulation. Long afterglow nanoprobe with good compatibility and low toxicity can be used to detect and trace specific tumor cells.

### 3. Summary and Prospect

Fluorescent probes have been widely used in biomedicine because of their unique advantages such as good chemical stability, low toxicity, long life span and good fluorescence performance. However, the current fluorescent probes have their own advantages and disadvantages. Fluorescent labeling techniques are limited to varying degrees. The sensitivity and photostability of organic fluorescent dyes need to be improved. Because of their defects, most quantum dot fluorescence probes are still in the preliminary stage of study, and reduce the toxicity and increase the yield. Further research is needed to enhance biological compatibility. Although long afterglow new nanoprobe At present, there are still many problems that need to be solved in the application of biological imaging. For example, nanoparticles are easy to agglomerate and cause particles to be too large, dispersity needs to be improved, surface modification is complex and targeting is low, and so on. However, its irreplaceable advantages have attracted great interest of researchers, and the research on long afterglow nanowires has emerged one after another. It is believed that with the continuous development of fluorescent marker technology and the combination of fluorescent probes and related probes, researchers will break through the existing limitations and make new fluorescent probes in the field of molecular biology. Many fields such as biomedical imaging and disease diagnosis are promising.

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