Review Article

Open Access

Jiafeng Wan, Xiaoyuan Zhang, Kai Zhang*, and Zhiqiang Su* Biological nanoscale fluorescent probes: From structure and performance to bioimaging

https://doi.org/10.1515/revac-2020-0119 received September 10, 2020; accepted October 27, 2020

Abstract: In recent years, nanomaterials have attracted lots of attention from researchers due to their unique properties. Nanometer fluorescent materials, such as organic dyes, semiconductor quantum dots (QDs), metal nanoclusters (MNCs), carbon dots (CDs), etc., are widely used in biological imaging due to their high sensitivity, short response time, and excellent accuracy. Nanometer fluorescent probes can not only perform in vitro imaging of organisms but also achieve in vivo imaging. This provides medical staff with great convenience in cancer treatment. Combined with contemporary medical methods, faster and more effective treatment of cancer is achievable. This article explains the response mechanism of threenanometer fluorescent probes: the principle of induced electron transfer (PET), the principle of fluorescence resonance energy transfer (FRET), and the principle of intramolecular charge transfer (ICT), showing the semiconductor QDs, precious MNCs, and CDs. The excellent performance of the three kinds of nano fluorescent materials in biological imaging is highlighted, and the application of these three kinds of nano fluorescent probes in targeted biological imaging is also introduced. Nanometer fluorescent materials will show their significance in the field of biomedicine.

Keywords: nano fluorescent probe materials, semiconductor quantum dots, metal nanoclusters, carbon dots, bioimaging

1 Introduction

For centuries, cancer has been a global problem that has plagued human health. It has become the leading cause of morbidity and mortality in developed countries. The development and research of low-toxicity, high-targeting, and high-selectivity drugs have become a hotspot for many scientists [1,2]. Nanoparticles have a unique size and shape-dependent physical and chemical properties [3,4]. They can be combined with targeting ligands to enter the body, target specific tumor cells, and realize cell imaging in vivo [5]. The development of nanotechnology can mostly help surgeons delineate the edges of tumors, identify residual tumor cells and micrometastasis, and determine whether the cancer is entirely removed [6].

Nanomaterials usually refer to materials with one dimension or more within the range of 0.1–100 nm in the three-dimensional scale of tiny particles [7,8]. Fluorescent nanomaterials include organic fluorescent dyes [9], fluorescent proteins [10], QDs, MNCs, CDs, etc. Organic fluorescent dyes are the most common nano fluorescent probe materials. Among them, rhodamine fluorescent dyes normally have a high quantum yield. They are widely used in biological applications, such as biomolecular labeling and detection, cancer diagnosis, and so on [11]. However, organic dyes and fluorescent proteins have poor fluorescence stability, weak photobleaching resistance, and short fluorescence lifetime, so they are greatly restricted in actual use [12–14]. Emerging nano fluorescent materials such as semiconductor QDs, precious MNCs, and CDs are not only used in biological imaging and targeting tumor cell applications due to their excellent physical and chemical properties, but also in drug and gene delivery, biosensors, medical treatment, photocatalysis, electrocatalysis, etc. have been widely used [1,15–17].

This article mainly introduces three materials: semiconductor QDs, precious MNCs, and CDs as nanometer fluorescent probes. First, we introduce the response mechanism of nano fluorescence probes. Then we briefly explain the basic concepts and related properties of the three materials and introduced each material in detail. Finally, we

^{*} **Corresponding author: Kai Zhang**, Dental Medical Center, China-Japan Friendship Hospital, Beijing 100029, People's Republic of China, e-mail: zkzryhyy@126.com

^{*} Corresponding author: Zhiqiang Su, State Key Laboratory of Chemical Resource Engineering, Beijing Key Laboratory of Advanced Functional Polymer Composites, Beijing University of Chemical Technology, 100029 Beijing, China, e-mail: suzq@mail.buct.edu.cn Jiafeng Wan, Xiaoyuan Zhang: State Key Laboratory of Chemical Resource Engineering, Beijing Key Laboratory of Advanced Functional Polymer Composites, Beijing University of Chemical Technology, 100029 Beijing, China

DE GRUYTER

show the specific application of three kinds of nano fluorescent probes in bioimaging and tumor cell targeting.

2 Design principle of nanometer fluorescent probe

Fluorescent probes are usually composed of three parts, namely recognition genes, linkers, and signal reporter genes. The recognition gene can react with the analyte, and the signal reporter gene (also called fluorophore) can convert the chemical environment change caused by the reaction of the recognition gene with the analyte into an output model that can be easily observed. The linker, as its name implies, connects the recognition gene and the signal reporter gene, becoming the hub of molecular recognition. The analyte reacts with the recognition gene. This reaction is transmitted to the signal reporter gene through the linker. The reaction is reflected by the fluorescent signal change of the signal reporter gene. This path is the response mechanism of the fluorescent probe [18]. There are generally three design mechanisms for the fluorescence recognition process: the principle of photoinduced electron transfer (PET), the principle of fluorescence resonance energy transfer (FRET), and the principle of intramolecular charge transfer (ICT). These design principles are shown in Figure 1. Below we introduce these design mechanisms in detail.

2.1 Photoinduced electron transfer (PET)

In PET, there is a linker between the receptor and the fluorophore. The linker connects the fluorophore and the receptor, while also separating the two during fluorophore excitation. Electrons are transferred from the highest occupied molecular orbital (HOMO) of the acceptor (or donor) to the semi-occupied molecular orbital (SOMO) of the fluorophore (or acceptor), resulting in fluorescence quenching. However, if the non-bonded electron pair of the donor is combined with the analyte, the redox potential of the donor increases, and the HOMO energy is lower than the fluorophore, which will cause the fluorescence to be switched on, and that limits the PET process [19,20].

2.2 Fluorescence resonance energy transfer (FRET)

FRET refers to the process in which the excitation energy of a donor transfers to the acceptor when the emission spectrum



Figure 1: Design principle of nanometer fluorescent probe: (a) PET, (b,c) ICT, (d) FRET. Reprinted image with permission from Ref. [20], Copyright 2019, Elsevier B.V.

of the donor overlaps with the excitation spectrum of the acceptor, causing the acceptor to emit fluorescence. This is a non-radiation intermolecular dipole effect. The key factors that determine the fluorescence resonance energy transfer are mainly two points: (1) the emission spectrum of the donor and the excitation spectrum of the acceptor effectively overlap; (2) the distance between the donor and the acceptor needs to be maintained between 1–10 nm. The third one is the relative orientation of donor emission dipole moment and acceptor absorption dipole moment [21].

2.3 Intramolecular charge transfer (ICT)

In ICT system, the fluorophore and the acceptor are directly connected to a single entity. This single entity can provide electrons as an electron donor or accept electrons as an electron acceptor, so it has two functions. The addition of analytes will cause the intensity of the dipole on one side of a single entity to change, thereby affecting the overall intensity change and spectral shift. When the analyte interacts with the acceptor end, the electron-withdrawing property of the acceptor increases, and the emission spectrum shifts red; when the analyte interacts with the donor segment, the electron-donating properties of the donor decrease, and the emission spectrum shifts blue. Compared with the PET mechanism, there is no spectral shift during fluorescence quenching in the PET process, and a significant spectral change occurs in the ICT mechanism [19,20].

3 Classification of nanofluorescent probes

3.1 Semiconductor quantum dots (QDs)

Semiconductor QDs are novel fluorescent probes for biological and medical applications, usually composed of nanoscale II-VI and III-V elements. QDs are a kind of nanoparticles with quantum domain limiting effect, whose size is usually between 1–10 nm and nearly spherical [22–24]. Due to the development of quantum confinement, semiconductor QDs have unique optical properties. Compared with organic dyes, QDs have many advantages in fluorescence properties [25]. Firstly, QDs have a broad and continuous excitation spectrum and a narrow and symmetrical fluorescence emission spectrum (Figure 2) [26]. The size of QDs determines the wavelength of fluorescence emission.



Figure 2: Physical properties of QDs. (a) The fluorescence emission spectrum of QDs is adjustable, and multiple QDs can be excited by a single light source. (b) The emission spectrum of QDs can range from near-infrared region to the ultraviolet region. Reprinted image with permission from Ref. [30], Copyright 2015, American Chemical Society.

By changing the size and chemical composition of QDs, the fluorescence emission spectrum of QDs can be adjusted from near-ultraviolet to near-infrared, with the wavelength range of 400-2000 nm [27]. Second, compared with organic dyes, QDs have a more massive stokes shift, which means that the difference between the peak of the excitation wavelength of QDs and the height of the emission wavelength is massive. In this way, the overlap of the excitation spectrum with the emission spectrum and its influence on the fluorescence detection in low signal detection can be avoided [28,29]. Third, QDs have high fluorescence intensity and good stability. Unlike conventional organic fluorescent dyes, which are easily bleached by light, QDs are 100 to 1000 times more resistant to light bleaching than organic dyes and fluorescent proteins [25,26]. In addition, the fluorescence yield of QDs is high, and the fluorescence life generated by QDs is long, and their excited state life lasts 20-50 ns. This enables the QDs to obtain fluorescence signals without background interference during use [22,30]. QDs are used in bioimaging, drug delivery, biosensor, cell tracking, and other fields due to their excellent optical properties [31].

In 1985, Brus first discovered QDs in colloids: ZnS and CdS particles [32]. Then in 1998, Alivisatos and Nie made the first successful use of semiconductor QDs as fluorescent probes in biology [33,34]. Since the synthesized QDs contain heavy metal elements (such as cadmium), they cannot be directly used in biological applications, so they need to be treated with water solubility [33]. Nie et al. used ZnS to encapsulate CdSe and then added a thioglycolic acid solution (Figure 3). On one hand, the sulfhydryl group in thioglycolic acid can be combined with Zn atoms. On the other hand, the carboxylic acid group of thioglycolic acid makes QDs water-soluble. Studies have shown that the optical properties of the obtained QDs have not been changed [34]. At present, there are three main types of methods for synthesizing QDs, namely physical method, chemical method, and biological method. Material synthesis methods include laser physical vapor deposition and laser irradiation of large particles. However, QDs prepared by this method have low yield and poor stability. Therefore, many researchers use chemical processes to synthesize QDs [35]. Chemical synthesis method is mainly divided into an organic synthesis method and an aqueous synthesis method. QDs obtained by this method have good monodispersity and optical properties [30].

3.2 Metal nanoclusters (MNCs)

Fluorescent MNCs are likely to replace organic dyes or QDs [17]. Noble MNCs are typically smaller than 2 nm and consist of several or dozens of atoms [36]. Similar to semiconductor QDs, emission wavelength of noble MNCs can be adjusted from UV-Visible light to near-infrared light, and a single light source can excite the emission wavelength of multiple colors. Emission energy of MNCs can be regulated by determining the number of constituent atoms and ligands. Similarly, noble MNCs have high fluorescence intensity and broad stokes shift [17,37]. However, unlike semiconductor QDs, noble MNCs exhibit low toxicity and ultrafine size [38]. Large nanoparticles can quickly cause kidney barrier effect and cause serious side effects in the body. Noble MNCs can be effectively removed in the body due to their small size [37]. Noble metal nanoparticles have a strong surface plasmon resonance effect due to the electron excitation of the continuous conductive band. In contrast, noble MNCs, due to their small size close to the Fermi wavelength of electrons,



Figure 3: (a) Schematic diagram of CdSe QD capped by ZnS covalently coupled with thioglycolic acid and protein. (b) TEM of QD-transferin (an iron transport protein) conjugates. Reprinted image with permission from Ref. [34], Copyright 1998, Science.



Figure 4: Properties of Au NCs. Reprinted image with permission from Ref. [40], Copyright 2012, American Chemical Society.

will lose their surface plasmon resonance effect, showing discrete electronic structure and molecular properties [17,39,40]. It is important to note that colloid and cluster are often used interactively by researchers. Generally speaking, metal nanoparticles larger than 10 nm are called colloid, while metal nanoparticles smaller than 2 nm are called MNCs [41]. Noble MNCs also have some properties related to size, such as HOMO-LUMO transition, oxidizing reduction, magnetism, and handshape (as is shown in Figure 4) [37,40,41]. Besides, noble MNCs also have an essential property, which can be used as a new type of nano-catalysts in the fine chemical industry, medicine, food additives, and other fields [42]. The ultra-fine size and low toxicity of noble MNCs make their applications in biological imaging and fluorescence labeling very attractive [43].

In the 1960s, Malatesta firstly reported the synthesis of Au NCs using phosphine as a ligand. However, the structure of the Au NCs was still unknown [44]. After continuous research, Malatesta deduced the crystal structure of the Au NCs [45]. The precious MNCs were prepared by the template method. They had good biocompatibility and were quickly modified to adapt to specific applications. Therefore, the template method has become the primary method to prepare precious MNCs [46]. At present, proteins, DNA, polymers, dendrimers, and small sulfhydryl molecules are used to prepare noble MNCs.

3.3 Carbon dots (CDs)

In 2004, Xu et al. inadvertently isolated a kind of fluorescent nanoparticles when preparing carbon nanotubes by arc discharge method [47]. Later, in 2006, the Sun research group used the laser ablation method to prepare the fluorescent nanoparticles and named them CDs [48]. CDs are carbon nanoparticles with a particle size of less than 10 nm [49]. CDs are also referred to as carbon quantum dots (CQDS) in some literature. CDs have excellent photoluminescence properties. They show high fluorescence quantum yield, anti-swelling, and anti-bleaching photoluminescence decay, and long concentration range. They could be adjusted from deep ultraviolet to near infrared [50–53]. Besides, compared with QDs, CDs have good biocompatibility and low toxicity, which can almost be ignored.

Moreover, at a high concentration of CDs, the damage of CDs to organisms is shallow [53]. There are functional groups such as the hydroxyl group, carboxyl group, and carbonyl group on the surface of CDs. These functional groups cause CDs have good water solubility and provide the possibility of functionalization of CDs, which improves the effectiveness of CDs in practical application [51,54]. Due to the high price and large size of QDs, which affect the observation in vivo, QDs are subject to many limitations in practical application [27]. However, CDs with small size, low production costs, and low prices can be produced on a large scale, which is considered as a superior substitute for semiconductor QDs [54].

Synthesis methods of CDs are generally divided into two types: top-down and bottom-up. CDs obtained by the top-down approach tend to have high crystallinity and complete structure [51,55]. However, CDs with amorphous carbon core, large surface functional groups, and doping sites may be obtained by the bottom-up method (Figure 5) [51,55]. Therefore, according to the different carbon cores, CDs are generally divided into graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs). GQDs are made up of one or more layers of graphene with edges connected by chemical groups and with a degree of crystallinity. CNDs are generally spherical carbon nanoparticles with no lattice. PDs are usually polymerized, assembled, or cross-linked by a linear nonconjugated polymer [56,57]. The photoluminescence mechanism of CDs has long been controversial among researchers. Three theories have been discussed the most, namely quantum confinement theory, molecular state theory, and surface state theory [52,58,59]. The difference of the synthesis method, prepared CDs structure, and performance will be different, so it is important to understand the preparation method of CDs [58].



Figure 5: Synthesis method of CDs. Reprinted image with permission from Ref. [57], Copyright 2015, Springer Nature.

4 Applications of nanoscale fluorescent probes in biotargeting and bioimaging

4.1 QDs in biological targeting and biological imaging

Traditional fluorescent materials (such as organic dyes, fluorescent proteins, etc.) are prone to photobleaching, and the signal intensity is low. Multiple light sources are required to excite different fluorophores and other defects, which are subject to many restrictions in actual use [14,60,61]. QDs have vigorous fluorescence intensity and stability and have massive stokes shifts, photobleaching resistance, and controllable optical properties. Therefore, in biomedical applications such as bioimaging and targeting labels, QDs are very attractive to researchers [62]. In general, QDs can enter cells four ways: (a) passive absorption through endocytosis; (b) binding or modification with biopolymers, and then internalization through interaction with cell membranes and endocytosis; (c) direct physical manipulation of the cells (such as microinjection); (d) use of several uptake modes at the same time in combination [63].

In the past ten years, research on the biological imaging of QDs has significantly been developed [30]. Generally speaking, directly prepared QDs have poor biocompatibility, have specific toxicity, and are prone to non-specific adsorption and aggregation during use in the biological environment. Therefore, they need to be modified in biomedicine (usually using natural coupling [64] or encapsulation [65]). Then they can possess good water solubility, biocompatibility, and low cytotoxicity during use [14,66,67]. Gao et al. used amphiphilic triblock copolymers to improve QDs and used them for in vivo imaging in mice and targeted imaging of human prostate cancer cells. Passive targeted imaging is achieved through the penetration and retention of QDs at the tumor site. At the same time, QDs can also be combined with antibodies, and the targeted effect of antibodies and antigens can be used to achieve actively targeted imaging. However, the speed and efficiency of active targeting are significantly higher than the rate and efficiency of passive targeting (Figures 6) [28].

QDs are also used in multi-mode imaging [62,68], multicolor imaging [60,69], and multiphoton imaging [70]. Among them, multicolor imaging can identify individual malignant tumors in a complex biological tissue environment. This application provides detailed molecular information and morphological features in clinical medical diagnosis [60]. Multi-mode imaging



Figure 6: (a) Passive targeted imaging of quantum dots; (b) active targeted imaging of quantum dots . Reprinted image with permission from Ref. [28], Copyright 2004, Springer Nature.

combines QDs imaging technology with other imaging technologies (such as nuclear magnetic resonance imaging, computed tomography, etc.) [30]. Shibu et al. used nuclear magnetic resonance imaging technology and near-infrared fluorescence imaging technology to track the fate of modified QDs in mice, and found that kidneys can effectively excrete QDs within 48 h [68]. QDs have more excellent resolution under two-photon excitation and also have higher temperature sensitivity. Highquality thermal gradient images can be recorded in the fluid, and the internal temperature changes of individual cells can also be measured [70].

4.2 Noble MNCs in biotargeting and bioimaging

Although QDs have been successfully applied to cancer targeting and cell imaging in living animals, the mechanism of biodegradation in animals remains unclear due to the toxicity of QDs because of their heavy metal components [71,72]. In addition, the size of QDs is relatively large. Although this allows QDs to have extremely high resolution, it also reduces imaging efficiency and sensitivity, thus limiting their application in clinical imaging [73]. Compared with QDs, noble MNCs not only have the advantages of QDs in biological imaging but also have the characteristics of small size, low toxicity, and good biocompatibility. Therefore, they become more attractive fluorescent probes in biological imaging [72,74,75]. Wu et al. were the first group to use precious MNCs in near-infrared fluorescence imaging. They found that noble MNCs avoided uptake of the reticuloendothelial system due to their small size.

Thereby, they passively targeted Hela tumors in tumorbearing mice through the retention effect (EPR) [73].

Among the precious MNCs, the properties of Au NCs have been studied the most extensively [46,76]. However, due to the small size of Au clusters, the yield of Au NCs, synthesized by traditional methods, is low. Fluorescence intensity is not enough to achieve biological imaging. Therefore, Sun et al. used iron oxidase to assemble two Au NCs. The fluorescence intensity of the cluster is enhanced, and the fluorescence emission is adjustable. This kind of fluorescent probe can be used for whole-body imaging, especially kidneys [77]. Researchers not only use the fluorescence intensity of precious MNCs for biological imaging [78] but also use their fluorescence lifetime for imaging. Shang et al. developed the Fluorescence Lifetime Imaging (FLIM) technology. Through life-gating, cell imaging can be achieved without autofluorescence. FLIM images can not only show the uptake of MNCs by cells but also provide information on the environmental changes of NCs [79].

Due to the small size of precious MNCs, a suitable template preparation (such as glutathione) is chosen to make them quickly cleared by the kidneys and are therefore discharged out of the body after biological imaging [80,81]. While targeting tumor cells, precious MNCs can also provide sufficient information on cancer cells by detecting other substances in the organism (such as a high concentration of reactive oxygen species [82], PH value [83], etc.). Like fluorescent probe imaging with QDs, noble MNCs probe in vivo imaging can also be used with other imaging methods (such as computed tomography, magnetic resonance imaging technology) to achieve multi-mode imaging and provide more tumor information [84], as is shown in Figure 7.



Figure 7: (a) Schematic diagram of Au Gds preparation; (b) Au Gds for trimodal tumor imaging. Reprinted image with permission from Ref. [80], Copyright 2011, WILEY-VCH Verlag GmbH & Co.

4.3 CDs in biotargeting and bioimaging

CDs are used as an excellent fluorescent bio-imaging agent due to their broad absorption spectrum, narrow emission spectrum, adjustable photoluminescence, good chemical stability, and light stability, and photobleaching resistance [85,86]. Compared with QDs and noble MNCs, CDs have the advantages of low toxicity, good biocompatibility, low cost, facile synthesis, easy surface modification, and large-scale preparation [86–90]. The physical and chemical properties of CDs can be controlled by the reactants, reaction products, and reaction by-products [86]. Passivating CDs by using different organic substances and placing the prepared CDs in an oxidized state can improve the fluorescence quantum yield of the CDs, thereby obtaining higherquality biological imaging [91].

There are various functional groups on the surface of the CDs. These functional groups not only cause CDs to have good biocompatibility but also provide the possibility of surface modification of the CDs [92]. Hua et al. combined the surface functional groups of CDs with protoporphyrin IX (PpIX) to obtain multifunctional fluorescent CQDs. This kind of CDs can not only perform high-quality nucleolus imaging of fixed cells but it can cause that cells are imaged with high-quality nucleoli

(Figure 8) [88]. Due to poor biocompatibility and high toxicity, quantum dots are rarely used for drugs and gene delivery. CDs can realize the dual application of non-viral gene vectors and biological imaging probes at the same time, so that genetic material can be transferred to specific cells of patients to achieve gene therapy [93,94]. Zhou et al. modified the surface of CDs to make it have a positive surface charge, so that the prepared CD can be used as a nanoprobe for biological imaging, and as an efficient nonviral gene carrier to achieve drug and gene transfer. They also found that CDs have high transfection efficiency and cannot enter the nucleus during the transfection process. They speculate that the high transfection efficiency of CDs comes from the strong aggregation of macromolecular pDNA, which prevents its enzymatic hydrolysis during transportation. On the other hand, small size of the prepared CDs and their positive surface charge comtribute to high transfection rate [94].

Some researchers are interested in the development of carbon points emitting red fluorescence and their application in near-infrared biological imaging [95–97]. Li et al. attached electron acceptor groups (rich in sulfoxide/carbonyl groups) to the outer layer and edges of the CDs. These groups lead to increased surface oxidation and discontinuous energy levels, thereby enhancing the infrared absorption band and near-infrared



Figure 8: CDs synthesis schematic diagram and targeting the nucleolus imaging mechanism. Reprinted image with permission from Ref. [88], Copyright 2018, American Chemical Society.

fluorescence emission of CDs. They then successfully applied the prepared CDs to the imaging of the stomach of live mice [95].

5 Conclusions and outlook

In summary, nano fluorescent probes have great potential in biomedicine (Table 1). They can quickly target tumor cells and image biological cells. This provides excellent opportunities for medical personnel in cancer treatment. While targeting tumor cells, nanometer fluorescent probes can be combined with drugs and genes to accurately transport therapeutic drugs or expressed genes to tumor cells, thereby achieving the effect of cancer treatment. Through cell imaging in organisms, it is possible to understand relevant information of tumor cells better and help medical personnel to treat cancer better. Semiconductor QDs, precious MNCs, CDs, and other nano fluorescent materials all have adjustable emission spectra, good fluorescence, chemical stability, and vigorous fluorescence intensity will become the leading research direction of nanometer fluorescent probes.

Compared with the three materials, QDs and MNCs were developed earlier as nanoscale fluorescent probes, and the synthesized QDs have certain toxicity due to the presence of heavy metal elements. Therefore, in the process of using quantum dots in vivo, it is necessary to modify them, and improve the biocompatibility of quantum dots to reduce toxicity by means of biological coupling or ligand exchange. Compared to the other two materials, the size of the QDs is larger, which reduces the efficiency and sensitivity of imaging, which makes it more restricted in actual use. The size of MNCs is small, and the presence of the protective agent provides MNCs with good biocompatibility and low cytotoxicity. It is very noteworthy that the fluorescence properties of precious metal nanoclusters and precious metal nanoparticles are slightly different, which is easy to be confused in some literature. Noble metal nanoparticles have a surface plasmon resonance effect due to their large size, so their fluorescence is weak. However, the size of the noble metal nanoclusters is close to the Fermi wavelength of electrons, and the surface plasmon resonance effect disappears, showing strong fluorescence properties. CDs were discovered relatively late and have been extensively studied in the past decade. They have been used in various fields such as biomedicine, photocatalysis, electrocatalysis, and biosensors. Compared with QDs, CDs are smaller in size, have good biocompatibility, and are less toxic; compared with MNCs, CDs have a wider source of raw materials and are easily obtained from ordinary biomass materials. In addition, CDs have low cost, can be mass-produced, simple synthesis, and easy surface modification. CDs are likely to become excellent green fluorescent materials.

Types	Materials	Methods	Fluorescent emission	Applications	Results	Refs
Quantum dots	PbQDs	Water phase method	Near -infrared	NIR-II Fluorescence Imaging	Detection of bacterial infection and the dynamic balance of the immune system in mice	[65] [98]
	(dBSA) -CuInS ₂ /ZnS	Water phase method	Near -infrared	Targeted bioimaging of macrophages, liver cancer cells, and pancreatic cells	Produce a red fluorescent signal	[99]
	MoS ₂ QDs	Water phase method	Blue	Detect Cr ³⁺ concen- tration in water or biological samples	MoS ₂ QDs exhibits a linear fluores- cence quenching response with the increase of Cr ³⁺ ion concentration	[100]
Metal nano clusters	Peptide@Ag/ Au NCs	Peptides as templates	Red	Hypochlorite (ClO) lysosome targeted imaging	Fluorescent probes have intense cell penetration, strong targeting ability to lysosomes, and high sensitivity to ClO	[75]
	Au NCs	Peptides as templates	Near -infrared	NIR-II Fluorescence Imaging	Detect many small blood vessels for brain imaging	[78]
	AuNCs@ NMOFs	Small molecules of mercaptol as templates	Red	Detection of highly reactive oxygen species (hROS)	High sensitivity and selectivity to hROS in cells	[81]
Carbon dots	GQDs	Microwave method	Near -infrared	NIR Fluorescence Imaging	Effectively internalized by Hela cells and successfully imaged in vivo and in vitro	[97]
	CPDs	Hydrothermal method	Red	In vivo cell imaging in mice	Produce high-efficiency red fluores- cence without toxicity	[96]
	PNF -GQDs	Electrochemistry method	Blue	Label Hela tumor cells	Have higher labeling efficiency, can target and image at the same time	[101]

Table 1: Application and research results of different nanometer fluorescent probes

Due to the late discovery of CDs, there are many synthesis methods, so the photoluminescence mechanism of CDs has not been fully understood. Currently, quantum confinement theory, molecular state theory and surface state theory are more common. Researchers continue to explore the photoluminescence mechanism of CDs. In addition, for CDs, there is a need to develop green synthesis methods. To achieve this goal, one can start from two aspects: raw materials and the synthesis process of CDs. Raw materials of CDs can be selected from renewable natural fuels, such as organisms, biological wastes and protein products to replace common reaction precursors such as graphene and carbon nanotubes. Synthesis methods of CDs are mainly divided into two types: top-down method and bottom-up method. Relatively speaking, top-down method requires more stringent conditions, and since raw materials are more expensive, this method is not suitable for large-scale production. Therefore, bottom-up method has become the mainstream method for CDs synthesis. It can be considered to meet the

requirement of synthetic CDs under the condition of reducing energy use. As a relatively young nano fluorescent material, CDs still have a lot of potential for researchers to develop.

Acknowledgments: The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (NSFC, Grant no. 51873016), the Fundamental Research Funds for the Central Universities (JD2014) and the Joint Project of BRC-BC (Biomedical Translational Engineering Research Center of BUCT-CJFH) (XK2020-11).

Conflict of interest: The authors have declared no conflict of interest.

References

[1] Wei W, Zhang X, Zhang S, Wei G, Su Z. Biomedical and bioactive engineered nanomaterials for targeted tumor photothermal therapy: A review. Mater Sci Eng C Mater Biol Appl. 2019;104:109891.

- [2] Belkahla H, Herlem G, Picaud F, Gharbi T, Hemadi M, Ammar S, Micheau O. TRAIL–NP hybrids for cancer therapy: a review. Nanoscale. 2017;9(18):5755–68.
- [3] Qi P, Zeng J, Tong X, Shi J, Wang Y, Sui K. Bioinspired synthesis of fiber-shaped silk fibroin-ferric oxide nanohybrid for superior elimination of antimonite. J Hazard Mater. 2021;403:123909.
- [4] Wang J, Fang T, Li J, Yan Y, Li Z, Zhang J. Precise Mesoscopic Model Providing Insights into Polymerization-Induced Self-Assembly. Langmuir. 2020;36(27):8009–16.
- [5] Swierczewska M, Lee S, Chen X. Inorganic Nanoparticles for Multimodal Molecular Imaging. Mol Imaging. 2011;10(1): 3–16.
- [6] Singhal S, Nie S, Wang MD. Nanotechnology applications in surgical oncology. Annu Rev Med. 2010;61:359–73.
- [7] Jin R, Zeng C, Zhou M, Chen Y. Atomically Precise Colloidal Metal Nanoclusters and Nanoparticles: Fundamentals and Opportunities. Chem Rev. 2016;116(18):10346–413.
- [8] Zhang Y, Fang T, Hou Q, Li Z, Yan Y. Water desalination of a new three-dimensional covalent organic framework: a molecular dynamics simulation study. Phys Chem Chem Phys. 2020;22(29):16978–84.
- [9] Tang B, Xing Y, Li P, Zhang N, Yu F, Yang G. A Rhodamine-Based Fluorescent Probe Containing a Se–N Bond for Detecting Thiols and Its Application in Living Cells. J Am Chem Soc. 2007;129(38):11666–7.
- [10] Van Huy L, Tanaka C, Imai T, Yamasaki S, Miyamoto T. Synthesis of 12-O-Mono- and Diglycosyl-oxystearates, a New Class of Agonists for the C-type Lectin Receptor Mincle. ACS Med Chem Lett. 2019;10(1):44–9.
- [11] Niu G, Liu W, Wu J, Zhou B, Chen J, Zhang H, et al. Aminobenzofuran-fused rhodamine dyes with deep-red to near-infrared emission for biological applications. J Org Chem. 2015;80(6):3170–5.
- [12] Zrazhevskiy P, Gao X. Multifunctional quantum dots for personalized medicine. Nano Today. 2009;4(5):414–28.
- [13] Chakraborty A, Boer JC, Selomulya C, Plebanski M. Amino Acid Functionalized Inorganic Nanoparticles as Cutting-Edge Therapeutic and Diagnostic Agents. Bioconjugate Chem. 2017;29(3):657–71.
- [14] Dubertret B, Skourides PA, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. In Vivo Imaging of Quantum Dots Encapsulated in Phospholipid Micelles. Science. 2002;298(5599):1759–62.
- [15] Sharma S, Dutta V, Singh P, Raizada P, Rahmanisani A, Hosseinibandegharaei A, Thakur VK. Carbon quantum dot supported semiconductor photocatalysts for efficient degradation of organic pollutants in water: A review. J Clean Prod. 2019;228:755–69.
- [16] Mattoussi H, Palui G, Na HB. Luminescent quantum dots as platforms for probing in vitro and in vivo biological processes. Adv Drug Deliver Rev. 2012;64(2):138–66.
- [17] Yu P, Wen X, Toh Y, Ma X, Tang J. Fluorescent Metallic Nanoclusters: Electron Dynamics, Structure, and Applications. Part Part Syst Char. 2015;32(2):142–63.
- [18] Bissell RA, De Silva AP, Gunaratne HQN, Lynch PLM, Maguire GEM, Sandanayake KRAS. Molecular fluorescent signalling with 'fluor-spacer-receptor' systems: approaches to sensing

and switching devices via supramolecular photophysics. Chem Soc Rev. 1992;21(3):187–95.

- [19] Jung HS, Verwilst P, Kim WY, Kim JS. Fluorescent and colorimetric sensors for the detection of humidity or water content. Chem Soc Rev. 2016;45(5):1242–56.
- [20] Bigdeli A, Ghasemi F, Abbasimoayed S, Shahrajabian M, Fahimikashani N, Jafarinejad S, et al. Ratiometric fluorescent nanoprobes for visual detection: Design principles and recent advances - A review. Anal Chim Acta. 2019;1079:30–58.
- [21] Zhang X, Hu Y, Yang X, Tang Y, Han S, Kang A, et al. FOrster resonance energy transfer (FRET)-based biosensors for biological applications. Biosens Bioelectron. 2019;138:111314.
- [22] Gao X, Yang L, Petros JA, Marshall FF, Simons JW, Nie S. In vivo molecular and cellular imaging with quantum dots. Curr Opin Biotechnol. 2005;16(1):63–72.
- [23] Smith AM, Gao X, Nie S. Quantum dot nanocrystals for in vivo molecular and cellular imaging. Photochem Photobiol. 2004;80(3):377–85.
- [24] Mal J, Nancharaiah YV, Van Hullebusch ED, Lens PNL. Metal chalcogenide quantum dots: biotechnological synthesis and applications. RSC Adv. 2016;6(47):41477–95.
- [25] Smith AM, Duan H, Mohs AM, Nie S. Bioconjugated quantum dots for in vivo molecular and cellular imaging. Adv Drug Deliver Rev. 2008;60(11):1226–40.
- [26] Kairdolf BA, Qian X, Nie S. Bioconjugated Nanoparticles for Biosensing, in Vivo Imaging, and Medical Diagnostics. Anal Chem. 2017;89(2):1015–31.
- [27] Bailey RE, Smith AM, Nie S. Quantum dots in biology and medicine. Physica E. 2004;25(1):1–12.
- [28] Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol. 2004;22(8):969–76.
- [29] Zrazhevskiy P, Sena M, Gao X. Designing multifunctional quantum dots for bioimaging, detection, and drug delivery. Chem Soc Rev. 2010;39(11):4326–54.
- [30] Zhou J, Yang Y, Zhang CY. Toward Biocompatible Semiconductor Quantum Dots: From Biosynthesis and Bioconjugation to Biomedical Application. Chem Rev. 2015;115(21):11669–717.
- [31] Bonilla JC, Bozkurt F, Ansari S, Sozer N, Kokini JL. Applications of quantum dots in Food Science and biology. Trends Food Sci Tech. 2016;53(53):75–89.
- [32] Rossetti R, Hull R, Gibson JM. Excited electronic states and optical spectra of ZnS and CdS crystallites in the ≅15 to 50 Å size range: Evolution from molecular to bulk semiconducting propert. J Chem Phys. 1985;82(1):552–9.
- [33] Bruchez MP, Moronne MM, Gin P, Weiss S, Alivisatos AP. Semiconductor Nanocrystals as Fluorescent Biological Labels. Science. 1998;281(5385):2013–6.
- [34] Chan WCW, Nie S. Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection. Science. 1998;281(5385):2016–8.
- [35] Zeng H, Du X, Singh SC, Kulinich SA, Yang S, He J, Cai W. Nanomaterials via Laser Ablation/Irradiation in Liquid: A Review. Adv Funct Mater. 2012;22 (7):1333–53.
- [36] Park S, Kim H, Lim SC, Lim K, Lee ES, Oh KT, et al. Gold nanocluster-loaded hybrid albumin nanoparticles with fluorescence-based optical visualization and photothermal conversion for tumor detection/ablation. J Control Release. 2019;304 :7–18.

- [37] Song X, Goswami N, Yang H, Xie J. Functionalization of metal nanoclusters for biomedical applications. Analyst. 2016;141(11):3126–40.
- [38] Diaz SA, Hastman DA, Medintz IL, Oh E. Understanding energy transfer with luminescent gold nanoclusters: a promising new transduction modality for biorelated applications. J Mater Chem B. 2017;5(39):7907–26.
- [39] Reiner AT, Fossati S, Dostalek J. Biosensor platform for parallel surface plasmon-enhanced epifluorescence and surface plasmon resonance detection. Sensors Actuat B-chem. 2018;257:594–601.
- [40] Qian H, Zhu M, Wu Z, Jin R. Quantum Sized Gold Nanoclusters with Atomic Precision. Accounts Chem Res. 2012;45(9):1470–9.
- [41] Saha K, Agasti SS, Kim C, Li X, Rotello VM. Gold Nanoparticles in Chemical and Biological Sensing. Chem Rev. 2012;112(5):2739–79.
- [42] Fang J, Zhang B, Yao Q, Yang Y, Xie J, Yan N. Recent advances in the synthesis and catalytic applications of ligand-protected, atomically precise metal nanoclusters. Coordin Chem Rev. 2016;322:1–29.
- [43] Lin C-AJ, Lee C-H, Hsieh J-T, Wang H-H, Li JK, Shen J-L, et al. Synthesis of Fluorescent Metallic Nanoclusters toward Biomedical Application: Recent Progress and Present Challenges. J Med Biol Eng. 2009;29(6):276–83.
- [44] Naldini L, Cariati F, Simonetta G, Malatesta L. Gold-tertiary phosphine derivatives with intermetallic bonds. Chem Commun. 1966;(18):647–48.
- [45] Mcpartlin M, Mason R, Malatesta L. Novel cluster complexes of gold(0)–gold(I). Journal of The Chemical Society D: Chem Commun. 1969;(7):334.
- [46] Zhang Y, Zhang C, Xu C, Wang X, Liu C, Waterhouse GIN, et al. Ultrasmall Au nanoclusters for biomedical and biosensing applications: A mini-review. Talanta. 2019;200:432–42.
- [47] Xu X, Ray R, Gu Y, Ploehn HJ, Gearheart LA, Raker K, Scrivens WA. Electrophoretic Analysis and Purification of Fluorescent Single-Walled Carbon Nanotube Fragments. J Am Chem Soc. 2004;126(40):12736–7.
- [48] Sun Y, Zhou B, Lin Y, Wang W, Fernando KAS, Pathak P, et al. Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence. J Am Chem Soc. 2006;128(24):7756–7.
- [49] Patel KD, Singh RK, Kim H. Carbon-based nanomaterials as an emerging platform for theranostics. Mater Horiz. 2019;6(3):434–69.
- [50] Boakyeyiadom KO, Kesse S, Opokudamoah Y, Filli MS, Aquib, Joelle MMB, et al. Carbon dots: Applications in bioimaging and theranostics. Int J Pharm. 2019;564:308–17.
- [51] Hu C, Li M, Qiu J, Sun Y. Design and fabrication of carbon dots for energy conversion and storage. Chem Soc Rev. 2019;48(8):2315–37.
- [52] Mintz KJ, Zhou Y, Leblanc RM. Recent development of carbon quantum dots regarding their optical properties, photoluminescence mechanism, and core structure. Nanoscale. 2019;11(11):4634–52.
- [53] Mohammadinejad R, Dadashzadeh A, Moghassemi S, Ashrafizadeh M, Dehshahri A, Pardakhty A, et al. Shedding light on gene therapy: Carbon dots for the minimally invasive image-guided delivery of plasmids and noncoding RNAs - A review. J Adv Res.2019;18:81–93.

- [54] Molaei MJ. A review on nanostructured carbon quantum dots and their applications in biotechnology, sensors, and chemiluminescence. Talanta. 2019;196:456–78.
- [55] Li K, Liu W, Ni Y, Li D, Lin D, Su Z, Wei G. Technical synthesis and biomedical applications of graphene quantum dots. J Mater Chem B. 2017;5(25):4811–26.
- [56] Li K, Zhao X, Wei G, Su Z. Recent Advances in the Cancer Bioimaging with Graphene Quantum Dots. Curr Med Chem. 2017;25(25):2876–93.
- [57] Zhu S, Song Y, Zhao X, Shao J, Zhang J, Yang B. The photoluminescence mechanism in carbon dots (graphene quantum dots, carbon nanodots, and polymer dots): current state and future perspective. Nano Res. 2015;8(2):355–81.
- [58] Liu ML, Chen BB, Li CM, Huang CZ. Carbon dots: synthesis, formation mechanism, fluorescence origin and sensing applications. Green Chem. 2019;21(3):449–71.
- [59] Xiao L, Sun H. Novel properties and applications of carbon nanodots. Nanoscale Horiz. 2018;3(6):565–97.
- [60] Liu J, Lau SK, Varma V, Moffitt RA, Caldwell ML, Liu T, et al. Molecular Mapping of Tumor Heterogeneity on Clinical Tissue Specimens with Multiplexed Quantum Dots. ACS Nano. 2010;4(5):2755–65.
- [61] Prasanna APS, Venkataprasanna KS, Pannerselvam B, Asokan V, Jeniffer RS, Venkatasubbu GD. Multifunctional ZnO/SiO2 Core/Shell Nanoparticles for Bioimaging and Drug Delivery Application. J Fluoresc. 2020;30(5):1075–83.
- [62] Stasiuk GJ, Tamang S, Imbert D, Poillot C, Giardiello M, Tisseyre C, et al. Cell-Permeable Ln(III) Chelate- Functionalized InP Quantum Dots As Multimodal Imaging Agents. ACS Nano. 2011;5(10):8193–201.
- [63] Breger JC, Delehanty JB, Medintz IL. Continuing progress toward controlled intracellular delivery of semiconductor quantum dots. Wires Nanomed Nanobi. 2015;7(2):131–51.
- [64] Kakizuka T, Ikezaki K, Kaneshiro J, Fujita H, Watanabe TM, Ichimura T. Simultaneous nano-tracking of multiple motor proteins via spectral discrimination of quantum dots. Biomed Opt Express. 2016;7(7):2475–93.
- [65] Chen J, Feng S, Chen M, Li P, Yang Y, Zhang J, et al. In Vivo Dynamic Monitoring of Bacterial Infection by NIR-II Fluorescence Imaging. Small. 2020;16(34):e2002054.
- [66] Hong H, Shi J, Yang Y, Zhang Y, Engle JW, Nickles RJ, et al. Cancer-targeted optical imaging with fluorescent zinc oxide nanowires. Nano Lett. 2011;11(9):3744–50.
- [67] Wu X, Liu H, Liu J, Haley K, Treadway JA, Larson JP, et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol. 2003;21(1):41–6.
- [68] Shibu ES, Ono K, Sugino S, Nishioka A, Yasuda A, Shigeri Y, et al. Photouncaging Nanoparticles for MRI and Fluorescence Imaging in Vitro and in Vivo. ACS Nano. 2013;7(11):9851–9.
- [69] Zrazhevskiy P, Gao X. Quantum dot imaging platform for single-cell molecular profiling. Nat Commun. 2013;4(1):1619.
- [70] Maestro LM, Rodriguez EM, Rodriguez FS, La Cruz MCI, Juarranz A, Naccache R, et al. CdSe Quantum Dots for Two-Photon Fluorescence Thermal Imaging. Nano Lett. 2010;10(12):5109–15.
- [71] Chen H, Li B, Ren X, Li S, Ma Y, Cui S, Gu Y. Multifunctional near-infrared-emitting nano-conjugates based on gold

clusters for tumor imaging and therapy. Biomaterials. 2012;33(33):8461–76.

- [72] Sun H, Sakka Y. Luminescent metal nanoclusters: controlled synthesis and functional applications. Sci and Technol Adv Mat. 2014;15(1):014205.
- [73] Wu X, He X, Wang K, Xie C, Zhou B, Qing Z. Ultrasmall nearinfrared gold nanoclusters for tumor fluorescence imaging in vivo. Nanoscale. 2010;2(10):2244–9.
- [74] An Y, Ren Y, Bick M, Dudek A, Hong-Wang Waworuntu E, Tang J, et al. Highly fluorescent copper nanoclusters for sensing and bioimaging. Biosens Bioelectron. 2020;154:112078.
- [75] Jia M, Mi W, Guo S, Yang QZ, Jin Y, Shao N. Peptide-capped functionalized Ag/Au bimetal nanoclusters with enhanced red fluorescence for lysosome-targeted imaging of hypochlorite in living cells. Talanta. 2020;216:120926.
- [76] Zhang W, Lin D, Wang H, Li J, Nienhaus GU, Su Z, et al. Supramolecular Self-Assembly Bioinspired Synthesis of Luminescent Gold Nanocluster-Embedded Peptide Nanofibers for Temperature Sensing and Cellular Imaging. Bioconjugate Chem. 2017;28(9):2224–29.
- [77] Sun C, Yang H, Yuan Y, Tian X, Wang L, Guo Y, et al. Controlling Assembly of Paired Gold Clusters within Apoferritin Nanoreactor for in Vivo Kidney Targeting and Biomedical Imaging. J Am Chem Soc. 2011;133(22):8617–24.
- [78] Liu H, Hong G, Luo Z, Chen J, Chang J, Gong M, et al. Atomic-Precision Gold Clusters for NIR-II Imaging. Adv Mater. 2019;31(46):e1901015.
- [79] Shang L, Azadfar N, Stockmar F, Send W, Trouillet V, Bruns M, et al. One-Pot Synthesis of Near-Infrared Fluorescent Gold Clusters for Cellular Fluorescence Lifetime Imaging. Small. 2011;7(18):2614–20.
- [80] Zhou C, Long M, Qin Y, Sun X, Zheng J. Luminescent Gold Nanoparticles with Efficient Renal Clearance. Angew Chem. 2011;50(14):3168–72.
- [81] Cao X, Cheng S, You Y, Zhang S, Xian Y. Sensitive monitoring and bioimaging intracellular highly reactive oxygen species based on gold nanoclusters@nanoscale metal-organic frameworks. Anal Chim Acta. 2019;1092:108–16.
- [82] Chen T, Hu Y, Cen Y, Chu X, Lu Y. A dual-emission fluorescent nanocomplex of gold-cluster-decorated silica particles for live cell imaging of highly reactive oxygen species. J Ame Chem Soc. 2013;135(31):11595–602.
- [83] Ding C, Tian Y. Gold nanocluster-based fluorescence biosensor for targeted imaging in cancer cells and ratiometric determination of intracellular pH. Biosens Bioelectron. 2015;65:183–90.
- [84] Xu C, Wang Y, Zhang C, Jia Y, Luo Y, Gao X. AuGd integrated nanoprobes for optical/MRI/CT triple-modal in vivo tumor imaging. Nanoscale. 2017;9(13):4620–8.
- [85] Zhang P, Zhao X, Ji Y, Ouyang Z, Wen X, Li J, et al. Electrospinning graphene quantum dots into a nanofibrous membrane for dual-purpose fluorescent and electrochemical biosensors. J Mater Chem B. 2015;3(12):2487–96.
- [86] Fang Y, Guo S, Li D, Zhu C, Ren W, Dong S, Wang E. Easy Synthesis and Imaging Applications of Cross-Linked Green Fluorescent Hollow Carbon Nanoparticles. ACS Nano. 2012;6(1):400–9.
- [87] Hsu P, Shih Z, Lee C, Chang H. Synthesis and analytical applications of photoluminescent carbon nanodots. Green Chem. 2012;14(4):917–20.

- [88] Hua X, Bao Y, Wu F. Fluorescent Carbon Quantum Dots with Intrinsic Nucleolus-Targeting Capability for Nucleolus Imaging and Enhanced Cytosolic and Nuclear Drug Delivery. ACS Appl Mater Inter. 2018;10(13):10664–77.
- [89] Zou W, Ji Y, Wang X, Zhao Q, Zhang J, Shao Q, et al. Insecticide as a precursor to prepare highly bright carbon dots for patterns printing and bioimaging: A new pathway for making poison profitable. Chem Eng J. 2016;294:323–32.
- [90] Pappalardo JS, Macairan JR, Macina A, Poulhazan
 A, Quattrocchi V, Marcotte I, Naccache R. Effects of polydopamine-passivation on the optical properties of carbon dots and its potential use in vivo. Phys Chem Chem Phys. 2020;22(29):16595–605.
- [91] Hu L, Sun Y, Li S, Wang X, Hu K, Wang L, et al. Multifunctional carbon dots with high quantum yield for imaging and gene delivery. Carbon. 2014;67:508–13.
- [92] Santos CIM, Rodríguez-Pérez L, Gonçalves G, Pinto SN, Melle-Franco M, Marques PAAP, et al. Maçôas EMS. Novel hybrids based on graphene quantum dots covalently linked to glycol corroles for multiphoton bioimaging. Carbon. 2020;166:164–74.
- [93] Pierrat P, Wang R, Kereselidze D, Lux M, Didier P, Kichler A, et al. Efficient in vitro and in vivo pulmonary delivery of nucleic acid by carbon dot-based nanocarriers. Biomaterials. 2015;51:290–302.
- [94] Zhou J, Deng W, Wang Y, Cao X, Chen J, Wang Q, et al. Cationic carbon quantum dots derived from alginate for gene delivery: One-step synthesis and cellular uptake. Acta Biomater. 2016;42:209–19.
- [95] Li D, Jing P, Sun L, An Y, Shan X, Lu X, et al. Near-Infrared Excitation/Emission and Multiphoton-Induced Fluorescence of Carbon Dots. Adv Mater. 2018;30(13):1705913.
- [96] Liu J, Li D, Zhang K, Yang M, Sun H, Yang B. One-Step Hydrothermal Synthesis of Nitrogen-Doped Conjugated Carbonized Polymer Dots with 31% Efficient Red Emission for In Vivo Imaging. Small. 2018;14(15):e1703919.
- [97] Hasan MT, Gonzalez-Rodriguez R, Lin CW, Campbell E, Vasireddy S, Tsedev U, et al. Rare-Earth Metal Ions Doped Graphene Quantum Dots for Near-IR In Vitro/In Vivo/Ex Vivo Imaging Applications. Adv Opt Mater. 2020, published ahead of print; DOI:10.1002/adom.202000897.
- [98] Kong Y, Chen J, Fang H, Heath G, Wo Y, Wang W, et al. Highly
 Fluorescent Ribonuclease-A-Encapsulated Lead Sulfide
 Quantum Dots for Ultrasensitive Fluorescence in Vivo
 Imaging in the Second Near-Infrared Window. Chem Mater.
 2016;28(9):3041–50.
- [99] Hu S, Zhang B, Zeng S, Liu L, Yong KT, Ma H, Tang
 Y. Microfluidic chip enabled one-step synthesis of biofunctionalized CuInS2/ZnS quantum dots. Lab Chip.
 2020;20(16):3001–10.
- [100] Desai ML, Jha S, Basu H, Saha S, Singhal RK, Kailasa SK. Simple hydrothermal approach for synthesis of fluorescent molybdenum disulfide quantum dots: Sensing of Cr(3+) ion and cellular imaging. Mater Sci Eng C Mater Biol Appl. 2020;111:110778.
- [101] Su Z, Shen H, Wang H, Wang J, Li J, Nienhaus GU, et al. Motif-Designed Peptide Nanofibers Decorated with Graphene Quantum Dots for Simultaneous Targeting and Imaging of Tumor Cells. Adv Funct Mater. 2015;25(34):5472–8.