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The Fluorescence Bioassay Platforms on Quantum Dots Nanoparticles

Tiancai Liu,[1,3] Bisen Liu,[2] Haili Zhang,[1] and Ying Wang[1]

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In this paper, we present the optical properties and the platforms on fluorescent quantum dots for biological labeling, biomedical engineering and biosensor in molecular imaging. Quantum dots possess several properties that make them very attractive for fluorescent tagging: broad excitation spectrum, narrow emission spectrum, precise tunability of their emission peak, longer fluorescence lifetime than organic fluorophores and negligible photobleaching. We describe how to take such advantages of quantum dots to develop the technology and employ it to build assay platforms. Finally, ultrasensitivity, multicolor, and multiplexing of the technology of semiconductor quantum dots open up promising and interesting possibilities for bioassay platform.

KEY WORDS: Quantum dot; nanocrystals; biolabeling; bioprobe; bioassay platform.

INTRODUCTION

There are many conventional techniques for biolabeling and biosensor using isotopic elements, organic dyes, and so on. But due to the disadvantage of radioactive detection of isotopic detection system, researchers have developed many nonisotopic detection techniques [1–5]. Current nonisotopic detection technology is mainly based on organic dyes, which has helped researchers to obtain much success. This technology, however, cannot meet the needs for long-time, ultrasensitivity, multicolors, multiplex bioassay due to that organic dyes have vital photobleaching and defective spectrum with narrow emission and broad excitation. Now, researchers have developed newly technology using quantum dots. These quantum dots of II–VI and III–V semiconductor in the 2–12 nm size range have attracted a great deal of research interest in the past few years in the field of physics, engineering, chemistry and biology [6–14]. Dramatically different from the bulk state, properties of QDs are dependent upon quantum confinement effects [15–17] in all three spatial dimensions. So far, the platforms based on this technology owing to properties of these semiconductor QDs demonstrate great potential in applications for biology and biomedicine. In this paper, we focus on the properties and technology of QDs.

OPTICAL PROPERTIES OF SEMICONDUCTOR QUANTUM DOTS

Semiconductor quantum dots (QDs) are 10^{-9}-meter scale nanocrystals (Fig. 1a) that are neither small molecules nor bulk solids and smaller than their exciton Bohr radii. Their composition and small size consisting of hundreds to thousands of atoms give these dots extraordinary optical properties that can be readily controlled by changing the size [18–21] or composition [14,22] of the dots in the synthesis procedure. On absorbing light, semiconductor QDs quickly re-emit the light but in a different color with longer wavelength, which is fluorescent. These fluorescent semiconductor nanocrystals combine the most sought-after characteristics, such as multiple colors and

[1] College of Life Science & Technology, Huazhong University of Science and Technology, Wuhan, P. R. China, 430074.
[2] School of Applied Mathematics, University Electronic Science and Technology of China, Chengdu, P. R. China.
[3] To whom correspondence should be addressed. E-mail: tcliu2000@163.com

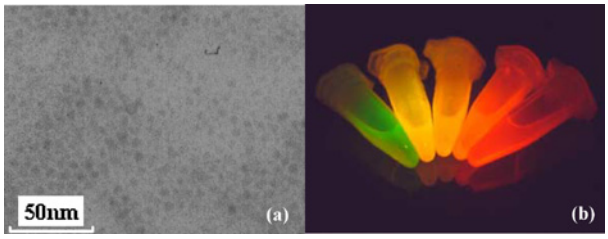

Fig. 1. Semiconductor ZnS@CdSe quantum dots. (a) Transmission electron microscopy (TEM) shows ZnS@CdSe quantum dots, (b) ZnS@CdSe quantum dots with different size excited by the same light source in chloroform solution.

brightness, offered by either fluorescent dyes or semiconductor light emitting diodes. In addition, QD particles have many unique optical properties that are found only in this material. And the most striking property is that the color of QDs—both in absorption and emission—can be "tuned" to any chosen wavelength by simply changing their size. The principle behind this unique property is the quantum confinement effect. This leads to different-sized QDs emitting light of different wavelengths that are shorter as the decrease of their sizes (Fig. 1b). Although other organic and inorganic materials exhibit the fluorescence, semiconductor QDs are the ideal fluorophore that are bright, non-photobleaching with narrow, symmetric emission spectra (Fig. 2), and have multiple resolvable colors that can be excited simultaneously using a single excitation wavelength (Fig. 1b). By using only a small number of semiconductor materials and an array of different sizes, we can make QDs with colors that span the spectrum, from ultraviolet to infrared.

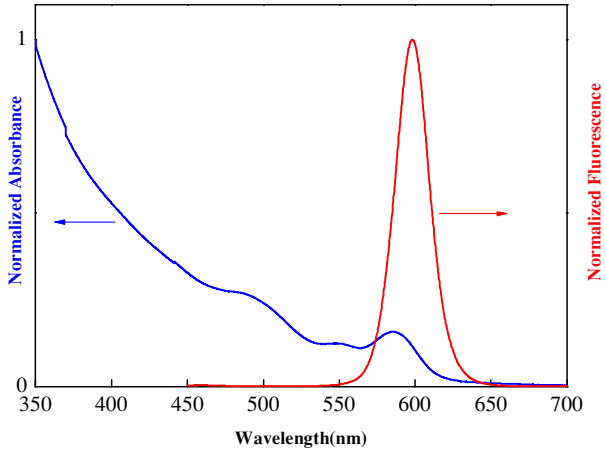

Fig. 2. The absorption and emission spectra of QDs (4 nm core). The wavelength of first absorption peak is 585 nm and that of the emission peak is 598 m with full width at half maximum (FWHM) 26 nm and quantum yield 31%.

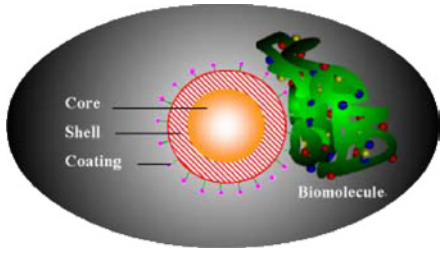

Fig. 3. Cartoon of magnified view of a single QD probe.

TECHNOLOGY ABOUT SEMICONDUCTOR QUANTUM DOTS

QD Optical Bioprobes

One of the strategies on the technology is quantum dot optical bioprobes made from quantum dot, which are solubilized and conjugated to affinity molecules, results from the anatomy of probes composed of three parts generally (Fig. 3). The first part of the QD nanocrystal synthesis is the creation of the semiconductor "core-shell." The composition (e.g. cadmium selenide, cadmium telluride, and indium arsenide) and size of the spherical core determine the optical properties of the quantum dot. The core material is selected to coarsely control the emission wavelength region. The size is then used to fine-tune the exact wavelength desired and the emission width is controlled by the size distribution, and now the newly techniques allow us to prepare very monodisperse samples with emission-peak full widths at half max (FWHM) in the 20–35 nm range (Fig. 2).

The second part is the shell which is inorganic materials overcoating the core and involves providing novel surface coatings to link the QD particles to biomolecules. This shell serves to protect the core, amplify the optical properties, and insulate the core from environmental effects. A single monolayer of this shell is capable of amplifying the quantum yield and photostability of these materials tenfold. Additional shell monolayers are typically added to enhance chemical stability. However, there are some "bare" QDs probes without any shell, which attach to biomolecules with the help of their water-soluble organic coating mentioned below.

To link QD nanocrystals to proteins, nucleic acids or small molecules, a third layer is an organic surface coating. There are several synthetic strategies that result in QD nanocrystals that are buffer-soluble, have reactive groups for linking to biomolecules, and reduce nonspecific binding (Fig. 4). A variety of organic molecules and polymers can be used to cover the nanocrystals, both protecting them from the environment and providing a chemical handle to

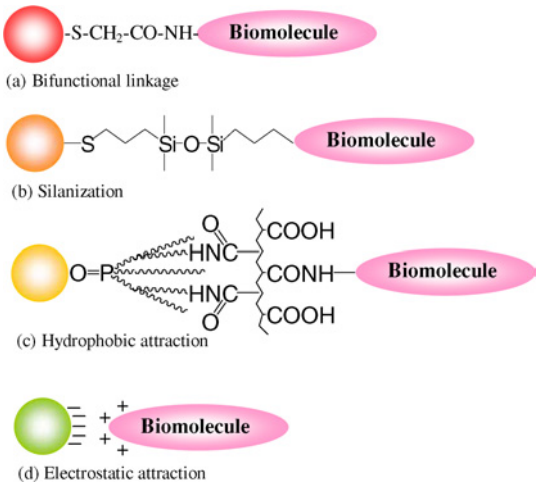

Fig. 4. Synthetic strategies of QD Probes, (a) use of a bifuntional ligand such as mercaptoacetic acid for linking QDs to biomolucules [5]. (b) tri-n-octylphosphine oxide-capped QDs bound to a modified acrylic acid polymer by hydrophobic forces [4]. (c)QD solubilization and bioconjugation using a mercaptosilane compound [23]. (d)Positively charged biomolecules are linked to negatively charged QDs by electrostatic attraction [24].

link them with other molecules. Moreover, novel coatings are developed for these materials based on a variety of hydrophilic and hydrophobic coating strategies, and employ these chemical techniques to couple antibodies, streptavidin, lectins, and nucleic acids to the QDs. The biomolecules coupled to the quantum dot retain their biological activity, and can be used in assays with very little change to existing protocols.

Additionally, QDs can be applied to bioprobes technology because of their size scale. Comparing to that of commonly used fluorescent proteins, QDs differ significantly in size from the small green fluorescent protein to the much larger phycoerythrin.

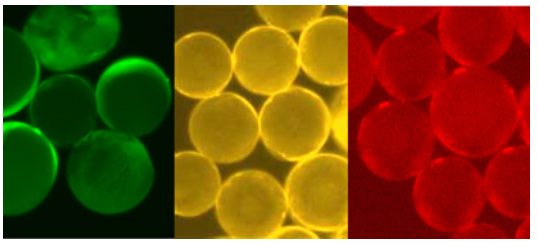

Fig. 6. 100 μm-sized fluorescence polystyrene beads respectively coded by green, yellow, and red quantum dots ($\lambda_{ex} = 515$ nm).

QD Optical Codes

Another strategy is quantum dot bar codes. QDs can be used as bar codes to create highly multiplexed assays. Generating unique color combinations of QDs, and capturing them in particles such as cells [25] or polystyrene beads [26], can distinctively identify each cell or particle by a spectral code. When excited with blue light, the emitted light spectrum determines the spectral code. Therefore, with QDs used as bar codes, highly multiplexed assays can be developed.

First is QDs code particles. Because of their unique optical properties, QDs can be used as building blocks for a novel and flexible encoding platform utilizing latex microspheres such as polystyrene beads, that is to say the QDs bar code particles platform. By mixing various combinations of spectrally distinct QDs, together with the much larger latex beads (Fig. 5), it is possible to generate many thousands of resolvable codes by wavelength and intensity of fluorescence spectra of QDs used to be trapped

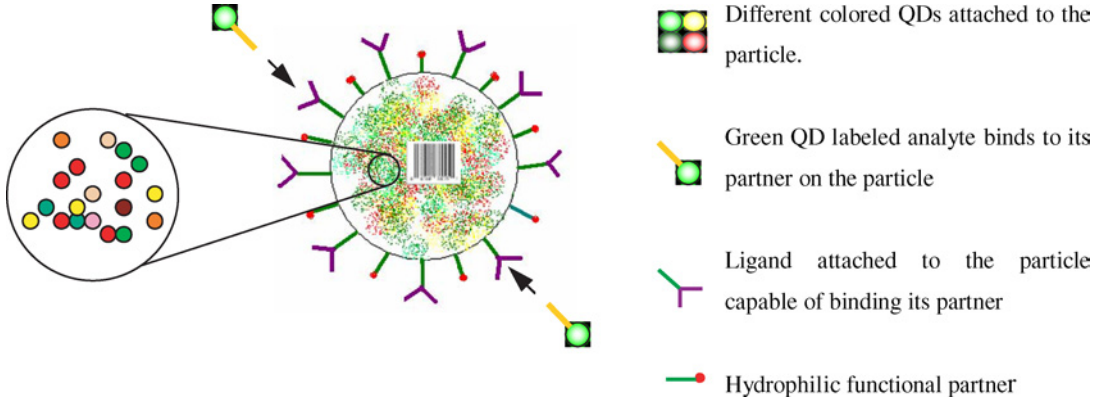

Fig. 5. Cartoon of QD code bead strategy.

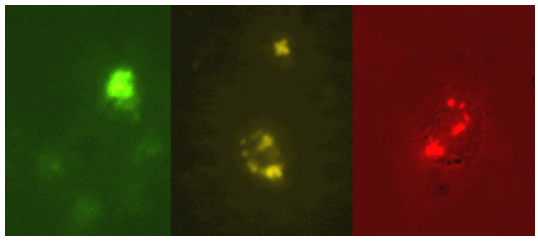

Fig. 7. Fluorescent phagocytes respectively coded by green, yellow and red quantum dots ($\lambda_{ex} = 510$ nm).

inside or on the outside of beads, which enable the bead to be identified and the code detected (Fig. 6). After that, by attaching a different biological ligand to these uniquely, spectrally encoded latex beads many assays can be analyzed simultaneously. These QDs code particles have the advantage of near solution-phase kinetics, can be used for many types of assays from immunoassays to SNP detection, are easy to handle, and can be detected using a variety of methods.

QDs also can be used to encode cells. we can deliver QDs into live or fixed cells for cellular encoding. These cellular bar codes can be used in a variety of whole cell assays important to the pharmaceutical industry. For example, a compound's potency and selectivity for a receptor can be simultaneously screened against a variety of receptor subtypes by encoding each cell line, and corresponding subtype, with a unique quantum dot code. The encoded cell lines are mixed and added to assay wells for screening by flow cytometry or fluorescence microscopy. The following (Fig. 7) is one of the examples of QDs code cell technology.

Platforms of Biotechnology About QDs

Based on such biotechnology about semiconductor QDs, many platforms can be built about bioanalysis and diagnostics. First is the QD labeling platform based on the QD probes technology; and in recent years, it has been applied widely in biology including microarray labeling, microscopy, flow cytometry, diagnostic tests in vitro or in vivo, and others.

Second is the QD code particle system which fits analysis in gene expression, protein profiling, SNP analysis, diagnostics. Last is QD code cell system and this platform can help researchers with tools in drug specificity screening, toxicology and others. The model of QD code platform is schemed as the following (Fig. 8).

CONCLUSION AND PROSPECTIVES

The novel optical properties of quantum dot optical probes make them extremely easy to detect using simple instrumentation, which absorb light so efficiently over a broad spectral range that it is easy to excite them with simple, inexpensive, illumination sources. Thus, traditional light sources such as lamps, lasers, and LEDs are excellent excitation sources for such optical probe platforms, which allows us to design simple and inexpensive optical systems for detection of ultra-sensitive, multiplexed assays, resulting a dramatic increase in sensitivity and throughput coupled with a significant decrease in cost. In addition, powerful QD code platforms developed are capable of both decoding beads or cells and reading assay reporters with a high level of sensitivity. Moreover, we are developing several methods in applications of the platforms of biotechnology about semiconductor QDs geared toward genomic, proteomic, and drug discovery solutions. That is to say the technology is promising.

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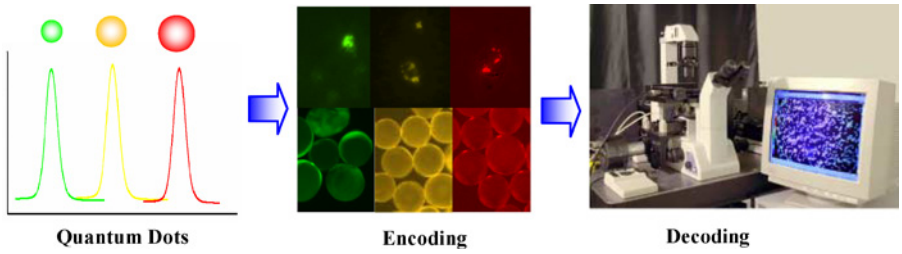

Fig. 8. Model of quantum dots code assay platform.

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