ACS APPLIED MATERIALS & INTERFACES

Review

www.acsami.org

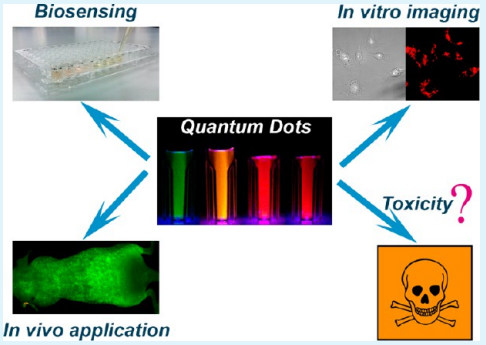
Functionalized Quantum Dots for Biosensing and Bioimaging and Concerns on Toxicity

Yucheng Wang,[†] Rui Hu,[†] Guimiao Lin,[†] Indrajit Roy,[‡] and Ken-Tye Yong*[,†]

[†]School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore 639798, Singapore

[‡]Department of Chemistry, University of Delhi, Delhi 110007, India

ABSTRACT: Considerable efforts have been devoted to the development of novel functionalized nanomaterials for bio-oriented applications. With unique optical properties and molar scale production, colloidal photoluminescent quantum dots (QDs) have been properly functionalized with controlled interfaces as new class of optical probes with extensive use in biomedical research. In this review, we present a brief summary on the current research interests of using fine engineered QDs as a nanoplatform for biomedical sensing and imaging applications. In addition, recent concerns on the potential toxic effects of QDs are described as a general guidance for the development on QD formulations in future studies.



KEYWORDS: *quantum dots, biosensing, bioimaging, biofunctionalization, toxicity*

1. INTRODUCTION

Quantum dots (QDs) are semiconductor nanocrystals whose electrons and holes are quantum-confined in all three spatial dimensions. They have attracted much attention during the past few decades due to their unique physical properties as distinguished from the corresponding bulk materials and have shown great potential in the fabrication of next generation optoelectronic devices.[1,2] Since their first demonstration as optical probes for cell labeling in the late 1990s,[3,4] QDs have emerged as a new class of fluorescent contrast agents for biomedical applications. In contrast with conventional organic dyes or fluorescent proteins, QDs possess unique optical properties. They have broad absorption band, narrow emission band, size-tunable emission from visible to near infrared (NIR) range, superior brightness, long fluorescent lifetime, and more importantly, they are highly resistant to photobleaching and have relatively large surface area for biofunctionalization.[5] With these advantages, many novel applications based on QD formulations have been developed for biosensing and bioimaging, such as cell tracking and multichannel and multimodal imaging.

The solution-phase synthesis method has revolutionized the QD fabrication technology for its capability of molar scale production of highly luminescent QDs with narrow emission bandwidth and excellent optical and colloidal stability. To date, QDs of various semiconductor materials have been obtained through solution phase synthesis, of which cadmium-based QDs had caught most of the attention and subsequently they dominated the biomedical research field for quite a few years, before other non-heavy metal based ones appeared. For most of the biosensing and bioimaging applications, there are two major issues to consider, i.e., the colloidal stability in aqueous environment and the potential toxicity. Since a large portion of the QDs are obtained in organic solvents, a phase transfer procedure is usually needed before applying them for biomedical applications. The toxicity issue is to some extent complicated. It is mainly because of the intrinsic potential toxic nature of the semiconductor materials themselves. Other factors, such as the surface modification, size and shape effect, delivery methods, and dosage are also important and should be comprehensively considered.

Lots of efforts have been proposed to improve the performance of QDs in biomedical applications through proper surface modification. A high band gap material zinc sulfide (ZnS) is widely used to shell-coat the QD cores creating a Type I core/shell structure to improve the physiochemical and optical performance of the QDs, as well as their biocompatibility in biological system. In addition, manifold state-of-the-art surface engineering methods have been applied to the QDs enabling them for enormous spectacular applications across the biomedical research field, especially in biosensing and bioimaging. The synthesis and functionalization of QDs have been well developed over the past decade and numerous reviews can be found covering the topic. In this contribution, we focus our attention in reviewing the biomedical applications of the functionalized QDs. More specifically, section 2 discusses the applications of QDs for biosensing, followed by a comprehensive introduction of using functionalized QDs for

Special Issue: Forum on Biomedical Applications of Colloidal Photoluminescent Quantum Dots

Received: September 18, 2012
Accepted: February 8, 2013
Published: February 8, 2013

in vitro cell imaging in section 3. Section 4 focuses on the in vivo applications of QDs. A brief discussion on QDs toxicity is given in section 5. Finally, problems and research barriers related to toxic effects are briefly described.

2. FUNCTIONALIZED QDS FOR BIOSENSING

In comparison with organic dyes and protein fluorophores, QDs exhibit unique photophysical and photochemical properties and thus enjoy great advantages as fluorescence probes in sensitive optical biosensing applications over a wide range, such as immunoassays, nucleic acid detection, biomolecule sensing, and catalysis monitoring. Developments in surface engineering and bioconjugation strategies will keep on benefiting the specificity and versatility of QD-based in vitro sensing methods.

2.1. Immunoassay. Immunoassay is a very useful tool in clinical tests, disease diagnostics, and other biomedical applications. Developments in QD surface engineering have enabled them to be used as novel fluorescent probes for sensing and analyzing a wide variety of chemical and biological analysts. For example, competitive fluoroimmunoassay based on QD-antibody conjugates has been developed for detection of different targets ranging from small molecules to proteins, virus and bacteria.[6,7]

In addition to the analyzing abilities of QDs, recent advances in QD-based immunoassay with ultrahigh sensitivity or rapid screening capability have also benefited the developments of novel readout methods, such as electrochemical detection, barcode, microfluidics technique and other readout strategies.[8,9] For example, ultrasensitive western blot analysis based on QD-monoclonal antibody conjugates was carried out with electrophoresis to screen protein expression in cell or tissue with ultrahigh specificity and throughput.[10] Zou et al. have integrated immunochromatographic test strip assay (ITSA) with fluorescent QD label for rapid and sensitive detection of small molecules. In their work, a portable assay for 3,5,6-trichloropyridinol (TCP) detection was demonstrated with high speed, clinically accuracy, and quantitative testing ability.[11] More recently, a simple method was created for protein kinase activity analysis, where unmodified CdTe QDs was used as fluorescent probes and tedious labeling and recognition treatment procedures were avoided.[12] As shown in Figure 1, peptide phosphorylation catalyzed by protein kinase (PKA) was able to change the QDs surface charge and in turn resulted in selective aggregation of the unmodified QDs. The change in the fluorescence was due to QD aggregation that shows an indication of the kinase activity. In a recent work by Jie et al., biofunctionalized QDs were employed for cancer cell electrochemiluminescence (ECL) assay.[13] In this work, capture DNA was designed with high affinity against cancer cells and dendrimer/QDs-DNA biocomplex was fabricated as optical probes. In order to obtain significantly enhanced sensitivity, the authors also introduced DNA device cycle-amplifying technique on magnetic microbeads, as shown in Figure 2. Specificity and quantitative testing ability of Ramos cancer cell detection were confirmed by cell type and concentration dependent ECL intensity. Despite the high sensitivity and rapid sensing response, the ability for multiplexed detection is an important function to be included in the QD-based immunoassay. Multichannel detection of toxins, drug chemical residues or cancer biomarkers has been recently demonstrated with QD-based sandwich immunoassay employing different multicolored antibody-QD conjugates.[14] Although, multichannel detection can be easily achieved by introducing different color QDs with narrow emission spectra, efforts are still required to address the possible cross-reaction between molecule probes and other non-specific issues, which may limit the number of analyte species in a simultaneous detection set-up.

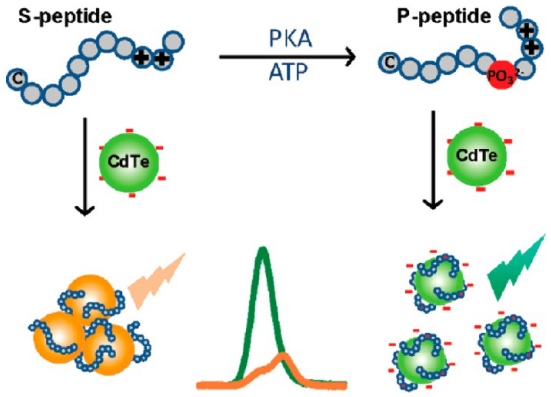


Figure 1. Concept of the fluorescence kinase activity assay based on QD aggregation. When carboxyl-coated green CdTe QDs (emission peak at 550 nm) are mixed with cationic S-peptides, the anchoring of peptides significantly decreases the negative charge density on each QD surface and induces aggregation of QDs. The interparticle interactions quench the QDs fluorescence and change the emissive wavelength to 575 nm (yellow). After the treatment of PKA, the phosphate groups are transferred from ATP to S-peptide, generating electrically neutral P-peptides phosphorylated peptide (P-peptide), which have a slight influence on surface charge of QDs and maintain the QDs dispersion and the green color fluorescence. Reproduced with permission from ref 12. Copyright 2011 American Chemical Society.

2.2. Nucleic Acid Detection. The use of QDs in nucleic acid detection has caught great attention in the past decade. DNA or RNA segments, acting as recognition moieties, are conjugated on QD surface to form fluorescent probes for genetic target analysis. The high specificity of hybridization between multicolored QD-DNA probes and the target strand with complementary sequence forms the basis for multiplexing of detection. For example, after coupling with DNA sequence probe, QDs with different emission colors were applied for multiplexed detection of the complementary sequences that are immobilized on a microarray platform.[15] The barcode configuration is another popular approach, especially for multiplexed sensing purpose. In this case, polymeric microbeads functionalized with different oligonuleotide probes are optically coded with distinguishable fluorescence colors or intensity levels by loading with different QD populations.[16,17] In a recent work by Giri et al, up to nine different gene fragments from pathogens such as syphilis, HIV, malaria, hepatitis B and C, were simultaneously detected with high fidelity by using nine QD barcodes.[17] This result suggests that QD barcode is a powerful toolkit for rapid gene mapping and infectious disease detection. It is worth mentioning that, in some multiplexed DNA detection schemes, some target analytes are free from fluorophore labeling and this kind of design processes have great potential for ultrasensitive genetic targets analysis with QD bioconjugates.[18] Furthermore, QD combination with techniques such as RT-PCR (real-time polymerase chain reaction) amplification and electrochemical (EC) assay or ECL with low background noise have been used to enhance the detection sensitivity.[19] For example, a high sensitive ECL detection of sequence-specific DNA target was recently reported, where ECL intensity was further enhanced by using nanoporous gold leaf (NPGL) electrode.[20]

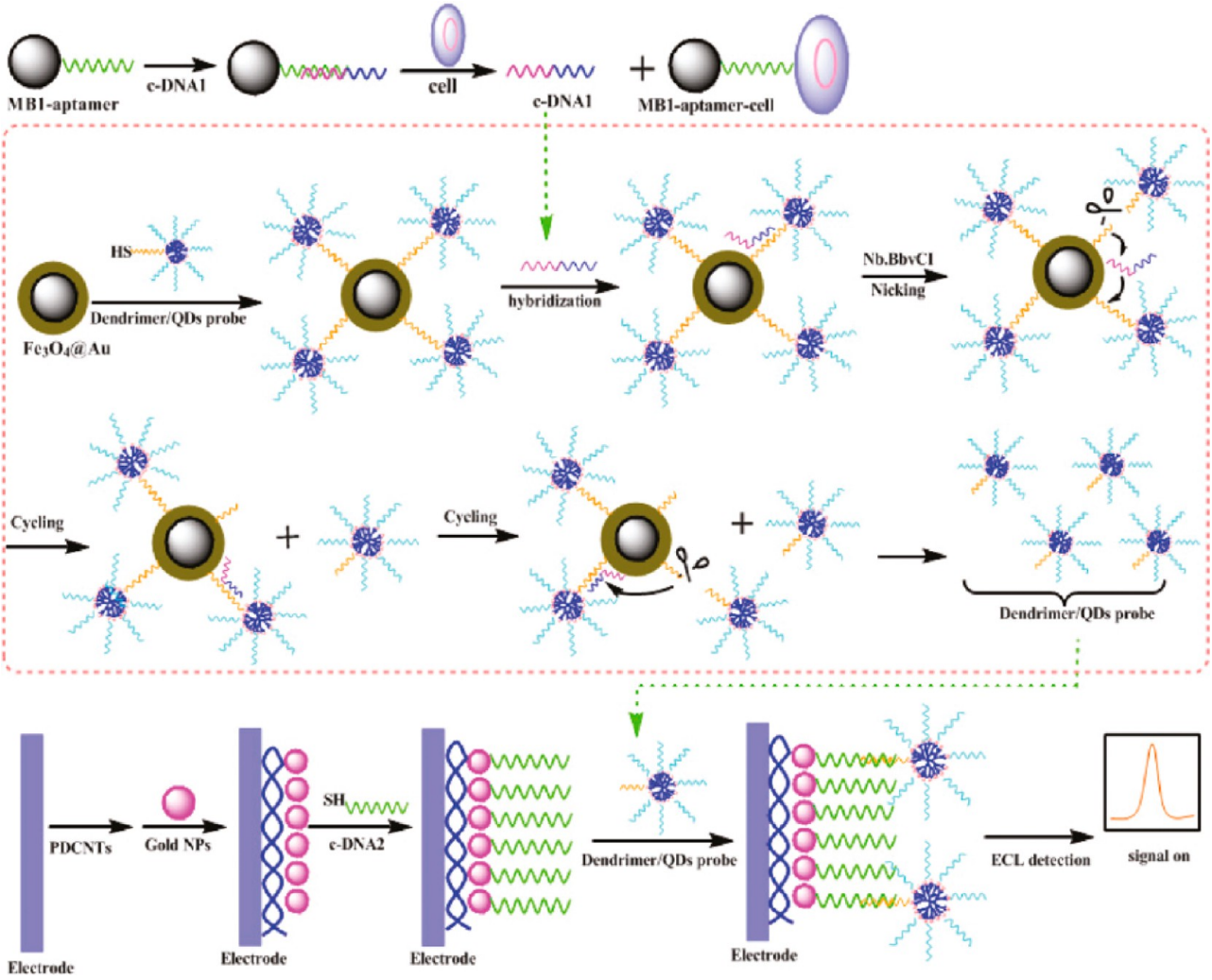


Figure 2. Schematic representation of the strategy for cell assay based on the MB1-aptamer biocomplex and DNA cycle-amplifying technique and ECL detection based on the dendrimer/QDs-DNA signal probe. Reproduced with permission from ref 13. Copyright 2011 American Chemical Society.

Besides those analytical applications, QD-based optical probes have also been developed to study cellular and subcellular gene expression. For example, fluorescence in situ hybridization (FISH) assays was employed together with hydroxylated QD-oligonucleotide probes for the labeling of Y chromosome in human sperm cells.[21] Later, multiplex FISH assay together with QDs were used to study the mRNA subcellular localization, and sensitive determination of housekeeping genes with QD-DNA conjugates was also demonstrated.[22,23] More recently, an on-chip ECL protocol has been developed together with QDs formulation for detection of mRNA targets in tumor cells and the system exhibits ultrahigh sensitive detection in comparison to FISH method.[24] As shown in Figure 3, QD was employed as an optical traceable transfection carrier to mediate the cellular internalization of reporter DNA probes. After intracellular hybridization process, expression of target mRNA in tumor cell can be indirectly evaluated by the ECL detection of reporter DNA liberated from cell lysates.

Furthermore, single-molecule level detection has been developed for high resolution mapping of protein binding sites on DNA strands using single QDs.[25] In a proof-of-concept experiment, multiple DNA binding proteins, such as transcription factors (TFs) were first anchored onto linear double-strand DNA at specific binding sites. Subsequently, the TFs were targeted by spectrally distinct QDs bearing TF-antibodies and precisely located by fluorescent microscopy. The authors predicted that the ability of QD for single molecule level detection will be helpful towards better understanding of cellular processes such as gene expression and regulation.

2.3. Quantum-Dot-Based FRET Sensing Application. Förster (or Fluorescence) resonance energy transfer (FRET) describes a mechanism of energy transfer from donor excited states to a proximal acceptor chromophore molecule through nonradiative dipole−dipole coupling. The energy transfer efficiency depends on the donor−acceptor distance, relative orientation, and the overlap between donor emission and acceptor absorption spectra.[26] Classical FRET sensing strategies based on fluorescent proteins have been developed as a tool for fundamental biochemical research, such as analyst detection and monitoring of molecular level interaction (e.g., molecule binding or conformation change).[27] In comparison to those conventional fluorophores, QDs are considered as spectacular FRET donor candidates for bio-sensing applications. Specifically, the narrow emission bandwidth of QDs is mostly desired for multiplexed FRET sensing, where donor emission of each

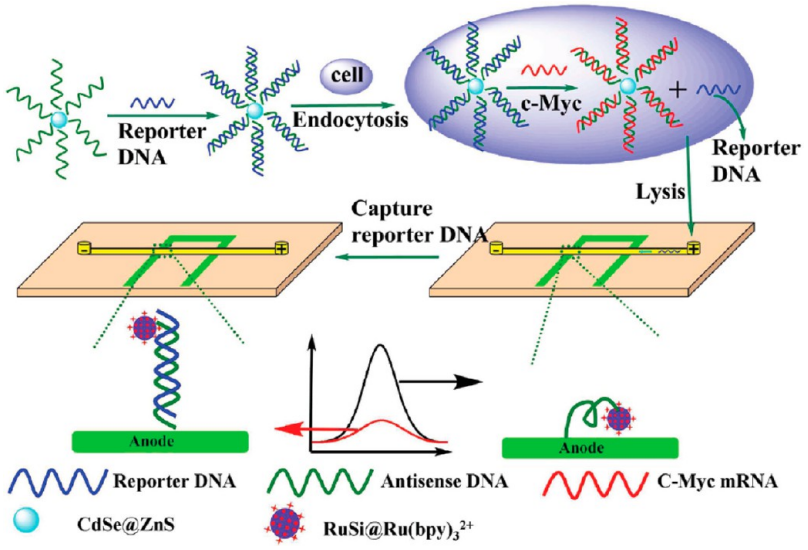
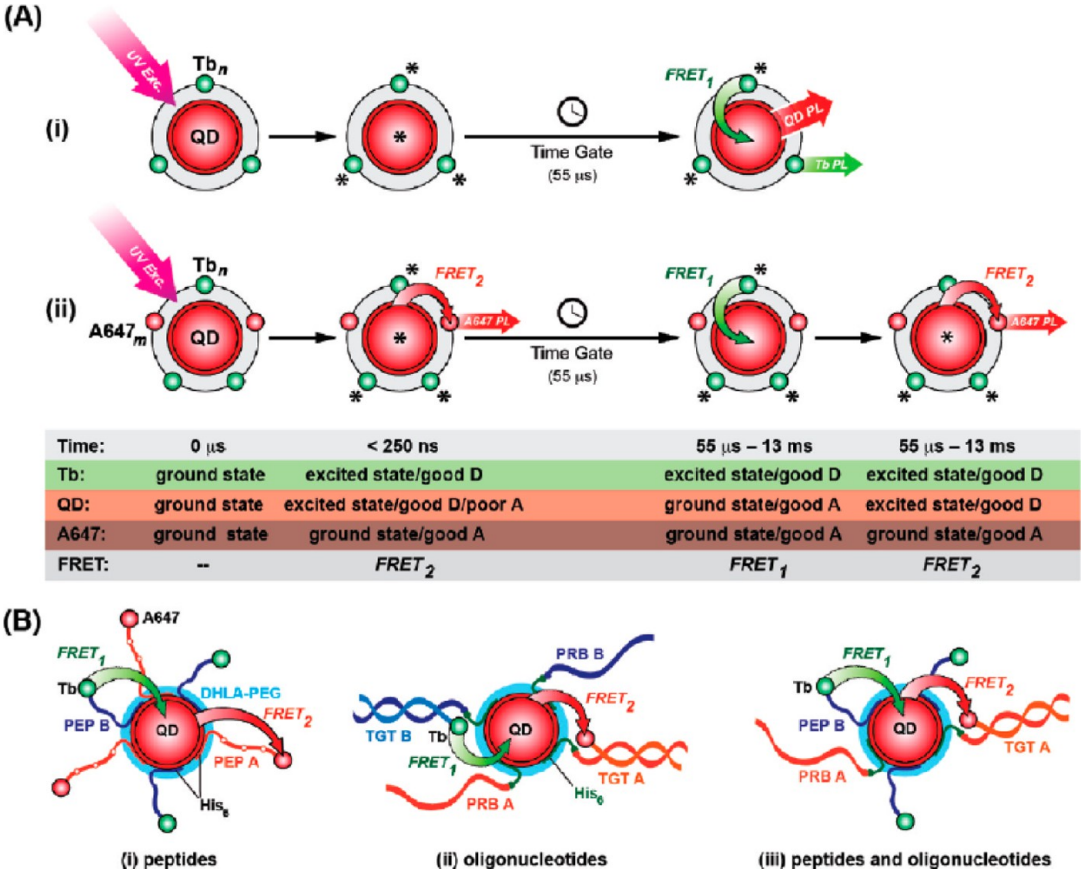


Figure 3. Schematic representation of intracellular c-Myc mRNA detection based on the wireless ECL biosensor. CdSe@ZnS QD-DNA conjugates provided an intracellular hybridization process that correlated with the relative levels of intracellular c-Myc mRNA. Dehybridized reporter DNA was liberated from cell lysates and detected by the ECL biosensor. Upon hybridization to the reporter DNA target, the formation of a rigid rodlike structure of dsDNA caused RuSi@ Ru(bpy)$_3^{2+}$ to be located away from the electrode, leading to a decrease of the ECL signal. Reproduced with permission from ref 24. Copyright 2012 American Chemical Society.



Time:	0 μs	< 250 ns	55 μs – 13 ms	55 μs – 13 ms
Tb:	ground state	excited state/good D	excited state/good D	excited state/good D
QD:	ground state	excited state/good D/poor A	ground state/good A	excited state/good D
A647:	ground state	ground state/good A	ground state/good A	ground state/good A
FRET:	--	FRET$_2$	FRET$_1$	FRET$_2$

Figure 4. (A) (i) Time-gated FRET sensitization of QD PL via FRET$_1$. Tb and QD are initially excited by a flash of UV light; QD serves as FRET acceptor for a proximal long-lifetime Tb donor. (ii) Time-gated sensitization of A647 PL via FRET$_1$ and FRET$_2$. The coassembly of a fluorescent dye, A647, with the Tb around a QD permits a two-step energy transfer relay with the QD as an intermediary. (B) The QD serves as a nanoscaffold for assembly of biomolecules labeled with Tb and A647: (i) peptide assembly, (ii) oligonucleotide assembly and hybridization, and (iii) both peptide and oligonucleotide assembly/hybridization. Reproduced with permission from ref 29. Copyright 2012 American Chemical Society.

channel should be well-distinguished. Also, attribute to the broad absorption profile of QDs, light for donor excitation can be selected wisely to avoid direct excitation of the acceptor dyes, while different color QD donors can be simultaneously

excited. On the basis of this strategy, both excitation and analysis systems can be simplified, and more importantly, the photobleaching from the acceptor dye can be suppressed when the excitation is located away from its own absorption peak. Furthermore, because of the large two-photon absorption cross-sections of QDs, NIR two-photon excitation can be applied to perform intracellular FRET detection with higher signal-to-background ratio.[28]

FRET sensing using QD as the donor has been extensively reported for DNA/RNA sequence monitoring and non-genetic molecule detection. For DNA/RNA detection, QDs functionalized with nucleic acids have been commonly used for multiplexed hybridization assay or even for DNA/RNA intracellular behavior monitoring. For example, nucleic acids hybridization assay in both solution and solid phase have been demonstrated by Algar and coworkers, where multiple color QDs and dye acceptors are introduced into the system for multiplexing. However, in a more recent work by the same group, spectrotemporal FRET detection was used for multiplexed oligonucleotide and peptide sensing without the need of multiple colors of QD (Figure 4).[29] In this novel approach, QDs served as donor and acceptor simultaneously within time-gated FRET relays, while orthogonal FRET channels were formed between QD and fluorescent dye or luminescent terbium(III) complex (Tb) that produced long fluorescence lifetime. In another recent report, a step-wise FRET approach was reported as a novel sensing system to simultaneously and non-invasively analyze DNA condensation and stability using QD bioconjugates (Figure 5). In this work, by introducing an intermediate acceptor DNA dye, a two-step FRET process was constructed to identify the release and degradation process of the DNA analyte condensation.[30] Additionally, after conjugation with aptamer or functional protein, QDs have also been demonstrated as FRET donors for detection of various molecular targets[31] and specific analyzing of protease activity.[32] QD-FRET are also widely studied for pH and ion sensing.[33,34]

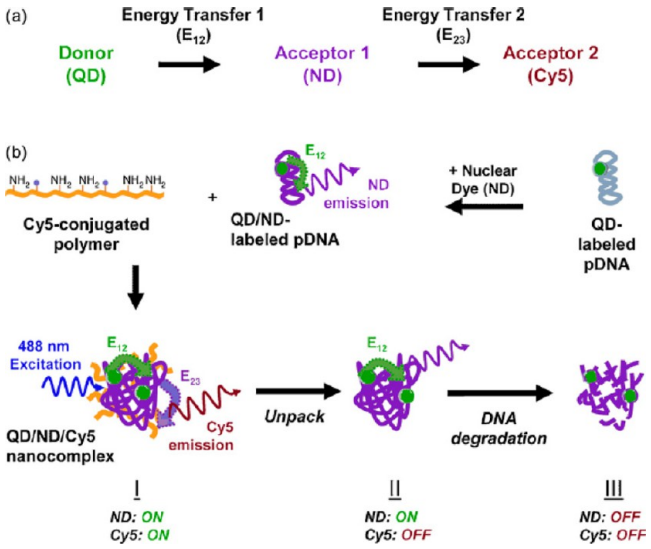


Figure 5. (a) Schematic of two-step QD-FRET. Excitation of the QD donor drives stepwise energy transfer (E_{12}) through the nuclear dye (ND) which serves as the first acceptor/donor (or relay) for energy transfer to the second acceptor Cy5 (E_{23}). (b) Plasmid DNA is double-labeled with QD and nuclear dyes before complexation with a Cy5-labeled cationic polymer to form nanocomplexes by self-assembly. Three distinct states of plasmid DNA (pDNA): (I) condensed within a nanocomplex, (II) released and intact, and (III) degraded, are distinguished by relative ND and Cy5 emission and calculated E_{12} and E_{23} efficiencies. Reproduced with permission from ref 30. Copyright 2009 Elsevier.

Although QDs have been extensively used for FRET sensing, several limitations should be mentioned. For instance, QD with larger size or inappropriate capping strategy may lead to an increase in the center-to-center distance between QD and the attached acceptor molecules, which substantially cause relatively low energy transfer efficiency and degrade the sensitivity.[35] To improve the situation, a two-step FRET is developed. By introducing a "mediator fluorophore" between donor and acceptor, a long distance step-wise energy transfer was demonstrated with high efficiency.[35] This limitation can be circumvented by increasing the number of acceptor linked to QD[36] or by using multichromophore protein as acceptor.[37] It is worth highlighting that, in addition to organic dye or fluorescent protein, Au nanoparticle (AuNP) can also act as a quencher when they are paired with QD to form an energy transfer structure.[38] The size-tunable absorption of the AuNP can be used as a good parameter for controlling the donor quenching rate of QDs.[37]

Nevertheless, due to the broad absorption band and long life time, QDs are normally not ideal FRET acceptors.[39] However, these properties benefit QDs to be used as energy acceptors in developing self-illuminating optical probes. In this case, bioluminescence resonance energy transfer (BRET) or chemical energy transfer (CEF) can be activated without external light excitation, while the energy transferred to QDs is generated by chemical reactions (e.g., luciferase mediated oxidation of substrates).[40] Because of the absence of background noise caused by excitation illumination, these self-illuminating probes displayed high sensitivity for molecule detection and are promising contrast agents for in vivo imaging.[37]

3. BIOCONJUGATED QUANTUM DOTS FOR IN VITRO CELL IMAGING

3.1. Cell Imaging with QDs Only. With similar fluorescent behavior to organic dyes or fluorescent proteins, QDs have found themselves wide applications in cell labeling and other in vitro studies. More importantly, with extraordinary photo- and chemical-stability and other unique properties, QDs are preferable in some novel applications where conventional dyes or fluorescent proteins might not be applicable, such as long term optical tracking and 3D optical sectioning. Since the first demonstrations using QDs for cell labeling in 1998,[3,4] a variety of QD probes have been developed. After conjugation with recognition moieties, such as ligands or antibodies, QDs can be highly specific to label the corresponding ligand receptors and antigens on cell membrane, which enables one to visualize and optically monitor the dynamics of membrane proteins and lipids.[41,42] For example, QDs coupled with antibodies against Her2 receptor, which is over-expressed on many kinds of breast cancer cells, were prepared for specific targeting of Her2-positive cancer cells.[43] Similarly, in a recent work by Cho et al., fluorescent superparamagnetic nanoparticles for multimodal imaging and hyperthermia applications were targeted to prostate cancer cell by using antibody against PSMA (prostate specific membrane antigen) as recognition moieties.[44] On the other hand, QD-ligand conjugations with less cost enjoy similar ability to label and track membrane proteins. For instance, QDs conjugated with EGF (epidermal

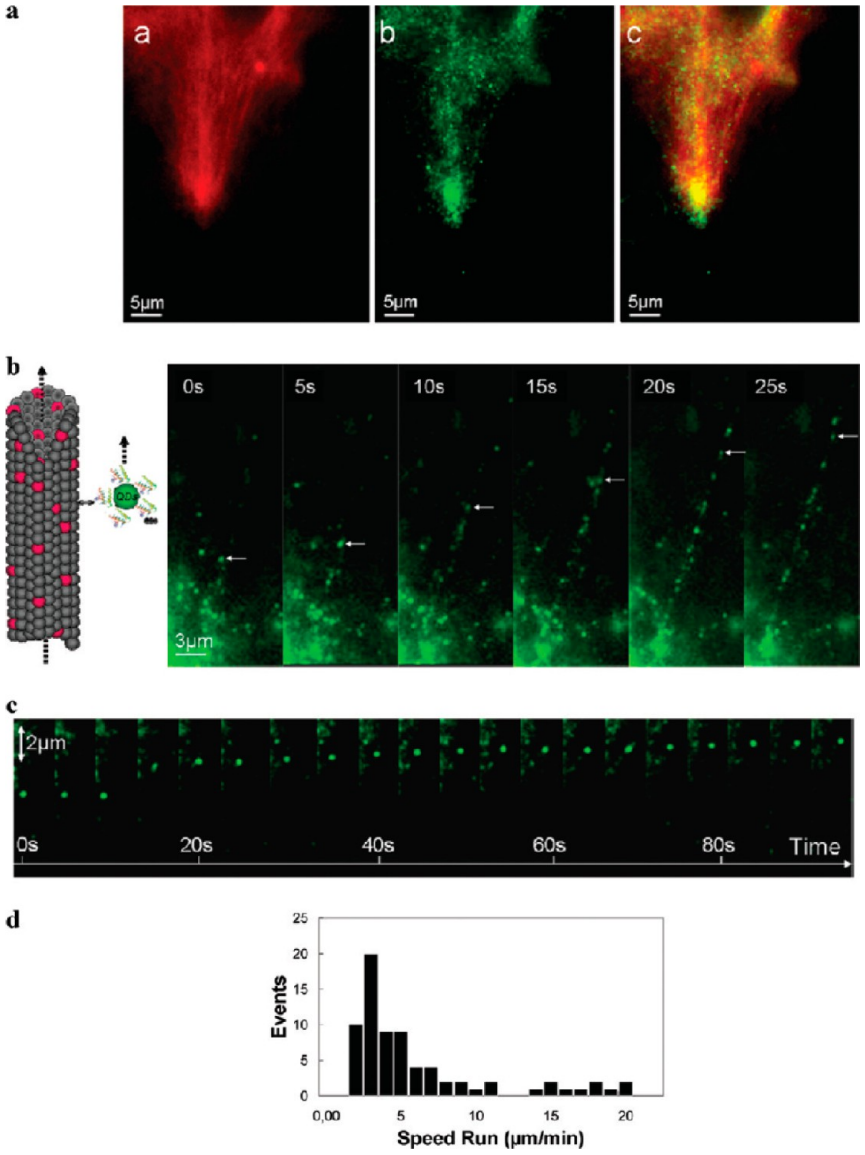


Figure 6. Tracking of QD-ND/EB1 on spindle structures following incubation of the QDs (λ = 545 nm) with cell extracts. (a) Rhodamine-labeled spindle structure (red channel) with QD-ND/EB1 (green channel) and the overlay of the two channels (shown in yellow). (b) Temporal image sequence (5 s/frame) of a single QD-ND/EB1 moving on a microtubule (see arrow). (c) A switch between a fast and slow movement. (d) Mean velocity histogram extracted from a collection of individual QD-ND/EB1 moving on the spindle structures (3 independent experiments, 3 structures). Reproduced with permission from ref 47. Copyright 2009 American Chemical Society.

growth factor) have the specificity to label EGFR membrane receptor over-expressed on various cancer cells.[45] Other ligands such as folic acid, RGD peptide, and aptamers have been demonstrated for labeling of folate receptor, $\alpha_v\beta_3$ integrin, PSMA and other membrane proteins.

In addition to cell labeling and membrane protein tracking, intracellular imaging with QDs has received great attention in the past decades. Numerous cell organelles and components have been labeled and monitored by QDs, such as intracellular molecules, nuclear antigens, microtubules, actin filaments and so on. For example, in a work by Wu et al., specific labeling of subcellular structures by QDs was achieved while the QD-based probes shown substantial advantages over organic dyes in multiplexed target detection.[43] More recently, monitoring of intracellular molecular motion with QDs, such as EB1 (microtubule-associated protein) and single myosin V (actin-associated protein), has also been demonstrated with high spatial and temporal resolution.[46,47] As shown in Figure 6, after conjugation with QDs, continuous movement of individual EB1 protein along the microtubule structure was monitored optically, while the mean velocity was also evaluated.[47] This result highlights the ability of QD for monitoring dynamic behavior of biomolecule over long periods of time, and their applications in studying complex biological structures and their functions.

Several ways are available to deliver the nanoparticles into cells through the lipid bilayer membrane. For example, cellular uptake of the quantum dots could be simply achieved by low-efficient nonspecific endocytosis,[48] whereas targeted and enhanced endocytosis can be achieved by receptor-mediated process when the dots are immobilized by membrane receptor.[49] However, in these cases, the internalized QDs can aggregate and be trapped in endolysosomal system without approaching to their targets.[50] Mechanical delivery by micro-

dx.doi.org/10.1021/am302030a | ACS Appl. Mater. Interfaces 2013, 5, 2786−2799

injection is a way to deliver homogeneously dispersed QDs into cytoplasm, without aggregation or getting trapped.[51] Although intracellular delivery of peptide conjugated QDs by microinjection for mitochondria and nucleus targeting has been demonstrated, unfortunately, this strategy is not feasible for large sample size. As an alternative, electroporation was applied for a large sample size by supplying pulsed electric field to increase membrane permeability, despite the fact that it is associated with cell death and aggregations.[51] Additionally, incorporation of QDs with cationic lipid/peptide, such as Lipofectamine and nona-arginine penetrating peptide (CCP), has proved to be an effective way for membrane transduction.[52] Duan et al. have developed cell-penetrating QDs employing hyperbranched copolymer PEI-g-PEG (PEG-grafted-polyethylenimine) as surface coating to escape from the endosomes.[53] In this method, the cationic polymer coating will induce proton sponge effect, where increased osmotic pressure swells and ruptures the endosomes, in turn release the QDs into cytoplasm. More recently, delivery of dispersed QDs into cytosol using pinocytosis method was demonstrated by Courty and coworkers. By incubating the cell with a hypertonic medium containing QDs, the cell membrane invagination induces giant pinocytotic vesicles formation in the cytoplasm with freely diffusing QDs trapped inside. After incubation in hypotonic solution, the QDs can be released because of increasing osmotic pressure and vesicle lysis.[54] By using this delivery method, individual kinesin motor proteins in HeLa cells were imaged by bioconjugated QDs at single molecule resolution.[55] Although different methods have been successfully demonstrated for organelle-level imaging, several problems have to be taken into consideration. For example, it is not possible to remove the nonspecific labeling from unbound probes in the cells by washing. As a result, additional strategies have to be incorporated such as designing the probes with initially off state and the fluorescence could be activated through targeting. New methods still need to be developed for future improvement in these fields.

3.2. Multimodal Imaging with QDs. In medical applications, imaging is an important technique for disease diagnosis as well as therapy. Many imaging techniques such as magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT) and optical imaging have been developed for clinical applications. Recently, the design and fabrication of imaging probes with multimodalities has become an active research area where different contrast agents can be fused into a single nanoparticle system.[56] QDs have been extensively investigated in this area because of their unique optical properties and the ease of conjugating additional contrast agents to them. In this section, several typical ways of realizing multimodal imaging probes based on QDs are discussed. To simplify our discussion, we focus on the development of QDs conjugated with MRI contrast agent for multimodal imaging.

Generally, there are mainly three popular ways to combine QDs with MRI contrast agents, namely, combine QDs with magnetic nanoparticles, paramagnetic ion doping, and conjugate QDs with paramagnetic complexes. To combine QDs with magnetic nanoparticles, several strategies have been conducted. First is a core/shell structure, examples being FePt particles coated with 3−5 nm CdSe shell from Gao et al, and Co-core coated with CdSe shell from Kim et al, of which both exhibited fluorescence emission while maintaining the superparamagnetic properties.[57,58] Heterodimers of QDs and superparamagnetic particles are another configuration. Gu and coworkers have obtained FePt−CdS heterodimers via transformation of FePt/CdS core/shell by heating.[59] The dimers consisting of FePt (2.5 nm) and CdS (3.5 nm) nanoparticles exhibit paramagnetic properties as well as fluorescence at 438 nm. In Selvan and coworkers' work, hetorodimers of CdS-(CdSe) QDs and $Fe_2O_3(Fe_3O_4)$ particles with tunable fluorescent emissions over 500−600 nm have been obtained by varying the growth time.[60] The dimers were then silica-shelled, functionalized and used for in vitro imaging of 4T1 mouse breast cancer cells to demonstrate the multimodality. In addition to direct attachment, separately prepared particles can also be incorporated together by using carrier materials,[61] such as silica and polymer. Although resulting large sizes (>50 nm) may impose limits in certain conditions, the benefits are distinct, including great payload and flexibility in probe design. For example, emission of the probe can be simply tuned by using QDs of different colors. Using the reverse microemulsion method, Yi et al. developed silica particles with multiple MPs and QDs entrapped.[62] In another report by Salgueirino-Maceira et al., layer-by-layer (LBL) assembly of polyelectrolytes and CdTe QDs onto the surface of silica-coated MPs was applied.[63] The resulting composite particles were further coated with a 20 nm thick outer silica layer to improve colloidal and chemical stability. These QD patterned magnetic silica spheres exhibit fluorescent properties and can be driven by an external magnetic field. Alternatively, polymer has become another choice as carrier matrix. Simultaneous encapsulation of QDs and magnetic nanoparticles in polyelectrolyte microcapsules with porous (∼10 nm) walls was first reported by Gaponik and coworkers.[64] The capsules loaded with nanoparticles showed red emission under excitation, and allowed manipulation by external magnetic field. Later, a new method was reported by Xie et al. to embed nanoparticles in copolymer nanospheres.[65] In this case, St-AAm nanospheres were synthesized with tunable dimension from 50 to 500 nm. The mesoporous surface of the nanospheres allowed entry of nanoparticles when swelling in chloroform/butanol solvent with QDs and magnetic nanoparticles. In vitro study suggested the nanospheres functionalized with folic acid were capable of targeting and separating cancer cell with high specificity.

Incorporating paramagnetic ion dopants into QDs could be another way to combine fluorescence and magnetic properties. Different synthesis methods have been developed for paramagnetic ion doping, including inverse micelle,[66] hot injection[67] and cluster method,[68] of which most were used for light-emitting devices and spintronics in the early research. Recently, Santra et al have demonstrated the potential of QDs doped with paramagnetic ions as multimodal probes for biological imaging.[69] In their work, CdS:Mn/ZnS QDs with yellow emission and magnetic response were synthesized by inverse micelle method. After silica shell coating and proper conjugation with HIV-1 TAT-peptide, the QDs successfully labeled carotid artery, showing the potential for in vivo imaging. However, reports show that metal element impurity may cause luminescence quenching.[70] Wang et al have developed a method to improve the situation.[71] Instead of doping the impurity into the QD's core, shell doping of Mn^{2+} ions to the CdSe/ZnS core/shell QDs were performed, which preserved the strong luminescence and meanwhile maximized the relaxivity for MRI. The probes were then used to treat macrophages and the uptake was verified by confocal microscopy and MRI (Figure 7). More recently, efforts have

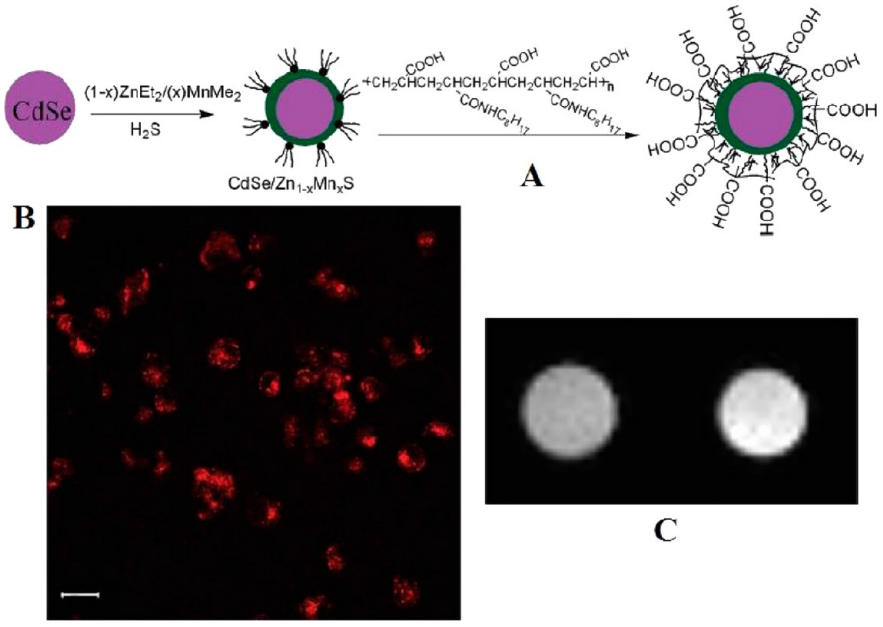


Figure 7. (A) Synthetic route to prepare water-soluble core/shell CdSe/$Zn_{1-x}Mn_xS$; (B) Confocal microscopy image of cells with sufficient multimodal quantum dots internalized, scale bar: 20μm; (C) T1-weighted images from the lysates of cells that have been incubated with quantum dots (right) show significant contrast enhancement as compared to cells that have not been exposed to quantum dots (left). Reproduced with permission from ref 71. Copyright 2007 American Chemical Society.

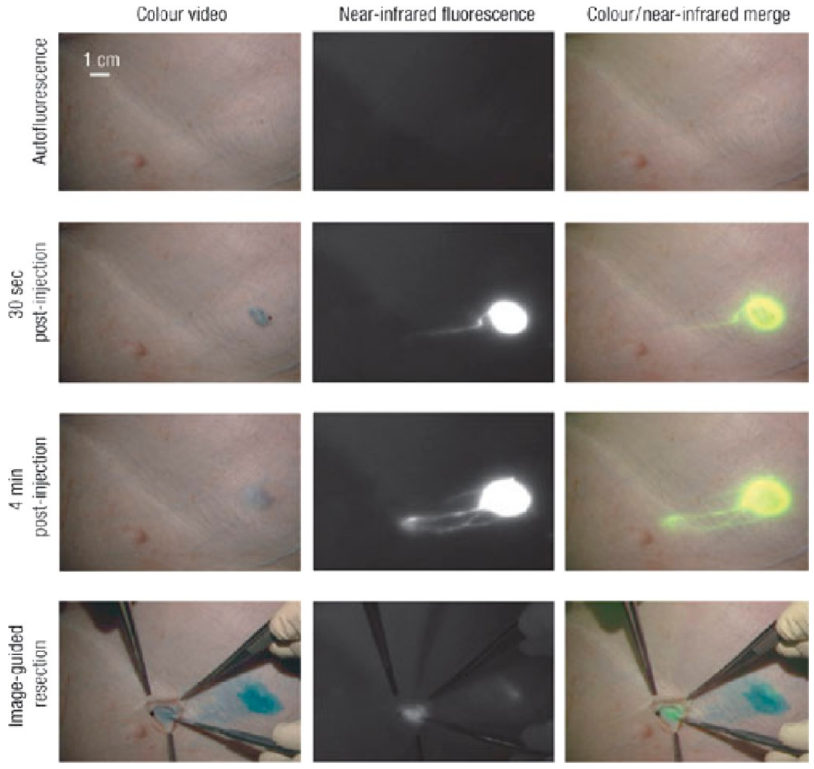


Figure 8. Images of the surgical field in a pig injected intradermally with 400 pmol of NIR QDs in the right groin. Top to bottom: before injection (autofluorescence), 30 s after injection, 4 min after injection, and during image-guided resection. Color video, NIR fluorescence and color-NIR merge images are shown from left to right. Reproduced with permission from ref 76. Copyright 2003 Nature Publishing Group.

also been made using heavy metal free materials. For example, Zhang et al. fabricated silicon QDs with Mn^{2+} dopants, allowing combinations of MRI and optical detections.[72]

Conjugating luminescent QDs with paramagnetic complexes is also a popular design. Paramagnetic ions for MRI, such as Gadolinium (Gd), are normally chelated with ligands, forming metal complexes to reduce the toxicity. The metal complexes, such as Gd-DTPA (diethylenetriaminepentaacetate) are then coupled to QDs through typical conjugation methods. Mulder et al have first demonstrated the use of CdSe/ZnS QDs conjugated with Gd-DTPA and RGD peptides as multimodal imaging probes for in vitro fluorescent and MR Imaging of

human umbilical vein endothelial cells (HUVEC), where the RGD peptides served as targeting molecules.[73] In order to raise the relaxivity of the probes, Prinzen and coworkers have shown a design with high Gd loading, where biotinylated Gd-wedges each with eight Gd-DTPA complexes are conjugated with streptavidin coated QDs.[74] The probes were further conjugated with biotinylated AnxA5 for targeting specificity, and used in MR and fluorescence imaging of apoptotic cells and injured murine carotid artery, showing the great potential in getting high resolution as well as real time imaging capabilities in biomedical applications.

4. BIOFUNCTIONALIZED QDS FOR IN VIVO APPLICATIONS

In vivo imaging is another important application area of QDs. Comparing with conventional imaging approaches in clinical dignostics, such as MRI (magnetic resonance imaging), PET (positron emission tomography), X-ray CT (X-ray computed tomography) and ultrasonography, in vivo optical imaging provides a more cost-effective way and is potential to offer a high resolution. To date, numerous in vivo imaging applications using functionalized QDs have been demonstrated, such as in vivo cell tracking,[75] vasculature imaging,[76,77] tumor imaging and targeted therapy.[75,78] Most importantly, these are in most cases non-invasive and in real time.

Cell development and movement in tissue is an interesting topic in the in vivo studies. QD labeling allows researchers to visualize the process in real time during a relatively long time frame of months scale. The first demonstration was performed on single frog (Xenopus) embryo, which was microinjected with QDs and the embryo as well as the development were imaged in real-time under continuous excitation.[79] The result suggested that QDs are proper fluorophores feasible for long-lasting intravital time-lapse studies and will help to unravel many open questions in the fields of embryology. In some other works, human cancer cells loaded with QDs were subcutaneously injected into mice and the developed tumor can be visualized directly under the skin through non-invasive optical imaging.[75] Additionally, QDs have also been introduced directly into the bloodstream for vasculature imaging. In the work by Larson et al, green-emitting QDs has been intravenously administered to mice and dynamically visualized in capillaries hundreds of micrometers deep in the skin.[80] In comparison with conventional contrast agents for vasculature imaging (e.g. FITC-dextran), QDs possess better contrast between vessels and the surrounding matrix, require much lower concentration,[81] and provide a chance to image single molecule targets in vessel.[82] Imaging of sentinel lymph nodes (SLNs) with QDs has caught great attention in the past decades because they play an important role in the immune system and cancer metastasis.[83] Kim et al. have reported the use of NIR QDs for labeling the SLNs in cancer surgery on animal models (mouse and pig), which provided the surgeon with direct visual guidance throughout the SLN mapping and resection procedure (Figure 8).[76] It should be noted that the size and surface coatings of QDs greatly affect their migrations in lymphatic system. Compared with small ones, QDs with larger hydrodynamic size cannot drain to a long distance,[84] and thereby are not suitable for lymphatic drainage mapping or image-guided resection. Targeted tumor imaging has been the most important in vivo application of QDs during the past few years, where different configurations of QD bioconjugates have been employed to improve the targeting efficiency. The first demonstration was carried out by Akerman and coworkers in 2002, in which visible QDs conjugated with three different peptides were intravenously injected into nude mice bearing breast cancer.[78] After several minute's circulation, fluorescence of histological section suggested specific distributions of QDs in tumor vasculature and organs. The first whole animal scale targeted imaging was reported by Gao et al in 2004.[75] In this work, antibody against PSMA was coupled with the QD for cancer targeting due to its high binding affinity to cancer-specific cell surface biomarkers. After intravenous injection and circulation, fluorescence image of the mice suggested an efficient accumulation of QDs in the subcutaneously implanted prostate tumor. In addition to these active targeting approaches, where specific ligands against cancer biomarkers are employed, targeted tumor imaging can also be achieved through a passive mode mediated by EPR effect (enhanced permeability and retention).[85,86] As a result of porous blood vessels formed in tumor tissue and also noneffective lymphatic drainage, QDs without bioaffinity can passively accumulate in tumor microenvironment and fluorescently label the tumor. Furthermore, attribute to its optical property and tumor targeting ability, QD has been incorporated with drug formulations, by either surface conjugation or loading them into polymer nanoparticles, to enable traceable drug delivery applications in vitro and in vivo.[44,87] These studies suggest that QDs can be served as a nano-platform that has great potential for in vivo imaging and future cancer diagnostics/therapy.

Because of the scattering and absorption, the penetration depth of the light is wavelength-dependent in the animal tissue. It was found that two spectral windows are preferred for in vivo imaging, namely 700−900 nm and 1200−1600 nm, where the tissue have minimal absorption, autofluorescence and decreased Rayleigh scattering.[88] For this reason, recent in vivo imaging studies were mostly carried out using NIR QDs, whereas multi-photon excitation of QDs using low intensity NIR light can further suppress the tissue autofluorescence.[89] Additionally, polyethylene glycol (PEG), a bioinert polymer, has become a popular biomaterial for passivating the QDs surface for minimizing the QDs uptake in the reticuloendothelial system (RES).[90] Detailed studies also shown that molecular weight, chain length and coverage degree of the PEG polymer greatly affect the in vivo behavior of QDs.[91] In addition to surface chemistry, biodistribution and clearance profiles of QDs are also associated with the overall hydrodynamic diameters of the nanoparticles. For example, size-dependent circulation lifetime of PEG-QD has been presented by Chan's group (Figure 9). Because nanoparticles with large and small sizes have distinct preferences for tumor tissue accumulation and penetration, respectively, the authors provided the design parameters of nanoparticles needed for optimized tumor imaging and drug delivery applications.[86] More recently, a multistage delivery system was demonstrated by Wong et al, where the size-changing nanoparticle facilitate the delivery of QDs in tumor tissue with both sufficient quantity and deep penetration,[92] thus providing great prospects in future tumor imaging and therapy. In this case, after efficient accumulation of QDs in tumor vasculature followed by extravasation, a size 'shrinking' of the QD gelatin nanoparticle (~100 nm) was triggered by proteases (MMP-2) that were highly expressed in tumor microenvironment (Figure 10, left). The release of QDs with small sizes (~10 nm) enhanced their diffusion in dense collagen matrix of the interstitial space (Figure 10, right). In general, renal clearance is a useful method to remove QDs from the body; but

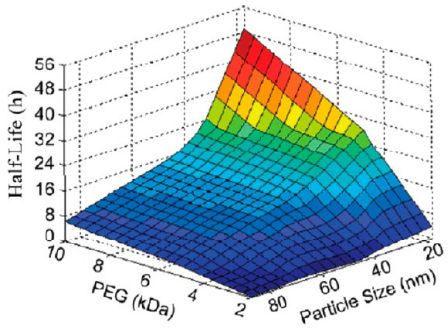


Figure 9. Combination of 5 GNP diameters and 3 mPEG molecular weight brush layers were used to determine blood half-life (hours) as a function of particle size (nm) and mPEG molecular weight (kDa). Half-life generally improved as particle diameter decreased and mPEG molecular weight increased. Reproduced with permission from ref 86. Copyright 2009 American Chemical Society.

this process is highly dependent on the particles size and surface coating. Typically, QDs with 5−6 nm can be easily removed by renal filtration; thus QDs with larger size may not be able to excrete effectively.[93] Recent studies have indicated that clearance of ultrasmall size of QDs via kidney can be evaluated by ICP-MS analysis or even fluorescent signal from the urine sample (Figure 11).[77,90]

5. QUANTUM DOT TOXICITY

A few years after QDs were introduced into the biomedical research area, the QD toxicity issue was raised by many biomedical researchers and clinical professionals because the material is made from heavy-metal elements. This issue has received great attention in the QDs community and many research groups have proposed various methods to either reduce cadmium-based QDs or replacing them with cadmium-free QDs. To date, QD toxicity assessment is an important research area and the findings will help to provide useful guidelines for translating QDs for clinical applications. So far, many QD toxicity studies have been carried out on various QD formulations to fully understand their impacts on the biological environment. However, there remain many discrepancies in the outcomes of QD toxicity assessments. Thus, it is difficult for one to fully understand the underlying mechanism of the QD toxicity in vitro and in vivo. For example, QDs with different surface coatings and sizes play important roles in the uptake of cells whereby causing different levels of toxic effects in vitro.[94] It was reported that the QD toxicity may also come from the surfactant molecules on the surface of QDs.[95] Despite the inconsistent results from in vitro models for toxicity evaluation such as proliferation, apoptosis, genetic variations, cellular morphology, or metabolic activity, the QD toxicity assessment may also be complicated by the different cell lines used for cytotoxicity testing, which may exhibit varying tolerance to QD-induced toxicity.[96]

Although there are still several ongoing debates in the QDs toxicity issue and many more systematic studies are still needed, the release of heavy metal ions from the breakdown of QDs was generally accepted to be the main culprit in causing the toxicity effects based on current in vitro findings. Some studies have shown that the heavy-metal QD core material can be encapsulated with polymer coating to enhance their chemical stability; degradation and release of heavy-metal ions is detected from these particles when they are exposed to photolytic and intracellular oxidative conditions.[97,98] The particle size is another major concern when evaluating the QD toxicity. This is because particle size is related to the potential of the breakdown of the QDs.[95] In some recent works, toxicity assessments of QDs and soluble Cd salt at similar Cd concentrations were compared to investigate the Cd ion induced cytotoxicity. The distinctly different effects observed between free Cd and QD suggest that toxicity of the QDs is indeed complicated by factors of bioaccumulation, abnormal local concentration, and nanoscale effects of the particles.[96,99,100]

In addition to chemical degradation, free radical generation is another concern for QD toxicity. Photosensitive QD transfers energy or electron to molecular oxygen and cause formation of singlet oxygen, which in turn react with water or other molecules and catalyze production of reactive oxygen/nitrogen

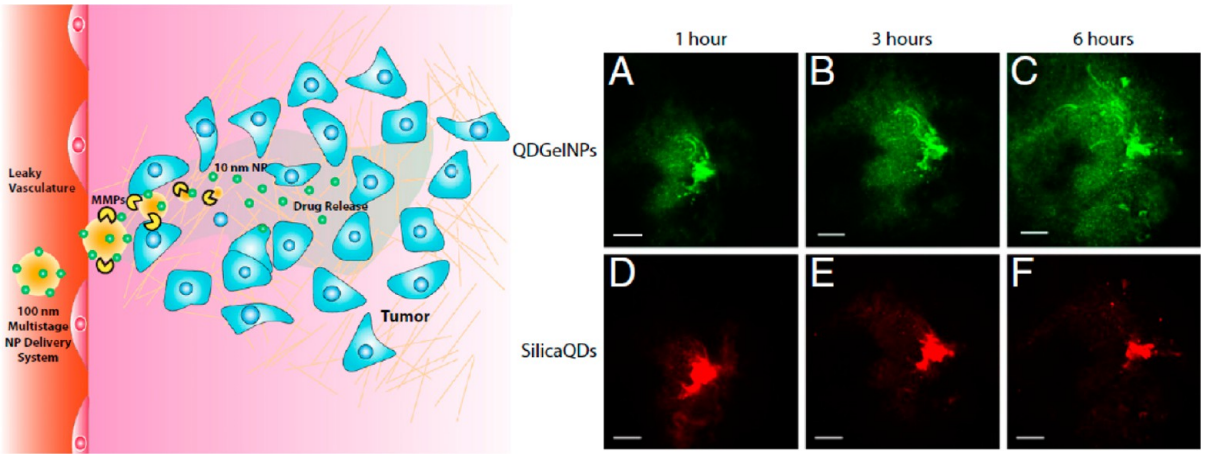


Figure 10. Left: Schematic depiction of the multistage nanoparticle drug delivery system. The initial 100 nm multistage nanoparticle delivery system accumulates preferentially around leaky vessels in tumor tissue. By cleaving away the gelatin scaffold with MMP-2, a protease highly expressed in tumor tissue, 100-nm QDGelNPs changing size to 10 nm QD NPs, which can deeply penetrate the dense collagen matrix of the interstitial space. Right: In vivo images of QDGelNPs and silica QDs (diameter = 105 nm) after intratumoral coinjection into the HT-1080 tumor. QDGelNPs imaged (A) 1, (B) 3, and (C) 6 h after injection. SilicaQDs imaged (D) 1, (E) 3, and (F) 6 h after injection. (Scale bar: 100μm). Reproduced with permission from ref 92. Copyright 2011 National Academy of Sciences, USA.

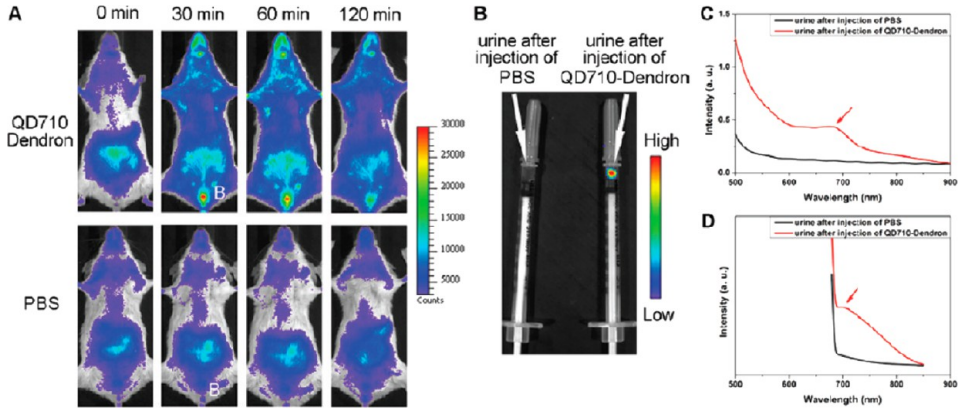


Figure 11. Renal clearance of QD710-Dendron. (A) Fluorescence imaging of mice ventral before and after tail-vein injection of QD710-Dendron (200 pmol) or PBS at 30, 60, and 120 min, respectively. The fluorescent signal originating from QD710-Dendron in the bladder (labeled as B) was visible, while there was no fluorescent signal in control mice. (B) The fluorescence imaging of urine samples collected after 90 min and the urine from the mice injected with QD710-Dendron had a strong fluorescent signal. (C) UV–vis absorption and (D) Fluorescence emission spectra of the urine samples show the characteristic absorption peak and emission maximum of QD710-Dendron. Reproduced with permission from ref 77. Copyright 2012 American Chemical Society.

species (ROS)/(RNS), such as hydroxyl radical (·OH), superoxide anion ($^{-}O_2$), hydrogen peroxide (H_2O_2), and peroxynitrite ($^{-}ONOO$).[101,102] Generation of these free radicals was reported to cause DNA nicking and break,[103,104] cell apoptosis and loss of metabolic functions.[105] However, recent study suggested that, oxidative stress is the leading mechanism of QD toxicity. With increasing concentration, Cd ions release becomes the major contributory factor for their adverse effects in vitro.[106] Also, it is worth mentioning that there are reports taking the advantage of this free radical generation process and using it for photodynamic therapy (PDT) aiming at targeted cancer treatment.[107]

Strategies have been explored to reduce the potential toxicity from the degradation and the free radical generation. The ZnS shell coating method is usually introduced, which slows down the oxidation process by limiting the transport of oxygen to the core surface. However, this method along with other coating strategies using small molecules, polymers, proteins or silicon dioxide do not fully solve the problem while ROS generation and degradation of the shells and consequently the core material still occurs. Nevertheless, for those applications where long term toxicity is not a major concern, the coating strategies offer a variety of possibilities to improve the biocompatibility and other performances of the QDs. More recently, progresses have been made in developing cadmium-free QDs, such as III–V (eg. InP),[108,109] Mn/Cu doped Zinc chalcogenide,[110,111] Si,[112,113] and others material based QDs (e.g., $CuInS_2$, $CuInSe_2$, $AgInS_2$).[114,115] These QDs, given the name "new generation QDs", provide competitive properties and make themselves as promising candidates for a wide range of future applications.

6. SUMMARY AND FUTURE OUTLOOK

In this review, QDs as a novel class of fluorescent nanomaterials for biosensing and bioimaging have been highlighted and discussed. Comparing to conventional organic fluorophores or fluorescent proteins, QDs prepared with wet chemical synthesis method possess many advantages, such as the tunable emission wavelength, broad absorption band, narrow emission bandwidth, and most importantly, high resistance to photobleaching. Flexible functionalization approaches can be used to modify the surface of QDs and allow them to be transformed into a versatile nanoplatform for conjugation of biomolecules and other modality contrast agents. The prepared QD bioconjugates can be used for biomedical applications ranging from biosensing to bioimaging.

Many useful results have been obtained using QDs for bioimaging applications, especially in the study of single-molecule monitoring, where the QD's long fluorescence lifetime and optical stability are useful for imaging the dynamics of a single biomolecule. Also, QDs are particularly suited for multiphoton imaging in vivo, which presents an opportunity for traceable drug delivery applications. We envision that in the coming few years a large number of QD formulations will be translated for in vivo applications and some may even selected for clinical use.

So far, there are a few reports suggesting that some QD formulations show cytotoxicity and causes alteration of cell functions. Some QD formulations have relatively large hydrodynamic sizes, which will reduce their ability for some selected in vitro and in vivo applications such as label cellular molecules and may reduce highly dense tissue penetration. Thus, it is important to engineer suitable sizes of QDs for specific applications. The toxicity and fate of QDs in vivo have been continuously studied for the past few years, particularly regarding distribution and breakdown of QDs in nonhuman primates. This study will provide many useful guidelines for creating biocompatible QD formulations aimed at long term in vivo applications.[116] In addition to this, we need to understand the impact of surface chemistry and physicochemical properties of QDs in biological environment so that it will allow us to engineer safer QD formulations. Currently, there are lots of assays integrated with QD formulations but many of these systems are still in the infancy stage and further development is needed to enhance their detection sensitivity.

The unique optical property and rich surface chemistry of QDs discussed here have clearly shown that QDs do have the potential for usefulness in biotechnology and medicinal applications. In the future, the use of QDs technology will have significant impact in clinical research areas ranging from molecular biological research to oncology.

AUTHOR INFORMATION

Corresponding Author

*Tel: +65-6790-5444. E-mail: ktyong@ntu.edu.sg.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Start-up grant (M4080141.040) from Nanyang Technological University, and Tier 1 Academic Research Funds (M4010360.040 RG29/10) from Singapore Ministry of Education and partially from the Singapore Ministry of Education under Tier 2 Research Grant MOE2010-T2-2-010 (M4020020.040 ARC2/11).

■ REFERENCES

(1) Klimov, V. I.; Ivanov, S. A.; Nanda, J.; Achermann, M.; Bezel, I.; McGuire, J. A.; Piryatinski, A. *Nature* **2007**, *447* (7143), 441−446.
(2) Schlamp, M. C.; Peng, X. G.; Alivisatos, A. P. *J. Appl. Phys.* **1997**, *82* (11), 5837−5842.
(3) Chan, W. C. W.; Nie, S. M. *Science* **1998**, *281* (5385), 2016−2018.
(4) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281* (5385), 2013−2016.
(5) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4* (6), 435−446.
(6) Kale, S.; Kale, A.; Gholap, H.; Rana, A.; Desai, R.; Banpurkar, A.; Ogale, S.; Shastry, P. *J. Nanopart. Res.* **2012**, *14*, 3.
(7) Yang, L. J.; Li, Y. B. *Analyst* **2006**, *131* (3), 394−401.
(8) Klostranec, J. M.; Xiang, Q.; Farcas, G. A.; Lee, J. A.; Rhee, A.; Lafferty, E. I.; Perrault, S. D.; Kain, K. C.; Chan, W. C. W. *Nano Lett.* **2007**, *7* (9), 2812−2818.
(9) Yang, M. H.; Javadi, A.; Gong, S. Q. *Sens. Actuators, B* **2011**, *155* (1), 357−360.
(10) Scholl, B.; Liu, H. Y.; Long, B. R.; McCarty, O. J. T.; O'Hare, T.; Druker, B. J.; Vu, T. Q. *ACS Nano* **2009**, *3* (6), 1318−1328.
(11) Zou, Z. X.; Du, D.; Wang, J.; Smith, J. N.; Timchalk, C.; Li, Y. Q.; Lin, Y. H. *Anal. Chem.* **2010**, *82* (12), 5125−5133.
(12) Xu, X. H.; Liu, X.; Nie, Z.; Pan, Y. L.; Guo, M. L.; Yao, S. Z. *Anal. Chem.* **2011**, *83* (1), 52−59.
(13) Jie, G. F.; Wang, L.; Yuan, J. X.; Zhang, S. S. *Anal. Chem.* **2011**, *83* (10), 3873−3880.
(14) Peng, C. F.; Li, Z. K.; Zhu, Y. Y.; Chen, W.; Yuan, Y.; Liu, L. Q.; Li, Q. S.; Xu, D. H.; Qiao, R. R.; Wang, L. B.; Zhu, S. F.; Jin, Z. Y.; Xu, C. L. *Biosens. Bioelectron.* **2009**, *24* (12), 3657−3662.
(15) Gerion, D.; Parak, W. J.; Williams, S. C.; Zanchet, D.; Micheel, C. M.; Alivisatos, A. P. *J. Am. Chem. Soc.* **2002**, *124* (24), 7070−7074.
(16) Han, M. Y.; Gao, X. H.; Su, J. Z.; Nie, S. *Nat. Biotechnol.* **2001**, *19* (7), 631−635.
(17) Giri, S.; Sykes, E. A.; Jennings, T. L.; Chan, W. C. W. *ACS Nano* **2011**, *5* (3), 1580−1587.
(18) Ho, Y. P.; Kung, M. C.; Yang, S.; Wang, T. H. *Nano Lett.* **2005**, *5* (9), 1693−1697.
(19) Su, J.; Zhang, H. J.; Jiang, B. Y.; Zheng, H. Z.; Chai, Y. Q.; Yuan, R.; Xiang, Y. *Biosens. Bioelectron.* **2011**, *29* (1), 184−188.
(20) Hu, X. F.; Wang, R. Y.; Ding, Y.; Zhang, X. L.; Jin, W. R. *Talanta* **2010**, *80* (5), 1737−1743.
(21) Pathak, S.; Choi, S. K.; Arnheim, N.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, *123* (17), 4103−4104.
(22) Choi, Y.; Kim, H. P.; Hong, S. M.; Ryu, J. Y.; Han, S. J.; Song, R. *Small* **2009**, *5* (18), 2085−2091.
(23) Chan, P. M.; Yuen, T.; Ruf, F.; Gonzalez-Maeso, J.; Sealfon, S. C. *Nucleic Acids Res.* **2005**, *33*, 18.
(24) Wu, M. S.; Qian, G. S.; Xu, J. J.; Chen, H. Y. *Anal. Chem.* **2012**, *84* (12), 5407−5414.
(25) Ebenstein, Y.; Gassman, N.; Kim, S.; Antelman, J.; Kim, Y.; Ho, S.; Samuel, R.; Michalet, X.; Weiss, S. *Nano Lett.* **2009**, *9* (4), 1598−1603.
(26) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer: New York, 2006.
(27) Jares-Erijman, E. A.; Jovin, T. M. *Nat. Biotechnol.* **2003**, *21* (11), 1387−1395.
(28) Clapp, A. R.; Pons, T.; Medintz, I. L.; Delehanty, J. B.; Melinger, J. S.; Tiefenbrunn, T.; Dawson, P. E.; Fisher, B. R.; O'Rourke, B.; Mattoussi, H. *Adv. Mater.* **2007**, *19* (15), 1921.
(29) Algar, W. R.; Wegner, D.; Huston, A. L.; Blanco-Canosa, J. B.; Stewart, M. H.; Armstrong, A.; Dawson, P. E.; Hildebrandt, N.; Medintz, I. L. *J. Am. Chem. Soc.* **2012**, *134* (3), 1876−1891.
(30) Chen, H. H.; Ho, Y. P.; Jiang, X.; Mao, H. Q.; Wang, T. H.; Leong, K. W. *Nano Today* **2009**, *4* (2), 125−134.
(31) Chi, C. W.; Lao, Y. H.; Li, Y. S.; Chen, L. C. *Biosens. Bioelectron.* **2011**, *26* (7), 3346−3352.
(32) Prasuhn, D. E.; Feltz, A.; Blanco-Canosa, J. B.; Susumu, K.; Stewart, M. H.; Mei, B. C.; Yakovlev, A. V.; Loukov, C.; Mallet, J. M.; Oheim, M.; Dawson, P. E.; Medintz, I. L. *ACS Nano* **2010**, *4* (9), 5487−5497.
(33) Snee, P. T.; Somers, R. C.; Nair, G.; Zimmer, J. P.; Bawendi, M. G.; Nocera, D. G. *J. Am. Chem. Soc.* **2006**, *128* (41), 13320−13321.
(34) Wu, C. S.; Oo, M. K. K.; Fan, X. D. *ACS Nano* **2010**, *4* (10), 5897−5904.
(35) Medintz, I. L.; Clapp, A. R.; Mattoussi, H.; Goldman, E. R.; Fisher, B.; Mauro, J. M. *Nat. Mater.* **2003**, *2* (9), 630−638.
(36) Zhang, C. Y.; Yeh, H. C.; Kuroki, M. T.; Wang, T. H. *Nat. Mater.* **2005**, *4* (11), 826−831.
(37) Medintz, I. L.; Mattoussi, H. *Phys. Chem. Chem. Phys.* **2009**, *11* (1), 17−45.
(38) Quach, A. D.; Crivat, G.; Tarr, M. A.; Rosenzweig, Z. *J. Am. Chem. Soc.* **2011**, *133* (7), 2028−2030.
(39) Clapp, A. R.; Medintz, I. L.; Fisher, B. R.; Anderson, G. P.; Mattoussi, H. *J. Am. Chem. Soc.* **2005**, *127* (4), 1242−1250.
(40) So, M. K.; Xu, C. J.; Loening, A. M.; Gambhir, S. S.; Rao, J. H. *Nat. Biotechnol.* **2006**, *24* (3), 339−343.
(41) Roullier, V.; Clarke, S.; You, C.; Pinaud, F.; Gouzer, G.; Schaible, D.; Marchi-Artzner, V.; Piehler, J.; Dahan, M. *Nano Lett.* **2009**, *9* (3), 1228−1234.
(42) Lee, J.; Choi, Y.; Kim, K.; Hong, S.; Park, H. Y.; Lee, T.; Cheon, G. J.; Song, R. *Bioconjugate Chem.* **2010**, *21* (5), 940−946.
(43) Wu, X. Y.; Liu, H. J.; Liu, J. Q.; Haley, K. N.; Treadway, J. A.; Larson, J. P.; Ge, N. F.; Peale, F.; Bruchez, M. P. *Nat. Biotechnol.* **2003**, *21* (1), 41−46.
(44) Cho, H. S.; Dong, Z. Y.; Pauletti, G. M.; Zhang, J. M.; Xu, H.; Gu, H. C.; Wang, L. M.; Ewing, R. C.; Huth, C.; Wang, F.; Shi, D. L. *ACS Nano* **2010**, *4* (9), 5398−5404.
(45) Kawashima, N.; Nakayama, K.; Itoh, K.; Itoh, T.; Ishikawa, M.; Biju, V. *Chem.—Eur. J.* **2010**, *16* (4), 1186−1192.
(46) Pierobon, P.; Achouri, S.; Courty, S.; Dunn, A. R.; Spudich, J. A.; Dahan, M.; Cappello, G. *Biophys. J.* **2009**, *96* (10), 4268−4275.
(47) Dif, A.; Boulmedais, F.; Pinot, M.; Roullier, V.; Baudy-Floc'h, M.; Coquelle, F. M.; Clarke, S.; Neveu, P.; Vignaux, F.; Le Borgne, R.; Dahan, M.; Gueroui, Z.; Marchi-Artzner, V. *J. Am. Chem. Soc.* **2009**, *131* (41), 14738−14746.
(48) Zhang, L. W.; Monteiro-Riviere, N. A. *Toxicol. Sci.* **2009**, *110* (1), 138−155.
(49) Ding, H.; Yong, K. T.; Law, W. C.; Roy, I.; Hu, R.; Wu, F.; Zhao, W. W.; Huang, K.; Erogbogbo, F.; Bergey, E. J.; Prasad, P. N. *Nanoscale* **2011**, *3* (4), 1813−1822.
(50) Delehanty, J. B.; Mattoussi, H.; Medintz, I. L. *Anal. Bioanal. Chem.* **2009**, *393* (4), 1091−1105.
(51) Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. *Adv. Mater.* **2004**, *16* (12), 961.
(52) Liu, B. R.; Huang, Y. W.; Winiarz, J. G.; Chiang, H. J.; Lee, H. J. *Biomaterials* **2011**, *32* (13), 3520−3537.
(53) Duan, H. W.; Nie, S. M. *J. Am. Chem. Soc.* **2007**, *129* (11), 3333−3338.
(54) Muro, E.; Fragola, A.; Pons, T.; Lequeux, N.; Ioannou, A.; Skourides, P.; Dubertret, B. *Small* **2012**, *8* (7), 1029−1037.
(55) Courty, S.; Luccardini, C.; Bellaiche, Y.; Cappello, G.; Dahan, M. *Nano Lett.* **2006**, *6* (7), 1491−1495.
(56) Louie, A. Y. *Chem. Rev.* **2010**, *110* (5), 3146−3195.

(57) Gao, J. H.; Zhang, B.; Gao, Y.; Pan, Y.; Zhang, X. X.; Xu, B. *J. Am. Chem. Soc.* **2007**, *129* (39), 11928−11935.
(58) Kim, H.; Achermann, M.; Balet, L. P.; Hollingsworth, J. A.; Klimov, V. I. *J. Am. Chem. Soc.* **2005**, *127* (2), 544−546.
(59) Gu, H. W.; Zheng, R. K.; Zhang, X. X.; Xu, B. *J. Am. Chem. Soc.* **2004**, *126* (18), 5664−5665.
(60) Selvan, S. T.; Patra, P. K.; Ang, C. Y.; Ying, J. Y. *Angew. Chem., Int. Ed.* **2007**, *46* (14), 2448−2452.
(61) Koole, R.; Mulder, W. J. M.; van Schooneveld, M. M.; Strijkers, G. J.; Meijerink, A.; Nicolay, K. *Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol.* **2009**, *1* (5), 475−491.
(62) Yi, D. K.; Selvan, S. T.; Lee, S. S.; Papaefthymiou, G. C.; Kundaliya, D.; Ying, J. Y. *J. Am. Chem. Soc.* **2005**, *127* (14), 4990−4991.
(63) Salgueirino-Maceira, V.; Correa-Duarte, M. A.; Spasova, M.; Liz-Marzan, L. M.; Farle, M. *Adv. Funct. Mater.* **2006**, *16* (4), 509−514.
(64) Gaponik, N.; Radtchenko, I. L.; Sukhorukov, G. B.; Rogach, A. L. *Langmuir* **2004**, *20* (4), 1449−1452.
(65) Xie, H. Y.; Zuo, C.; Liu, Y.; Zhang, Z. L.; Pang, D. W.; Li, X. L.; Gong, J. P.; Dickinson, C.; Zhou, W. Z. *Small* **2005**, *1* (5), 506−509.
(66) Bhargava, R. N.; Gallagher, D.; Hong, X.; Nurmikko, A. *Phys. Rev. Lett.* **1994**, *72* (3), 416−419.
(67) Norris, D. J.; Yao, N.; Charnock, F. T.; Kennedy, T. A. *Nano Lett.* **2001**, *1* (1), 3−7.
(68) Hanif, K. M.; Meulenberg, R. W.; Strouse, G. F. *J. Am. Chem. Soc.* **2002**, *124* (38), 11495−11502.
(69) Santra, S.; Yang, H. S.; Holloway, P. H.; Stanley, J. T.; Mericle, R. A. *J. Am. Chem. Soc.* **2005**, *127* (6), 1656−1657.
(70) Biswas, S.; Kar, S.; Chaudhuri, S. *J. Phys. Chem. B* **2005**, *109* (37), 17526−17530.
(71) Wang, S.; Jarrett, B. R.; Kauzlarich, S. M.; Louie, A. Y. *J. Am. Chem. Soc.* **2007**, *129* (13), 3848−3856.
(72) Zhang, X.; Brynda, M.; Britt, R. D.; Carroll, E. C.; Larsen, D. S.; Louie, A. Y.; Kauzlarich, S. M. *J. Am. Chem. Soc.* **2007**, *129* (35), 10668−+.
(73) Mulder, W. J. M.; Koole, R.; Brandwijk, R. J.; Storm, G.; Chin, P. T. K.; Strijkers, G. J.; Donega, C. D.; Nicolay, K.; Griffioen, A. W. *Nano Lett.* **2006**, *6* (1), 1−6.
(74) Prinzen, L.; Miserus, R.; Dirksen, A.; Hackeng, T. M.; Deckers, N.; Bitsch, N. J.; Megens, R. T. A.; Douma, K.; Heemskerk, J. W.; Kooi, M. E.; Frederik, P. M.; Slaaf, D. W.; van Zandvoort, M.; Reutelingsperger, C. P. M. *Nano Lett.* **2007**, *7* (1), 93−100.
(75) Gao, X. H.; Cui, Y. Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. M. *Nat. Biotechnol.* **2004**, *22* (8), 969−976.
(76) Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Grand, A. M.; Lee, J.; Nakayama, A.; Parker, J. A.; Mihaljevic, T.; Laurence, R. G.; Dor, D. M.; Cohn, L. H.; Bawendi, M. G.; Frangioni, J. V. *Nat. Biotechnol.* **2004**, *22* (1), 93−97.
(77) Gao, J. H.; Chen, K.; Luong, R.; Bouley, D. M.; Mao, H.; Qiao, T. C.; Gambhir, S. S.; Cheng, Z. *Nano Lett.* **2012**, *12* (1), 281−286.
(78) Akerman, M. E.; Chan, W. C. W.; Laakkonen, P.; Bhatia, S. N.; Ruoslahti, E. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (20), 12617−12621.
(79) Dubertret, B.; Skourides, P.; Norris, D. J.; Noireaux, V.; Brivanlou, A. H.; Libchaber, A. *Science* **2002**, *298* (5599), 1759−1762.
(80) Larson, D. R.; Zipfel, W. R.; Williams, R. M.; Clark, S. W.; Bruchez, M. P.; Wise, F. W.; Webb, W. W. *Science* **2003**, *300* (5624), 1434−1436.
(81) Stroh, M.; Zimmer, J. P.; Duda, D. G.; Levchenko, T. S.; Cohen, K. S.; Brown, E. B.; Scadden, D. T.; Torchilin, V. P.; Bawendi, M. G.; Fukumura, D.; Jain, R. K. *Nat. Med.* **2005**, *11* (6), 678−682.
(82) Yu, X. F.; Chen, L. D.; Li, K. Y.; Li, Y.; Xiao, S.; Luo, X.; Liu, J.; Zhou, L.; Deng, Y. L.; Pang, D. W.; Wang, Q. Q. *J. Biomed. Opt.* **2007**, *12* (1), 014008.
(83) Parungo, C. P.; Soybel, D. I.; Colson, Y. L.; Kim, S. W.; Ohnishi, S.; DeGrand, A. M.; Laurence, R. G.; Soltesz, E. G.; Chen, F. Y.; Cohn, L. H.; Bawendi, M. G.; Frangioni, J. V. *Ann. Surg. Oncol.* **2007**, *14* (2), 286−298.
(84) Zimmer, J. P.; Kim, S. W.; Ohnishi, S.; Tanaka, E.; Frangioni, J. V.; Bawendi, M. G. *J. Am. Chem. Soc.* **2006**, *128* (8), 2526−2527.
(85) Tanaka, T.; Shiramoto, S.; Miyashita, M.; Fujishima, Y.; Kaneo, Y. *Int. J. Pharm.* **2004**, *277* (1-2), 39−61.
(86) Perrault, S. D.; Walkey, C.; Jennings, T.; Fischer, H. C.; Chan, W. C. W. *Nano Lett.* **2009**, *9* (5), 1909−1915.
(87) Yong, K. T.; Wang, Y. C.; Roy, I.; Rui, H.; Swihart, M. T.; Law, W. C.; Kwak, S. K.; Ye, L.; Liu, J. W.; Mahajan, S. D.; Reynolds, J. L. *Theranostics* **2012**, *2* (7), 681−694.
(88) Frangioni, J. V. *Curr. Opin. Chem. Biol.* **2003**, *7* (5), 626−634.
(89) Yong, K. T.; Roy, I.; Ding, H.; Bergey, E. J.; Prasad, P. N. *Small* **2009**, *5* (17), 1997−2004.
(90) Schipper, M. L.; Iyer, G.; Koh, A. L.; Cheng, Z.; Ebenstein, Y.; Aharoni, A.; Keren, S.; Bentolila, L. A.; Li, J. Q.; Rao, J. H.; Chen, X. Y.; Banin, U.; Wu, A. M.; Sinclair, R.; Weiss, S.; Gambhir, S. S. *Small* **2009**, *5* (1), 126−134.
(91) Daou, T. J.; Li, L.; Reiss, P.; Josserand, V.; Texier, I. *Langmuir* **2009**, *25* (5), 3040−3044.
(92) Wong, C.; Stylianopoulos, T.; Cui, J. A.; Martin, J.; Chauhan, V. P.; Jiang, W.; Popovic, Z.; Jain, R. K.; Bawendi, M. G.; Fukumura, D. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (6), 2426−2431.
(93) Choi, H. S.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J. P.; Ipe, B. I.; Bawendi, M. G.; Frangioni, J. V. *Nat. Biotechnol.* **2007**, *25* (10), 1165−1170.
(94) Lovric, J.; Bazzi, H. S.; Cuie, Y.; Fortin, G. R. A.; Winnik, F. M.; Maysinger, D. *J. Mol. Med.* **2005**, *83* (5), 377−385.
(95) Kirchner, C.; Liedl, T.; Kudera, S.; Pellegrino, T.; Javier, A. M.; Gaub, H. E.; Stolzle, S.; Fertig, N.; Parak, W. J. *Nano Lett.* **2005**, *5* (2), 331−338.
(96) Chen, N.; He, Y.; Su, Y. Y.; Li, X. M.; Huang, Q.; Wang, H. F.; Zhang, X. Z.; Tai, R. Z.; Fan, C. H. *Biomaterials* **2012**, *33* (5), 1238−1244.
(97) Hoshino, A.; Fujioka, K.; Oku, T.; Suga, M.; Sasaki, Y. F.; Ohta, T.; Yasuhara, M.; Suzuki, K.; Yamamoto, K. *Nano Lett.* **2004**, *4* (11), 2163−2169.
(98) Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. *Nano Lett.* **2004**, *4* (1), 11−18.
(99) Domingos, R. F.; Simon, D. F.; Hauser, C.; Wilkinson, K. J. *Environ. Sci. Technol.* **2011**, *45* (18), 7664−7669.
(100) Su, Y. Y.; Hu, M.; Fan, C. H.; He, Y.; Li, Q. N.; Li, W. X.; Wang, L. H.; Shen, P. P.; Huang, Q. *Biomaterials* **2010**, *31* (18), 4829−4834.
(101) Ipe, B. I.; Lehnig, M.; Niemeyer, C. M. *Small* **2005**, *1* (7), 706−709.
(102) Juzenas, P.; Generalov, R.; Juzeniene, A.; Moan, J. *J. Biomed. Nanotechnol.* **2008**, *4* (4), 450−456.
(103) Green, M.; Howman, E. *Chem. Commun.* **2005**, *1*, 121−123.
(104) Anas, A.; Akita, H.; Harashima, H.; Itoh, T.; Ishikawa, M.; Biju, V. *J. Phys. Chem. B* **2008**, *112* (32), 10005−10011.
(105) Cho, S. J.; Maysinger, D.; Jain, M.; Roder, B.; Hackbarth, S.; Winnik, F. M. *Langmuir* **2007**, *23* (4), 1974−1980.
(106) Li, K. G.; Chen, J. T.; Bai, S. S.; Wen, X.; Song, S. Y.; Yu, Q.; Li, J.; Wang, Y. Q. *Toxicol. In Vitro* **2009**, *23* (6), 1007−1013.
(107) Juzenas, P.; Chen, W.; Sun, Y. P.; Coelho, M. A. N.; Generalov, R.; Generalova, N.; Christensen, I. L. *Adv. Drug Delivery Rev.* **2008**, *60* (15), 1600−1614.
(108) Kim, S. W.; Zimmer, J. P.; Ohnishi, S.; Tracy, J. B.; Frangioni, J. V.; Bawendi, M. G. *J. Am. Chem. Soc.* **2005**, *127* (30), 10526−10532.
(109) Yong, K. T.; Ding, H.; Roy, I.; Law, W. C.; Bergey, E. J.; Maitra, A.; Prasad, P. N. *ACS Nano* **2009**, *3* (3), 502−510.
(110) Pradhan, N.; Battaglia, D. M.; Liu, Y. C.; Peng, X. G. *Nano Lett.* **2007**, *7* (2), 312−317.
(111) Geszke, M.; Murias, M.; Balan, L.; Medjandi, G.; Korczynski, J.; Moritz, M.; Lulek, J.; Schneider, R. *Acta Biomater.* **2011**, *7* (3), 1327−1338.
(112) Park, J. H.; Gu, L.; von Maltzahn, G.; Ruoslahti, E.; Bhatia, S. N.; Sailor, M. J. *Nat. Mater.* **2009**, *8* (4), 331−336.
(113) Erogbogbo, F.; Yong, K. T.; Roy, I.; Hu, R.; Law, W. C.; Zhao, W. W.; Ding, H.; Wu, F.; Kumar, R.; Swihart, M. T.; Prasad, P. N. *ACS Nano* **2011**, *5* (1), 413−423.

(114) Helle, M.; Cassette, E.; Bezdetnaya, L.; Pons, T.; Leroux, A.; Plenat, F.; Guillemin, F.; Dubertret, B.; Marchal, F. *PLoS One* **2012**, *7* (8), No. e44433.
(115) Cassette, E.; Pons, T.; Bouet, C.; Helle, M.; Bezdetnaya, L.; Marchal, F.; Dubertret, B. *Chem. Mater.* **2010**, *22* (22), 6117−6124.
(116) Ye, L.; Yong, K.-T.; Liu, L.; Roy, I.; Hu, R.; Zhu, J.; Cai, H.; Law, W.-C.; Liu, J.; Wang, K.; Liu, J.; Liu, Y.; Hu, Y.; Zhang, X.; Swihart, M. T.; Prasad, P. N. *Nat. Nanotechnol.* **2012**, *7* (7), 453−458.