Continuing progress toward controlled intracellular delivery of semiconductor quantum dots

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The biological applications of luminescent semiconductor quantum dots (QDs) continue to grow at a nearly unabated pace. This growth is driven, in part, by their unique photophysical and physicochemical properties which have allowed them to be used in many different roles in cellular biology including: as superior fluorophores for a wide variety of cellular labeling applications; as active platforms for assembly of nanoscale sensors; and, more recently, as a powerful tool to understand the mechanisms of nanoparticle mediated drug delivery. Given that controlled cellular delivery is at the intersection of all these applications, the latest progress in delivering QDs to cells is examined here. A brief discussion of relevant considerations including the importance of materials preparation and bioconjugation along with the continuing issue of endosomal sequestration is initially provided for context. Methods for the cellular delivery of QDs are then highlighted including those based on passive exposure, facilitated strategies that utilize peptides or polymers and fully active modalities such as electroporation and other mechanically based methods. Following on this, the exciting advent of QD cellular delivery using multiple or combined mechanisms is then previewed. Several recent methods reporting endosomal escape of QD materials in cells are also examined in detail with a focus on the mechanisms by which access to the cytosol is achieved. The ongoing debate over QD cytotoxicity is also discussed along with a perspective on how this field will continue to evolve in the future. © 2014 The Authors.

INTRODUCTION

Since the initial description of their specific use for biological labeling more than 15 years ago,†,‡ the use of luminescent semiconductor nanocrystals or quantum dots (QDs) in biological and related applications continues to grow at an almost exponential rate.†,5 Demonstrated utility within a biological framework to date includes use as cellular labels, in vitro and in vivo fluorophores and contrast agents for long-term tracking and deep-tissue imaging, use in drug or related selection schemes, photodynamic agents, molecular-scale platforms for the assembly of energy transfer based sensors, diagnostics, incorporation into bio-based information processors, biobarcodeing, logic biodevices, drug-delivery agents, theranostic materials, and light harvesting arrays, to name but a paltry few.†,5 All these, and indeed many other applications, seek to exploit the unique photonic and physiochemical properties of QDs within a targeted utility. Properties of interest include, but are not limited to: the ability to be bioconjugated

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Conflict of interest: The authors have declared no conflicts of interest for this article.

How to cite this article:
WIREs Nanomed Nanobiotechnol 2015, 7:131–151. doi: 10.1002/wnan.1281
to targeting molecules such as antibodies, the ability to chemically tune a narrow and symmetrical Gaussian photoluminescence (PL) peak emission (full-width-at-half-maximum of 25–40 nm) as a function of constituent core materials and quantum confinement effects (i.e., control the emission color from the UV to the near-IR); high quantum yields (QY ≈ 0.2–0.9); strong direct absorption (ε > 10⁶ M⁻¹ cm⁻¹) and some of the highest two-photon absorption cross sections (σTPA ≈ 10¹⁻¹⁰⁴ Goeppert Mayer units) available in a fluorophore.⁴⁻⁵ Cumulatively, these properties also allow QDs to be utilized in roles that require ‘deep’ multiplexing capabilities where many differentially-colored QDs can be simultaneously tracked or observed with minimal instrumental requirements. Benefits are also derived from the ability of QDs to engage in both Förster resonance energy transfer (FRET) and charge transfer with widely divergent materials in the designated role of donor or acceptor as desired.⁶⁻¹⁰,¹⁶⁻²¹ Changes in the efficiency of these transfer processes form the basis for signal transduction and monitoring when the QDs are used as sensing platforms that are actively incorporated into biomolecular interactions such as enzymatic activity. The nontrivial QD surface also directly contributes toward such utility as it allows the QD to act as a centralized nanoscaffold that can play host for multiple copies of targeting or sensing molecules or to carry biomolecular cargos; this can help increase localized avidity.²²,²³ Despite the breadth and variety of QD applications, one area continues to see the most consistent growth, namely cellular biology,²⁴ and it is here that we begin to focus the current discussion.

The Growing Role of QDs in Cellular Biology

Within the multi-encompassing descriptor of ‘cellular biology’, there are three primary subdivisions that can be used to characterize the function and potential applications of QDs in this area: (1) passive fluorophores, (2) active sensors, and (3) as potent theranostic research tools, see Figure 1. As passive fluorophores, QDs are mainly used to label cells or specific subcellular structures such as organelles for imaging and tracking applications. Here the primary property that is exploited is that of fluorescence where the intrinsically wide absorption allows for one, or multiple differentially emissive QDs, to be excited with a single excitation wavelength.³⁻⁵ This can be either in a direct excitation modality (e.g., UV lamp) or when using a multiphoton laser source. Additionally, use of QDs in this role does not preclude use of other organic or fluorescent protein fluorophores, and indeed, it most likely serves to augment these mixed labeling contexts.²⁷,²⁸ Introduction of QDs to the cells is achieved with all manner of cellular delivery techniques available ranging from passive exposure to postfixation immunocytochemistry. If specific targeting or labeling is required, the QDs may also be modified to display antibodies, peptides, nucleic acids, etc., which provide the QD with the requisite binding and recognition capabilities.

In contrast to the more passive fluorophore role briefly outlined above, QDs can also provide an active sensing function. This definition is meant to highlight that within this role the QD is providing several levels of utility. First, the QD serves as a central nanoscaffold that helps to assemble and display one or multiple copies of targeting or sensing biomolecules (again antibodies, peptides, nucleic acids, etc.) if required. The QD is also an active part of transducing a sensing event. To accomplish this, the QD may be further decorated with some type of chemical or biomolecule that acts as an energy transfer donor or acceptor. Biological events (e.g., proteolysis) or environmental changes alter the position or state of this secondary moiety which, in turn, alters the rate of energy transfer providing signal transduction. Examples of this utility include use of QDs decorated with dopamine-labeled peptides as sensors for pH changes during drug-induced intracellular alkalosis,²⁹ or use of a QD displaying a rhodamine-labeled peptide that is cleaved in response to intracellular caspase-1 activity.²⁶

The third major area of QD utility in cell biology is that of theranostics; the latter refers to the use of multifunctional nanoparticles (NPs) that are capable of targeted drug delivery while simultaneously providing contrast and sensing utility in certain circumstances. Theranostics are meant to overcome issues associated with systemic drug delivery along with creating a new generation of targeted medicines that serve multiple roles.¹³⁻¹⁵,³⁰⁻³³ Understanding how cells are targeted by theranostic agents along with how they are taken up and ultimately processed is at the heart of this developing field and it is here that QDs have much to offer. As most metallic and polymeric nanoparticulate materials are opaque without additional fluorescent labeling, QD utility for this role again includes strong PL coupled with amenability to energy transfer-based sensing, nontrivial surface area, well developed and controllable bioconjugation chemistry along with access to facile multiplexing formats. An early but highly representative example of such utility can be found in the work of Wang who used QD-based FRET to evaluate the
in intracellular stability and unpacking of DNA complexes in pursuit of optimizing transfection and gene delivery efficiencies. Another illustrative example was reported by Bagalkot and colleagues. Here a QD-aptamer-doxorubicin (central nanoplatform, targeting moiety, and drug, respectively) construct was demonstrated for directed targeting of prostate cancer cells. The QD could both be imaged inside the cells while simultaneously reporting the release of doxorubicin (Dox) through a FRET process. Although QDs may not be used in the final application, QD capabilities can clearly allow them to act as a generalized NP surrogate to unravel all the intricacies involved in these cellular and subcellular processes. It is important to note that controlled cellular delivery of QDs is at the intersection of these highlighted applications and will clearly be critical to further improvements in all. Moreover, even if in vivo imaging with QDs is the ultimate goal of a given experiment, cellular interactions will almost always be a pivotal component.

**Relevant Considerations**

There are several material/physicochemical properties that are important to consider for efficient QD cellular delivery. First, is insuring the best quality for the QD materials themselves. Although not proven unequivocally, the current consensus within the user community is that core/shell QDs, where a wider band-gap material such as ZnS is used to overcoat cores consisting of CdSe or CdTe, are preferable as they minimize Cd exposure and leakage/dissolution in the cellular environment. In conjunction with overcoating, the character of the ‘surface ligands’ used to make the QDs colloidal stable in an aqueous environment are critical since each comes with its own set of benefits and liabilities. For example, QDs surface-functionalized with amphiphilic or other similarly sized polymers tend to have high QYs, but this is at the cost of a large hydrodynamic size. Some ligands display certain functional groups such as amines or carboxyls which make them quite amenable to further bioconjugation reactions while others may be completely impervious and unsuitable; this would clearly affect the ultimate architecture of any desired QD bioconjugate. The surface ligands can also play a determining role in the delivery mechanism utilized, for example, overall net charge will be an issue for both electroporation and use of polymeric delivery agents along with potentially contributing

**FIGURE 1** Representative or schematic examples of quantum dots (QDs) use as passive fluorophores, active sensors, and theranostic tools. (a) Five-color immunohistochemical labeling of a mouse splenic tissue section simultaneously stained with QDs is used as a representative example for the passive fluorophore role. (Reprinted with permission from Ref 25. Copyright 2013 ACS) (b) For active sensing, a schematic describing the activity of a caspase-1 sensor is highlighted. This nanosensor is composed of QDs and rhodamine-B molecules, connected through a short peptide, cleavable by caspase-1. When the QDs are excited, they transfer their energy to the dye molecules by Förster resonance energy transfer (FRET) and the emission is observed at the wavelength specific for the dye. After enzymatic cleavage of the peptide molecules, the acceptor molecules are detached from the QDs which no longer provide an efficient energy transfer channel to them, and emission spectra changes back to that of QDs. Dissected mouse brain showing an example of fluorescence from this nanosensor. (Reprinted with permission from Ref 26. Copyright 2013 ACS). (c) Theranostic tools are highlighted on the right with a schematic of a nanoparticle (NP) bioconjugate. Each biological molecule would provide a different potential activity to the final conjugate. For example, the antibody would potentially provide targeting, the peptide – cellular uptake, the protein – sensing, the drug and nucleic acid could act as therapeutics. The central NP (read QD) acts as a central nanoscale platform, provides solubility through its polyethylene glycol or PEG layer, and can still contribute to imaging or sensing with inherent fluorescence or magnetic contrast depending upon its constituents and structure.
to toxicity. As no currently available ligand suffices for all intended uses of QDs in biological contexts, new ligand design continues to be an active area of research.\(^4,^{44}\) Lastly, how targeting moieties such as antibodies, peptides, aptamers, and the like are ‘bioconjugated’ onto the QDs must also be an important part of the consideration equation as this will directly affect the efficiency of delivery along with the hydrodynamic size of the final QD conjugate (Box 1).\(^{45,46}\)

**BOX 1**

**BIOCONJUGATION**

The attachment of biological molecules to NPs, i.e., bioconjugation, is a complex and growing research field in and of itself.\(^{45–47}\) It is now abundantly clear that the choice of bioconjugation chemistry utilized can have a profound effect on the QD-bioconjugate’s final structure and function.\(^{48}\) Regardless of whether the biological is a small drug or peptide or a large supramolecular protein complex, the bioconjugation technique utilized should ideally provide for control over: (1) the ratio of biological per QD (valence); (2) the orientation of the biological on the QD; (3) the separation distance between QD and biological; (4) affinity of their interaction; moreover, (5) the orientation should be homogeneous for all biologicals; and (6) the chemistry used should be applicable with all manner of QDs and biologicals.\(^{45–47,49,50}\) A wide variety of QD bioconjugation chemistries have been described to date and these range from the ubiquitous carbodiimide-catalyzed amide bond formation between amines and carboxyls to specialized linkages such as those provided by chemoselective ligations.\(^{1,51}\) The former borrows heavily from ‘classical’ protein modification and labeling chemistry while the latter draws from recent peptide modification/synthesis, ‘click’, and bioorthogonal chemistries. Almost all available QD bioconjugation chemistries cannot yet achieve a majority let alone a plurality of the above-described ‘ideal’ criteria, thus this also remains an active area of research. The interested reader is referred to several recent and comprehensive reviews on both the issue of NP bioconjugation along with that of the equally important issue of subsequent analysis and characterization.\(^{45,46,52,53}\)

The Endosomal Dilemma

With almost no unequivocal examples to the contrary, unless the QDs are directly inserted into the cellular cytosol using a technique such as microinjection, the primary route for intracellular delivery will involve some form of endocytosis as the critical intermediary step. Endocytosis itself refers to the process whereby the cellular membrane undergoes invagination, followed by vesicle formation, subsequent vesicle internalization into the cytosol and then trafficking and delivery to a variety of organelles, recycling, or, alternatively, cellular expulsion.\(^{54,55}\) This allows cells to selectively, or nonselectively, take up everything from nutrients in their environment to recycling receptors on their surfaces along with NPs that may be attached to, in, or just near their membranes. Endocytosis is an extremely complex process that is still not fully elucidated and that occurs via multiple interrelated and nonrelated pathways including, but not limited to, those that are clathrin-dependent/independent, mediated by caveolae or involve some form of pinocytosis or phagocytosis, etc., see Figure 2.\(^{54–56}\) It should be noted that this description, in and of itself, is a gross oversimplification.

More pertinently, this means that almost all QD materials are taken up into cells by endocytosis and thus remain sequestered in the endolysosomal system never achieving access to the cytosol.\(^{24,33,57–59}\) In formats where fluorescent cellular labeling is the goal, endosomal delivery may suffice until QD signal is diminished over time by cellular division or exocytosis. If endosomal localization is not the desired endpoint for the QDs within the cells, this can present a major dilemma in that further mechanisms are required to functionally effect endosomal release. Recent reports attempting to achieve this are described in detail below.

**CELLULAR DELIVERY OF QDS**

As a means of updating our previous review on the various modalities used for the cellular delivery of QDs,\(^{24}\) here we specifically limit our discussion of QD cellular delivery techniques to those that have been described during the 5-year time period that encompasses January 2009 through December 2013. As in our previous review, we delineate the delivery modalities into three categories based on their physicochemical nature. Passive QD delivery utilizes the inherent physical properties of the QD material (e.g., surface coating, charge) to facilitate uptake primarily by endocytosis. Facilitated QD delivery typically relies on the association or decoration of the QD surface with a polymer of biological (e.g., peptide, protein) to drive initial interactions of the QD with plasma membrane and ultimately its internalization by endocytosis again.
Active techniques involve the direct, physical manipulation of the cell (e.g., microinjection) to introduce the QD to the cellular environment. Finally, we consider examples of combinatorial QD delivery wherein several of the above uptake modalities are used simultaneously. Representative examples of passive, facilitated, and active QD delivery described in the sections that follow are summarized in Table 1.

Passive QD Delivery
The primary advantage of passive QD delivery is its simplicity; the QDs are merely incubated with cultured cells and the physicochemical nature of the QD surface functionalization drives cellular internalization. Several studies have examined the utility of this approach for QD delivery with a focus toward the resulting impact on cellular homeostasis. For example, Nagy et al. examined the role of QD surface charge on concomitant cytotoxicity and genotoxicity.60 Using CdSe core QDs (3 nm diameter) bearing either mercaptpropionic acid (MPA, negatively charged) or cysteamine (CYST, positively charged), the authors observed that while both QD species were efficiently internalized by endocytosis, disparate effects on cellular survival were noted. In normal human primary bronchial epithelial cells (NHBE), cytotoxicity was charge-dependent, with the positively charged CYST-QDs exhibiting two-fold higher cytotoxicity than the negatively charged MPA-QDs as determined by lactose dehydrogenase activity and mitochondrial function. Further, CYST-QDs induced significant levels of intracellular reactive oxygen species (ROS) while MPA-QDs did not. Additionally, while QDs bearing both surfaces induced DNA strand breaks, DNA damage caused by MPA-QDs was coupled with the activation of metallothionein-mediated DNA repair while CYST-QDs displaying an excess of positive surface charge did not exhibit this response, pointing to differential activation of cell survival pathways based on QD surface charge. Gosso et al. examined the effect of passively endocytosed carboxyl-coated CdSe-ZnS core–shell QDs on Ca2+ channel activity and Ca2+-dependent neurotransmitter secretion.61 In primary mouse chromaffin cells (isolated from adrenal gland), chronic (>24 h) exposure to QDs at ~20 nM yielded efficient QD internalization with no undermining of plasma membrane integrity (Figure 3(a)). A significant (~30%) decrease in Ca2+ currents, however, was observed as was the depolarization-evoked

&FIGURE 2| Endocytosis of nanoparticles (NPs). Model of endocytic mechanisms and intracellular transport with a focus on NP uptake into cells. NPs (green dots) and other substances taken up by endocytosis are enclosed within the early endosomes (EE), phagosomes, or macropinosomes (MP). These vesicles with particles then mature down the degradative pathway and become multivesicular bodies/late endosomes (MVB) which fuse with lysosomes (Lys). Alternatively, the NPs may be transported back to the cell surface either directly from EE or through the recycling endosomes (RE). The pH drops gradually from the cell surface to lysosomes where the pH is 4.0–5.5. The lysosomes contain proteases and other enzymes that degrade most biological substances. (Reprinted with permission from Ref 55. Copyright 2011 Elsevier)
TABLE 1 | Methods Utilized for the Intracellular Delivery of Quantum Dots

| Strategy             | Mechanism                        | Examples                                                                 | Targeted Cells                                                                 | References |
|----------------------|----------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------------|------------|
| Facilitated delivery | Peptide-mediated                 | His-Arg-rich peptide                                                    | A549 (lung adenocarcinoma; cytosol)                                        | 69,82      |
|                      |                                   | gH625 (Herpes simplex virus derived-peptide)                            | HeLa (cervical adenocarcinoma; cytosol)                                      | 70         |
|                      |                                   | JB577 peptide (palmitoylated)                                           | HEK, COS-1, A549, primary fibroblast, chick embryo, rat hippocampal neurons  | 8,9,57     |
|                      |                                   | Hph-1 (Arg-rich)                                                        | MDA-MB-435 (breast carcinoma)                                                 | 71         |
|                      |                                   | LAH, sweet arrow peptide                                                | COS-1 (African green monkey kidney)                                           | 64         |
|                      | Chemoselective peptides          | JB577 peptide (palmitoylated)                                           | HEK, COS-1, A549, primary fibroblast, chick embryo, rat hippocampal neurons  | 8,9,57     |
|                      | Protein                           | Positively charged protein domains                                      | CD133+, CD34+, CD14+, mesenchymal stem cells                                  | 74         |
|                      |                                   | SV40 virus particles                                                    | Vero cells                                                                    | 67         |
|                      |                                   | Baculovirus                                                             | U87 human glioma                                                              | 75         |
|                      | Polymer                           | Cross-linked methacrylate                                               | HeLa                                                                          | 85         |
|                      |                                   | Chitosan                                                                 | L929 (murine fibrosarcoma)                                                    | 76         |
|                      |                                   | Liposomes                                                                | B16F10 (mouse melanoma)                                                       | 78         |
|                      |                                   | Triblock copolymer                                                      | Panc-1                                                                        | 79         |
|                      | Small molecule                    | Lactose                                                                  | HeLa, Araki Sasaki (human corneal epithelium)                                | 80         |
|                      |                                   | Galactose                                                                | HepG2 (hepatocyte), MCF-7                                                     | 81         |
|                      |                                   | Gambogic acid                                                           | HepG2                                                                         | 88         |
| Active delivery      | Nanoneedle injection             |                                                                           |                                                                                | 88         |
|                      | Reversible membrane permeabilization |                                             |                                                                                | 89         |
|                      | Nanochannel electroporation      |                                                                           |                                                                                | 90         |
|                      | Nanoblade                         |                                                                           |                                                                                | 91         |
|                      | Microfluidic cell ‘squeezing’    |                                                                           |                                                                                | 92         |
| Passive uptake       | QD surface character/charge      |                                                                           |                                                                                | 60,61      |

exocytosis of catecholamine-containing secretory vesicles. In addition, the Ca\(^{2+}\)-dependence of overall exocytosis was reduced. Cumulatively these data suggest that passively internalized QDs can abrogate the exocytic machinery and ultimately catecholamine secretion within chromaffin cells.

Seeking to elucidate the relationship between QD diameter and ultimate intracellular fate, Williams et al. examined a variety of core and core–shell structures spanning 2.6–5.4 nm in hydrodynamic diameter ($H_D$); a further goal was to demonstrate the cell type-dependence of intracellular membrane-based barriers.\(^6\)\(^2\) By incubating the QDs with fixed/permeabilized cells, the authors eliminated the effects of QD uptake mechanisms on intracellular localization/migration. The smallest QDs (2.1 nm core/shell only hard diameter, $H_D$ 2.6 nm) were shown to enter the nuclei and nucleoli in all cell types tested (THP-1 (macrophage), HEp-2 (epithelial), AGS (gastric adenocarcinoma), and A549 (lung epithelial). However, larger QDs (4.4 nm hard diameter, $H_D$ 5.4 nm) displayed reduced penetration and each cell type exhibited its own cutoff size threshold for cytoplasmic and nuclear pore penetration. For example, in
controlled intracellular delivery of semiconductor QDs

THP-1 cells, 3.7 and 3.9 nm hard diameter QDs ($H_D$ 4.3 and 4.6, respectively) targeted the nucleus while in HEp-2 cells these same QDs remained resident on the plasma membrane (Figure 3(b)). It should be noted that this study is unique for the use of such small QD materials as commercial QD preparations are significantly larger. Lastly, Wong et al. demonstrated with an in vivo tumor model the ability of 10 nm diameter QDs to efficiently penetrate the dense collagen matrix of the tumor interstitial space once they were released from within a larger 100 nm collagen NP matrix.63 Taking advantage of the leakiness of the tumor vasculature combined with the presence of matrix metalloproteases allowed the intact 100 nm collagen NPs to be subsequently degraded by these proteases within the tumor resulting in the release and further extravasation of the smaller 10 nm QDs within the tumor’s interstitial space (Figure 3(c)). Cumulatively, these examples highlight the ability of the inherent physicochemical nature of the QD to be used for cellular or tissue localization without the need for further decoration of the QD surface with a targeting ligand.

Facilitated QD Uptake and Cytosolic Delivery

Peptides

Owing to their small size, ease of synthesis, and minimal immunogenicity, peptides have clearly emerged as one of the more popular ‘go-to’ biologicals for facilitating cellular QD delivery and over the past five years the number of studies detailing their use has grown steadily. The most prominent peptide used in this role...
remains the canonical HIV Tat protein-derived polypeptide motif. It is believed that this strong, localized positive charge facilitates initial interactions with negatively charged heparan proteoglycan moieties on the cell surface allowing for ultimate uptake of any peptide associated cargo by endocytosis.13,44,54–59 More recently, several groups have featured the QD as a model cargo in an effort to determine the efficiency of uptake when screening panels of candidate peptides. For example, our laboratory examined the cellular internalization efficiency of QDs mediated by four different cell penetrating peptide (CPP) motifs and found two peptides including an amphipathic, Leu/Ala-rich peptide motif derived from a membrane-inserting antimicrobial peptide and a second sweet arrow (SA) peptide/superoxide dismutase domain chimera could provide efficient endocytosis and cellular labeling in a COS-1 cell-based delivery model.66 We noted that both peptides provided for endocytosis of QDs in a ratiometric, valence-dependent manner (a recurring theme that has now become the general rule in peptide-facilitated cellular NP uptake—see Ref 65 and references therein) while eliciting minimal cytotoxicity.

Marin et al. employed square wave voltammetry to measure the cellular uptake of CdS QDs modified with glutathione and decorated with the proline-rich sweet arrow or SA-CPP.66 Electrochemical reduction of CdS QDs remaining in cell supernatants after incubation of QD-SA-CPP with HeLa (human cervical cancer) cell monolayers was used to quantify the remaining noninternalized QDs. When coupled with laser-scanning confocal microscopy, the monodisperse QDs were found localized just inside the inner leaflet of the membrane bilayer (Figure 4(a)). The Park group employed thermally sensitive QDs whose appended CPP moieties were capable of being ‘deshielded’ by exposure to elevations in temperature.71 A heat-responsive polyacrylamide polymer was used to shield the CPP peptides under physiological conditions while increases in temperature resulted in the presentation of the CPPs to the cell, mediating endocytosis of the QDs under controlled conditions. At lower temperatures (e.g., incubation at 25°C), the CPPs are shielded; the polymer shell starts to constrict at the transition temperature of 32°C, approaching the physiologic temperature (37°C), and the CPPs are exposed and help mediate enhanced uptake of the QDs. It should also be noted that even with the shielding, QD uptake was only reduced by ~50%, while deshielding yielded ~86% of the uptake of the positive control. This approach, in a similar vein as the low pH-mediated deshielding of drug-loaded poly(lactic-co-glycolic) acid NPs described by Sethuraman et al.72 demonstrate the ability of the QD (or other NP) to be controllably actuated in the presence of the appropriate cellular stimuli. For a recent discussion of the various schemes for realizing the controlled actuation of NP constructs, the interested reader is directed to Ref 14. Finally, more recent work by our group has demonstrated the utility of a chemoselective conjugation chemistry approach to realize pepotidyl motifs that direct QDs to specific subcellular organelles/structures. Here, a common poly-His starter peptide domain (to facilitate peptide-driven metal affinity coordination based self-assembly to the QD surface) was linked to any of a number of functional ‘organelle-targeting’ peptide motifs using chemoselective hydrazine chemistry.73 These included peptides for QD delivery to endosomes, the cytosol, mitochondria, and the plasma membrane. In COS-1 cells, the rapid and efficient delivery of QDs to these discrete cellular locations was demonstrated as was the ability to chemically assemble rather than directly synthesize relatively long peptide sequences (43 amino acids in the case of the mitochondrial delivery peptide). These results suggest this synthesis approach as an attractive scheme for evaluating new peptide sequences for QD and indeed other NP cellular delivery schemes.

**Proteins and Protein Scaffolds**

While peptides have taken a prominent role in QD cellular delivery, proteins and protein-based scaffolds (including those based on viruses) continue to be implemented for the same purposes in creative ways. Ranjbarvaziri et al. used positively charged carrier protein domains to characterize the uptake efficiency of various sized QDs (ranging from 12 to 20 nm) emitting across a range of wavelengths (525–800 nm) and noted efficient endocytosis and intracellular distribution of all QD species among primary CD133+, CD34+, CD14+, and mesenchymal stem cells (MSCs).74 The authors observed time- and size-dependent differences in QD uptake efficiency with the larger QDs displaying higher internalization efficiency in CD14+ cells while in MSCs no size-dependent uptake efficiency over a 1 h delivery regime was noted. Regardless of QDs size, when QD incubation times were extended to ≥2 h, all cell types exhibited comparable QD uptake, revealing an upper saturation limit to overall QD uptake. Further, toxicity assessment by LDH release and TUNEL assays showed no measurable effects on cell viability at the 10 nM concentrations used for QD delivery with any of the QD species. Li et al. used viral capsid-QD
hybrid particles to image virus behavior during infection of Vero cells. CdSe/ZnS QDs encapsidated with the major capsid protein of SV40 virus were used to track the time-resolved binding, entry and intracellular translocation of the virus over a 50-h experimental time window. Colocalization studies using endocytosis markers confirmed the involvement of caveolae-mediated endocytosis in the viral internalization process (Figure 4(b)). Baculoviral-mediated transduction of cells and tissues is an emerging gene therapy modality and Zhao et al. used CdTe QDs capped with glutathione to realize a noncovalent labeling strategy that availed the tracking of viral infection in real time in live cells. In human U87 glioma cells, QD luminescence in transduced cells confirmed no deleterious effects on viral entry in vitro. When injected intravenously or intraventricularly into mice, viral-mediated delivery of a transgene was confirmed by two-color whole-body imaging as the QD allowed for tracking of successfully transduced tissues in real time.

Polymers
Polymeric materials continue to be employed as vectors for the intracellular delivery of QDs. Recent examples here include the use of chitosan nanospheres conjugated with Ag2S QDs for the light-triggered release and imaging of nitric oxide release. Chitosan has also been used as a delivery vector for the cellular delivery of a probe capable of detecting pre-miRNA. The probe was comprised of a thiolated RNA bound to a gold NP on one end and to a QD on the other end. Thus, when intact, the QD luminescence was quenched by the proximal gold NP. Upon hybridization of the probe to the targeted pre-miRNA, cleavage by RNaseIII Dicer resulted in the release of the QD and a subsequent increase in its luminescence emission. Liposomes continue to play a role in cellular QD delivery as well. For example, Wen et al. performed a comparative analysis of various liposomal formulations (cationic, PEGylated, deformable) for their theranostic imaging and drug-delivery potential. Commercial carboxyl-capped QDs (800 nm emission) were impregnated into the liposomal bilayers

FIGURE 4 | Facilitated quantum dot (QD) delivery. (a) CdS QDs decorated with the proline-rich sweet arrow peptide localize just inside the inner leaflet of the plasma membrane of HeLa cells. Electrochemical analysis was used to quantify the QDs remaining in the cell culture supernatants. (Reprinted with permission from Ref 66. Copyright 2011 ACS) (b) Viral capsid-QD hybrids for real-time tracking of SV40 infection in Vero cells. QDs (red) encapsidated with SV40 major coat proteins colocalize with a marker of caveolae-mediated endocytosis (green) as evidenced by the yellow merged color. QD-viral protein hybrids were not localized with a transferrin marker of recycling endosomes (not shown). Scale bar, 20 μm. (Reprinted with permission from Ref 67. Copyright 2009 Wiley) (c) Cellular QD delivery facilitated by small molecule ligands. Cysteamine/gambogic acid-functionalized CdTe QDs internalized by HepG2 cells. Scale bar, 20 μm. (Reprinted with permission from Ref 68. Copyright 2013 Dove Press) (d) Cytosolic delivery of QDs using His-rich poly Arg peptides. His-Arg9 peptides self-assembled to carboxyl-capped QDs deliver QDs rapidly to the cytosol in A549 cells. QDs (green), actin (red) and nuclei (blue) show distribution of QDs in the cytosol. Magnification, 600×. (Reprinted with permission from Ref 69. Copyright 2011 Elsevier) (e) Use of herpes simplex virus derived peptide for cytosolic delivery of QDs. An amphiphilic peptide mediates efficient endocytosis and direct membrane translocation to the cytosol of covalently coupled QDs. Scale bar, 50 μm. (Reprinted with permission from Ref 70. Copyright 2011 Elsevier)
and were used for tracking the successful delivery of camptothecin and irinotecan (model anticancer drugs for treatment of melanomas) to a B16F10 mouse melanoma cell line. Finally, the Prasad group synthesized CdTe/ZnS core/shell QDs and encapsulated them in triblock copolymer Pluronic F127 micelle NPs for both in vitro and in vivo imaging applications. The polymer, bearing PEG termini, formed a hydrophilic shell that enabled stable aqueous dispersion of the QDs. When functionalized with folate for targeting to folate receptors displayed on Panc-1 (human pancreatic carcinoma) cells, specific uptake with concomitant cell viabilities of >90% were noted. Subsequent in vivo tests showed specific homing to tumor tissue with no detectable localization to off-target tissue sites (e.g., lung, liver, heart). These results clearly suggest this formulation may have a great deal of promise for delivery utility with other NP materials.

**Small Molecule Ligands**

A number of reports have documented the decoration of QDs with a variety of small molecule ligands meant to facilitate cellular uptake and we highlight a few examples here. Benito-Alfonso et al. functionalized CdSe/ZnS QDs with various length glycosylamines (lactose derivatives) to drive QD uptake by HeLa and Araki Sasaki (human corneal epithelium) cells. A QD surface coverage of ∼60% dimeric lactose produced the optimum combination of cellular uptake by endocytosis coupled with minimal perturbation of cellular metabolism and proliferation. Cai et al. synthesized biodegradable NPs composed of acid-labile segments and galactose grafts that were loaded with QDs for tracking the uptake and degradation of the polymeric NPs once internalized by HepG2 liver cells and MCF-7 breast cancer cells. The galactose moieties directly facilitated the initial binding to cells and the subsequent internalization of the QD-loaded NPs where the low pH environment of endosomes resulted in QD release from the NP conglomerate. Xu and coworkers employed CdTe QDs functionalized with cysteamine (for net positive surface charge) wherein the QD surface was also decorated electrostatically with the naturally occurring anticancer drug gambogic acid. The combination of charge and the presence of the small gambogic acid molecular ligand availed efficient cellular internalization within endocytic vesicles. The intracellular accumulation of the QD-appended drug significantly inhibited the proliferation of HepG2 cells in a dose-dependent manner compared to that of bare QD controls alone.

**Cytosolic Delivery of QDs**

As evidenced from the examples above, it has been repeatedly confirmed that the predominant route of QD uptake when delivered using facilitated means is via the endocytic pathway; this remains a critical technical challenge as the QD materials remain sequestered within the vesicular endolysosomal system. Still, a number of groups have demonstrated significant progress in devising schemes to mediate the escape of QDs from within this vesicular system and we highlight these examples here. Liu et al. developed a histidine-, arginine-rich peptide that delivered CdSe/ZnS core/shell QDs rapidly (∼5 min) to the cytosol of A549 cells. The speed of QD delivery and the fact that disruption of the cytoskeletal network did not inhibit the QD localization to the cytosol argued for a direct membrane translocation, however, the role of endocytosis in QD uptake could not be ruled out (Figure 4(d)). The same authors demonstrated the extended utility of this peptide for the subsequent delivery of carboxylated, PEG bifunctionalized InP/ZnS QDs. Falanga et al. reported on a membrane-perturbing domain derived from the gH glycoprotein of herpes simplex virus type 1. The gH625 peptide is primarily a hydrophobic motif that presents a number of polar amino acids when in a helical conformation, imparting an amphiphilic character to the peptide that aids in its association with lipid membranes. When covalently attached to QDs as a model drug cargo, this peptide mediated specific membrane binding and subsequent cytosolic delivery that was significantly higher than for QDs delivered with the canonical Tat peptide as observed when both QD-peptide species were incubated with HeLa cells at 50 nM (Figure 4(e)). On the basis of the observation that cytosolic delivery occurred at both 37°C and 4°C, the authors proposed an internalization mechanism that involved both endocytosis and direct membrane translocation for QD-peptide delivery to the cytosol.

We have also been actively pursuing the cytosolic delivery of a range of NP materials including QDs. During a screening analysis of various QD delivery methodologies, we identified a multifunctional, multidomain peptide (named JB577—previously referred to as Palm-1) that mediates the initial rapid endocytic uptake of QDs followed by a slower, sustained release of the QDs to the cytosol over a 48-h time period. Originally designed for the delivery of protein palmitoyl transferase 1 (PPT1) inhibitors across the blood–brain barrier, the peptide consists of the sequence \(\text{WG}_6\text{(DapPal)}_3\text{VKIKK}_6\text{GGH}_4\); where Pal is a palmitoyl group anchored to a synthetic
diaminopropionic acid (Dap) residue by a nonhydrolyzable amide linkage. This modular peptide (modules separated by ● in the above sequence) comprises multiple functional domains: (1) a positively charged lysine-rich domain for initial cellular binding; (2) a nonhydrolyzable palmitoyl moiety for interaction with membranes; (3) a polyproline motif to present domains 1 and 2 away from the QD surface; and (4) a polyhistidine tract for self-assembly of the peptide to the QD surface. A detailed structure/function analysis of the peptide revealed that JB577’s delivery of QDs to the cytosol was a cumulative function of the individual domains. Indeed, this was shown to be the case for the uptake and cytosolic delivery of QDs to established cell lines and primary cells as well as to discrete regions of the developing chick embryo nervous system and rat hippocampal neuronal slices (Figure 5).8,9 Additionally, these studies demonstrated the utility of the JB577 peptide for the cytosolic delivery of a range of other nanomaterials including proteins, gold NPs, and dendrimeric constructs. Finally, the Helms group has devised a cross-linked core–shell polymer colloid containing a pH-buffering methacrylate group has devised a cross-linked core–shell polymer colloid containing a pH-buffering methacrylate system.85 Surface-exposed primary amines allowed for the electrostatic self-assembly of streptavidin-coated QDs while tertiary amines within the core facilitated the proton-sponge effect to swell and rupture endosomes. This system allowed for the rapid (within hours) cytosolic delivery of picomolar concentrations of QDs in HeLa cells after the QDs had been first internalized via endocytosis.

BOX 2

MULTIFUNCTIONAL, MODULAR PEPTIDES

Utilizing peptides to facilitate cellular uptake or act as an enzymatic substrate essentially borrows all that is required for a critical function from a protein without the rest of the extraneous sequence and bulk. Most peptides used to facilitate NP uptake into cells can be considered, for all intents and purposes, bi-functional; they generally contain a minimum of two functionalities within their sequence. Using the many derivatives of the HIV Tat derived polyarginine CPP as an example, these peptides mostly contain a run of Arg₅ for the ‘business’ portion of facilitating cellular uptake and then, if required, another functional group or residue with the requisite group for attachment to a NP. Examples of the latter include biotin for binding to a streptavidin on the NP surface or a cysteine-thiol for coordinating to a gold NP surface.46 Recent work, however, indicates it is possible to combine multiple-different ‘modular’ functionalities within a single peptide sequence such that it can, in turn, impart multiple functionalities to the NP conjugate without requiring many different peptides or other forms of mixed labeling.9 In our work, the initial concepts emerged from designing modular protease substrate peptides that would: (1) self-assemble to QDs, (2) display an internal protease-recognized sequence, (3) terminate in a site that would be dye-labeled with a FRET acceptor for the QD, (4) and have a rigid intervening sequence that allowed the FRET acceptor to extend out away from the QD surface.86 The work of Boeman et al., described herein shows that CPP-like peptides can be designed to contain multiple different functionalities within their short sequence and suggests that this strategy can be extended to incorporate even more functions in a ‘mix and match’ modular approach.9 Moreover, structure/activity studies and combinatorial selection schemes can help improve the design and potential activity of such peptide sequences.

Active QD Delivery

The active delivery of QDs to cells involves the direct manipulation of the cell to translocate the QD across the plasma membrane barrier with the goal of targeting the cytosol or other subcellular structures. Initially, direct microinjection29 and electroporation87 were the standard approaches used here and in recent years several elegant variations of these techniques have been reported. In a modification of the microinjection technique, Yum and coworkers used microinjection coupled with electrochemical ‘controlled deconjugation’ to realize the on-demand release of QDs into the nucleus of living HeLa cells.88 A boron nitride nanoneedle/electrode (50 nm diameter) coated with a thin layer of gold was decorated with streptavidin-coated QDs by their conjugation to a self-assembled monolayer (SAM) on the needle surface. Once in position, application of an electrical potential to the needle/electrode caused desorption of the SAM and the release of discrete amounts of monodisperse QDs that could be tracked within the nucleus (Figure 6(a)). The authors noted several advantages of their scheme over traditional microinjection that relies on positive pressure with micron-bore injection tips; namely, the ability to achieve on-demand release of minute amounts of QD cargo to the cytosol and the delivery of single,
monodisperse QDs with minimal perturbation of the plasma or nuclear membranes.

Two other active delivery approaches rely on the generation of transient pores in the plasma membrane for QD entry. Medepalli et al. described a reversible permeabilization technique that combines osmosis-driven fluid transport in a hypotonic environment coupled with the activity of the membrane-permeabilizing plant glycoside, saponin. The use of the hypotonic environment was critical to provide unidirectional flow of QDs present in the surrounding medium into the cell while eliminating the loss of intracellular contents. Using this approach, the authors delivered well-dispersed polymer-coated CdSe/ZnS QDs to the cellular cytosol in as little as 2–5 min with minimal cell death observed (Figure 6(b)). Nanochannel electroporation was employed by the Lee group where lipoplexes (plasmid DNA:lipid assemblies) were introduced directly into the cytosol using a chip-based platform that electrophoretically injects the cationic lipoplexes by applying electrical pulses through a nanochannel. In contrast to standard lipofection, which relies on the endocytic pathway for lipoplex uptake, this approach bypasses the vesicular-based endolysosomal system and accesses the cytosol directly. When the authors encapsulated 605 nm-emitting QDs and Cy5-labeled antisense oligonucleotide into the lipoplexes, FRET confirmed the nonendosomal/cytosolic morphology of the lipoplexes and that the dissolution of the assemblies within the cytosol could be tracked in real time (Figure 6(c)). The Weiss group developed a novel active QD delivery technique referred to as ‘nanoblade’ based on use of pulsed laser-induced surface plasmons within the thin titanium coating on the tip of a glass capillary pipet. Plasmon absorption conducts heat into the surrounding liquid medium proximal to the metal creating nanosecond-short vapor bubbles at the plasma membrane, which results in the large transient pores or cuts in the membrane. When performed in the presence of a positively pressurized capillary, the outward flow of cargo-containing liquid allows direct delivery to the cytosol. In contrast to traditional microinjection, the nanoblade is held in close contact with the membrane without ever mechanically puncturing it. Using this approach the authors delivered tubulin-QD conjugates and imaged the morphology of QD-conjugated tubulin incorporation into the cytoskeleton in both
Active cellular quantum dot (QD) delivery. (a) Electrochemically controlled deconjugation for QD delivery to the nucleus. A boron nitride nanoneedle bearing streptavidin-coated QDs attached to a SAM layer are desorbed when a voltage is applied (left panel) which allowed for single QD tracking within the nucleus of a live HeLa cell (split right panel). The nucleus is denoted with the dashed line (left) alongside the brightfield image (right). Scale bar, 1 μm. (Reprinted with permission from Ref 88. Copyright 2010 Wiley) (b) Reversible permeabilization facilitates cellular QD entry. The 530 nm-emitting CdSe/ZnS QDs delivered intracellularly using combination of osmotic fluid transport and membrane-permeabilizing saponin. Image shows DIC (left) and QD (right) signal in H9C2 rat cardiomyocyte cells. (Reprinted with permission from Ref 89. Copyright 2013 IOP Publishing) Scale bar, 10 μm. (c) Nanochannel electroporation (NEP) transfection of QD-antisense-lipoplex assemblies monitored by Förster resonance energy transfer (FRET). NEP transfection of lipoplex NPs containing QDs delivers lipoplexes directly to the cytosol within 10 min in A549 cells. Note the QD (blue) and Cy5-antisense (red) signals are matched and separate from the endosomal label (green). QD-Cy5 FRET was used to monitor the dissolution of the QDs and Cy5-labeled antisense oligonucleotide from the assemblies over time. (Reprinted with permission from Ref 90. Copyright 2013 Wiley) (d) Nanoblade-mediated labeling of cytoskeleton with tubulin-QD conjugates. Laser-induced surface plasmons from a titanium-coated capillary induced transient pores in the plasma membrane allowing the intracellular influx of tubulin-QD conjugates (green, panel 1) that incorporate into the cytoskeletal network. Immuno-counterstaining of the tubulin network (red, panel 2) and merged images (panel 3) are shown to illustrate the high degree of overlap. Scale bar, 10 μm. (Reprinted with permission from Ref 91. Copyright 2012 ACS) (e) Microfluidic device-mediated cytosolic delivery of QD-dye FRET constructs. A QD-Rhodamine donor-acceptor pair joined by a glutathione-sensitive disulfide linkage is delivered to the cytosol via microfluidic-driven cellular deformation initially shows full energy transfer of the green QD to the red dye (0 h, red color, left panel). After 15 h, the cytosolic glutathione reduces the thiol linkage resulting in reemission of the QD donor (15 h, green color, right panel). Scale bar, 10 μm. (Reprinted with permission from Ref 92. Copyright 2012 ACS)

When compared to conventional microinjection, the nanoblade approach proved superior for both the successful delivery of the QD-tubulin conjugates and concomitant cell viability. Finally, the Bawendi group has described cytosolic delivery of QDs using a microfluidic device based on cell constriction or ‘squeezing’. Here, cells are rapidly deformed as they are passed through a microfluidic channel, resulting in transient membrane perturbations that are sufficient to allow QD payloads present in the surrounding medium to enter the cellular cytosol. QD delivery to the cytosol using this novel method was confirmed using a redox-sensitive FRET construct consisting of a QD donor and a Rhodamine acceptor joined by a glutathione-sensitive disulfide linkage. Glutathione-induced reduction of the linkage resulted in QD reemission in a time-dependent manner as the Rhodamine quencher/acceptor was displaced from the construct (Figure 6(e)). Further, imaging of blinking QDs confirmed the delivery of monodispersed, nonaggregated QDs to the cytosol using this platform.
In summary, all of the techniques described above, while they involve direct physical manipulation of the cell and often the plasma membrane, appear to exhibit minimal perturbation of cellular viability and thus potentially represent new and exciting tools that target QDs and any appended cargos directly to the cytosol in a rapid fashion. Moreover, they are equally applicable to a wide range of other NP materials and similarly functional constructs.

**Combinatorial QD Delivery**

In this section, we highlight select examples from the literature that have integrated multiple facets of QD delivery to realize the simultaneous cellular delivery of multiple QD-appended biologicals, drugs or other moieties or analogously the cellular uptake of hybrid QD-NP constructs. In many of these applications, the major recurring theme is theranostics-related focusing on either gene or drug delivery with the QD playing a central role as both a scaffold for assembly/delivery as well as a sensor for intracellular tracking during cellular uptake and trafficking. In particular, QD-mediated delivery of antisense oligonucleotides and small interfering RNA (siRNA) has been a common theme of such activity in recent years. For example, Zhang et al. used streptavidin-conjugated QDs to display biotinylated versions of the cellular uptake dodecapeptide p160 (YPWMEPAYQRFL) and an antisense oligonucleotide for selectively downregulating the expression of folate receptor-α in MCF-7 human breast cancer cells. When delivered to MCF-7 cells, the peptide mediated the endosomal uptake and subsequent release of the QD-antisense conjugates to the cytosol (Figure 7(a)). The authors noted a significant (~80%) reduction in folate receptor mRNA and a concomitant reduction in receptor protein levels that tracked with the concentration of delivered QD complex in a dose-dependent manner. Other examples where siRNA delivery featured the QD as a central scaffold have included the synthesis of a library of nontoxic QDs for the targeted knockdown of EGFP expression in U87 glioblastoma cells, the silencing of the human papillomavirus E6 gene in HeLa cells and the reduction of β-secretase expression to reduce accumulation in SK-N-SH neuroblastoma cells. QDs have also been used to deliver full length plasmid DNA encoding the herpes simplex virus thymidine kinase ‘suicide gene’; cells induced to express this gene metabolize ganciclovir to ganciclovir monophosphate (an analog of deoxyguanosine triphosphate) resulting in inhibition of DNA polymerase activity. Following delivery of the complexes using a commercial transfection reagent, the QD luminescence allowed the tracking of the complexes over a 96-h period in HeLa cells.

The Weil group took advantage of the PL quenching of QDs when complexed with plasmid DNA and a cationized bovine serum albumin (for facilitating cellular uptake) to visualize the diffusing of individual QDs within large endosomes in live cells. Fluorescence correlation spectroscopy demonstrated the superior stability of the QDs within endosomes in A549 cells over a 24-h period.

Delivery of therapeutic drugs in conjunction with other cargos has been another major theme enabled by the cellular delivery of QDs. Li and coworkers developed β-cyclodextrin (β-CD)-modified QDs assembled with both Dox and siRNA moieties directed against the MDR1 (multidrug resistance) gene to reverse its activity in HeLa cells. β-CD served as the host for the Dox guest while the siRNA was electrostatically assembled to positively charged cellular uptake peptides on the QD surface. Efficient uptake of the QDs and the appended cargos was observed experimentally and was coupled with a concomitant reduction in MDR1 protein expression (Figure 7(b)). The latter feature enabled enhanced Dox-induced apoptosis in HeLa cells compared to the free Dox-only control while the QDs allowed for the tracking of the complexes by confocal microscopy. QD-NP hybrids have also been used for the enhanced delivery of drugs and other compounds. Weng et al. devised liposomes decorated with anti-epidermal growth factor receptor Fab’ fragments (antibodies) that were conjugated to QDs. This hybrid system was used for convection-enhanced (pump-driven cannula) targeted delivery and imaging of liposome homing to human U-87 glioblastoma cells in vitro and brain tumor xenografts in vivo. Multiple QD delivery methods have also been ‘hybridized’ together and used in combination to realize the spatiotemporal labeling and tracking of distinct cellular locations/structures. Our laboratory employed microinjection, transfection, and peptide delivery to direct multiple, different color QD populations to the cytosol, early and late endosomes, and the plasma membrane, respectively, in A549 cells over 5 days in culture with no deleterious effects on cellular function noted (Figure 7(c)). In what is perhaps the most elegant implementation of combinatorial cellular delivery featuring QDs described to date, the Brinker group developed supported lipid bilayers (protocells) capable of carrying and delivering multiple, disparate model cargos including: dsDNA as an siRNA mimic, a fluorescent protein as a model toxin mimic, calcine as a drug surrogate, and QDs as a model NP cargo. When the surface of the loaded protocells were conjugated with targeting peptides for uptake by Hep3B cells and fusogenic peptides for
cytosolic delivery, each of the disparate cargos was distributed to its respective cellular location within 4 h (Figure 7(d)).

The examples of combinatorial cellular delivery utilizing QDs described above are by no means intended to serve as a comprehensive list of those demonstrated to date. Rather, they represent much of what combined NP-based diagnostics/therapeutics or ‘theranostics’ aims to accomplish; the incorporation of disparate functional nanomaterials into a single NP platform capable of cellular uptake, diagnosis/sensing and concomitant delivery of therapeutic agents. As exemplified by the representative reports highlighted above, QDs are already playing key roles in this capacity as both a prototypical NP and an easily visualized and tracked nanoplatform.

OUTLOOK
Toxicity
Since they were first considered for use in biological applications (particularly within the context of living cells), concerns about the toxic effects of QDs have persisted. Initially, these concerns were based...
primarily on the toxic nature of some of their core constituent materials (e.g., Cd, Te, Se) and while the inherent toxicity of these materials is without question, years of research have shed light on how to best minimize the toxic impact of QDs and their bioconjugate compositions. This section is by no means intended to be a comprehensive review of QD cytotoxicity (for that, the reader is directed to several excellent recent reviews on the subject\textsuperscript{102,103}). Rather, our goal here is to highlight two critical features to consider in the modulation of QD cytotoxicity: (1) the role of overcoating shells for QD core containment and (2) the influence of capping ligands used for solubilization. In the former case, studies have consistently demonstrated the important role of the overcoating shell in effectively reducing the leaching of Cd\textsuperscript{2+} ions throughout the cell.\textsuperscript{104,105} Indeed, recent work by the Fan group compared the Cd\textsuperscript{2+}-induced toxicity of CdTe core only, CdTe-CdS core–shell and CdTe-CdS-ZnS core–shell–shell structures and found the core–shell–shell materials to elicit no toxic effects while the core only material was responsible for significant metal-induced toxicity.\textsuperscript{103} In a similar vein, the surface character presented to the cell can contribute directly to cytotoxicity. While positively charged ligands on the QD surface can mediate membrane interactions and facilitate QD internalization, studies have shown that QDs (as well as other NPs) bearing an abundance of positive surface charge can significantly impact cellular proliferation\textsuperscript{106,107} as well as initiate any of a number of protective cellular survival pathways (e.g., metallothionein).\textsuperscript{108} A further lesson that has been gleaned from recent QD cellular studies is that one always needs to take into context the end goal of the experiment in which the QDs are playing a featured role. For example, if the purpose of the QD is to serve as a label for imaging/tracking, it is quite apparent that under the appropriate experimental conditions (e.g., optimized QD dose, delivery route, experimental time course, cell line, etc.) one can often expect QD bioconjugates to be as innocuous as traditionally employed fluorophores that are routinely used for these purposes. Indeed, recent work has shown that in the case of some fluorophores routinely used for cellular labeling and imaging (e.g., nucleic acid dyes), QDs can have less impact on cellular health over the timecourse of the experiment.\textsuperscript{109}

QD toxicity and indeed the entire field of engineered nanomaterial toxicity for that matter are extremely complex with no simple definitive answer or resolution in sight. That said, this concern should clearly not be the sole reason for whether QDs are utilized in a cellular experiment where their unique photophysical properties may be helpful or even ultimately required.

**Future**

What does the near term hold for the use of QDs within the context of cellular uptake and delivery? We suggest that it will be far more versatile and sophisticated examples of what has been described here. Using a foundation of ever more controllable bioconjugation chemistries,\textsuperscript{110} QDs will continue to be an important cellular label especially for multiplexing, a central scaffold and active component of energy transfer based sensors,\textsuperscript{111–113} and, perhaps most importantly, a prototypical NP platform material for developing and understanding all aspects of theranostics and NP-mediated drug delivery. This will again rely on concerted efforts in continuing the development of new QD ligands and surfaces.\textsuperscript{28,37,39,42–44,78,79,81,82,96,111,114} We can also expect far more elegant and novel mechanisms to be developed for facilitating QD cellular delivery. These will include new peptide sequences,\textsuperscript{25} new chemical and physical methods and new combinations of QD materials and techniques.\textsuperscript{115} The lessons learned from these endeavors, however, will extend far beyond that of just QD utility.

**ACKNOWLEDGMENTS**

The authors acknowledge the NRL NSI and Base Funding Program, and DTRA JSTO MIPR # B112582M.

**REFERENCES**

1. Chan WCW, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* 1998, 281:2016–2018.

2. Bruchez M Jr, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. *Science* 1998, 281:2013–2016.
3. Petryayeva E, Medintz IL, Algar WR. Quantum dots in bioanalysis: a review of applications across various platforms for fluorescence spectroscopy and imaging. *Appl Spectrosc* 2013, 67:215–252.

4. Algar WR, Susumu K, Delehanty JB, Medintz IL. Quantum dots in bioanalysis: crossing the valley of death. *Anal Chem* 2011, 83:8826–8837.

5. Rosenthal SJ, Chang JC, Kovtun O, McBride JR, Tomlinson ID. Biocompatible quantum dots for biological applications. *Chem Biol* 2011, 18:10–24.

6. Algar WR, Krull UJ. New opportunities in multiplexed optical bioanalyses using quantum dots and donor-acceptor interactions. *Anal Bioanal Chem* 2010, 398:2439–2449.

7. Algar WR, Massey M, Krull UJ. The application of quantum dots, gold nanoparticles and molecular switches to optical nucleic-acid diagnostics. *Trac-Trends Anal Chem* 2009, 28:292–306.

8. Walters R, Kraig RP, Medintz IL, Delehanty JB, Stewart MH, Susumu K, Huston AL, Dawson PE, Dawson G. Nanoparticle targeting to neurons in a rat hippocampal slice culture model. *ASN Neuro* 2012, 4:e00099.

9. Boeneman K, Delehanty JB, Blanco-Canosa JB, Susumu K, Stewart MH, Oh E, Huston AL, Dawson G, Ingle S, Walters R, *et al.* Selecting improved peptidyl motifs for cytosolic delivery of disparate protein and nanoparticle materials. *ACS Nano* 2013, 7:3778–3796.

10. Spillmann CM, Ancona MG, Buckhout-White S, Algar WR, Stewart MH, Susumu K, Huston AL, Goldman ER, Medintz IL. Achieving effective terminal exciton delivery in quantum dot antenna-sensitized multistep DNA photonic wires. *ACS Nano* 2013, 7:7101–7118.

11. Medintz IL, Farrell D, Susumu K, Trammell SA, Deschamps JR, Brunel FM, Dawson PE, Mattoussi HM. Multiplex charge transfer interactions between quantum dots and peptide-bridged ruthenium complexes. *Chem. Anal* 2009, 81:4831–4839.

12. Claussen JC, Algar WR, Hildebrandt N, Susumu K, Ancona MG, Medintz IL. Biophotonic logic devices based on quantum dots and temporally-staggered Förster energy transfer relays. *Nanoscale* 2013, 5:12156–12170.

13. Delehanty JB, Boeneman K, Bradburne CE, Robertson K, Medintz IL. Quantum dots: a powerful tool for understanding the intricacies of nanoparticle-mediated drug delivery. *Exp Opin Drug Deliv* 2009, 6:1091–1112.

14. Delehanty JB, Breger JC, Boeneman GK, Stewart MH, Medintz IL. Controlling the actuation of therapeutic nanomaterials: enabling nanoparticle-mediated drug delivery. *Ther Deliv* 2013, 11:1411–1429.

15. Delehanty JB, Susumu K, Manthe RL, Algar WR, Medintz IL. Active cellular sensing with quantum dots: transitioning from research tool to reality. *Anal Chim Acta* 2012, 750:63–81.

16. Medintz IL, Mattoussi H. Quantum dot-based resonance energy transfer and its growing application in biology. *Phys Chem Chem Phys* 2009, 11:17–45.

17. Algar WR, Kim H, Medintz IL, Hildebrandt N. Emerging non-traditional Förster resonance energy transfer configurations with semiconductor quantum dots: investigations and applications. *Coord Chem Rev* 2014, 263-263:65–85.

18. Algar WR, Malanoski AP, Susumu K, Stewart MH, Hildebrandt N, Medintz IL. Multiplexed tracking of coupled protease activity using a single color of quantum dot vector and a time-gated Förster resonance energy transfer relay. *Anal Chem* 2012, 84:10136–10146.

19. Algar WR, Malonski A, Deschamps JR, Blanco-Canosa JB, Susumu K, Stewart MH, Johnson BJ, Dawson PE, Medintz IL. Proteolytic activity at quantum dot-conjugates: kinetic analysis reveals enhanced enzyme activity and localized interfacial “hopping”. *Nano Lett* 2012, 12:3793–3802.

20. Stewart MH, Huston AL, Scott AM, Efros AL, Melinger JS, Gemmill KB, Trammell SA, Blanco-Canosa JB, Dawson PE, Medintz IL. Complex Förster energy transfer interactions between semiconductor quantum dots and a redox-active osmium conductor: *ACS Nano* 2012, 6:5330–5347.

21. Stewart MH, Huston AL, Scott AM, Oh E, Algar WR, Deschamps JR, Susumu K, Jain V, Prasuhn DE, Blanco-Canosa J, *et al.* Competition between Förster resonance energy transfer and electron transfer in stoichiometrically assembled semiconductor quantum dot-fullerene conjugates. *ACS Nano* 2013, 7:9489–9505.

22. Medintz IL, Pons T, Delehanty JB, Susumu K, Brunel FM, Dawson PE, Mattoussi H. Intracellular delivery of quantum dot-protein cargos mediated by cell penetrating peptides. *Bioconjug Chem* 2008, 19:1785–1795.

23. Prasuhn DE, Deschamps JR, Susumu K, Stewart MH, Boeneman K, Blanco-Canosa JB, Dawson PE, Medintz IL. Polyvalent display and packing of peptides and proteins on semiconductor quantum dots: predicted versus experimental results. *Small* 2010, 6:555–564.

24. Delehanty JB, Mattoussi H, Medintz IL. Delivering quantum dots into cells: strategies, progress and remaining issues. *Anal Bioanal Chem* 2009, 393:1091–1105.

25. Ravindran S, Snee PT, Eapen AS, Sundivakkam P, Tiruppathi C, George A. Acidic domains in DPP facilitate endocytosis: implications for targeted protein delivery. *J Biol Chem* 2013, 288:16098–16109.

26. Moquin A, Hutter E, Choi AO, Khatchadourian A, Castonguay A, Winnik FM, Maysinger D. Caspase-1 activity in microglia stimulated by pro-inflammagen nanocrystals. *ACS Nano* 2013, 11:9585–9598.
27. Delehanty JB, Bradburne CE, SusumU K, Boeneman K, Mei BC, Farrell D, Blanco-Canosa JB, Dawson PE, Mattoussi H, Medintz IL. Spatiotemporal multicolor labeling of individual cells using peptide-functionalized quantum dots and mixed delivery techniques. J Am Chem Soc 2011, 133:10482–10489.

28. Wu XY, Liu HJ, Liu JQ, Haley KN, Treadway JA, Larsson JP, Ge NF, Peale F, Bruchez MP. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 2003, 21:41–46.

29. Medintz IL, Stewart MH, Trammell SA, SusumU K, Delehanty JB, Mei BC, Melinger JS, Blanco-Canosa JB, Dawson PE, Mattoussi H. Quantum-dot/dopamine bioconjugates function as redox coupled assemblies for in vitro and intracellular pH sensing. Nat Mater 2010, 9:676–684.

30. Bao G, Mitragotri S, Tong S. Multifunctional nanoparticles for drug delivery and molecular imaging. Annu Rev Biomed Eng 2013, 15:253–282.

31. Kelkar SS, Reineke TM. Theranostics: combining imaging and therapy. Bioconjugate Chem 2011, 22:1879–1903.

32. Tan A, Yildirimer L, Rajadas J, De La Pena H, Pastorin G, Seifalian A. Quantum dots and carbon nanotubes in oncology: a review on emerging theranostic applications in nanomedicine. Nanomedicine 2011, 6:1101–1114.

33. Ho YP, Leong KW. Quantum dot-based theranostics. Nanoscale 2010, 2:60–68.

34. Ho YP, Chen HH, Leong KW, Wang TH. Evaluating the intracellular stability and unpacking of DNA nanocomplexes by quantum dots-FRET. J Control Release 2006, 116:83–89.

35. Bagalkot V, Zhang L, Levy-Nissenbaum E, Jon S, Kantoff PW, Langer R, Farokhzad OC. Quantum dot–aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on Bi-fluorescence resonance energy transfer. Nano Lett 2007, 7:3065–3070.

36. Brunetti V, Chibli H, Fiammengo R, Galeone A, Malvindi MA, Vecchio G, Cingolani R, Nadeau JL, Pompa PP. InP/ZnS as a safer alternative to CdSe/ZnS core/shell quantum dots: in vitro and in vivo toxicity assessment. Nanoscale 2013, 5:307–317.

37. Law WC, Yong KT, Roy I, Ding H, Hu R, Zhao WW, Prasad PN. Aqueous-phase synthesis of highly luminescent CdTe/ZnTe core/shell quantum dots optimized for targeted bioimaging. Small 2009, 5:1302–1310.

38. Chang E, Thekkkek N, Yu WW, Colvin VL, Drezek R. Evaluation of quantum dot cytotoxicity based on intracellular uptake. Small 2006, 2:1412–1417.

39. Oh JK. Surface modification of colloidal CdX-based quantum dots for biomedical applications. J Mater Chem 2010, 20:8433–8445.

40. Pons T, Uyeda HT, Medintz IL, Mattoussi H. Hydrodynamic dimensions, electrophoretic mobility and stability of hydrophilic quantum dots. J Phys Chem B 2006, 110:20308–20316.

41. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A. In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science 2002, 298:1759–1762.

42. Mei BC, SusumU K, Medintz IL, Delehanty JB, Mountziaris TJ, Mattoussi H. Modular poly(ethylene glycol) ligands for biocompatible semiconductor and gold nanocrystals with extended pH and ionic stability. J Mater Chem 2008, 18:4949–4958.

43. Mei BC, SusumU K, Medintz IL, Mattoussi H. Polyethylene glycol-based bidentate ligands to enhance quantum dot and gold nanoparticle stability in biological media. Nat Protoc 2009, 4:412–423.

44. SusumU K, Oh E, Delehanty JB, Blanco-Canosa JB, Johnson BJ, Jain V, Hervey WJ, Algar WR, Boeman K, Dawson PE, et al. Multifunctional compact zwitterionic ligands for preparing robust biocompatible semiconductor quantum dots and gold nanoparticles. J Am Chem Soc 2011, 133:9480–9496.

45. Algar WR, Prasuhn DE, Stewart MH, Jennings TL, Blanco-Canosa JB, Dawson PE, Medintz IL. The controlled display of biomolecules on nanoparticles: a challenge suited to bioorthogonal chemistry. Bioconjug Chem 2011, 22:825–858.

46. Sapsford KE, Algar WR, Berti L, Boeman Gemmill K, Casey BJ, Oh E, Stewart MH, Medintz IL. Functionalizing nanoparticles with biological molecules: developing chemistries that facilitate nanotechnology. Chem Rev 2013, 113:1904–2074.

47. Aubin-Tam ME, Hamad-Schifferli K. Structure and function of nanoparticle-protein conjugates. Biomed Mater 2008, 3:034001.

48. Boeman K, Deschamps JR, Buckhout-White S, Prasuhn DE, Blanco-Canosa JB, Dawson PE, Stewart MH, SusumU K, Goldman ER, Ancona M, et al. Quantum dot DNA bioconjugates: attachment chemistry strongly influences the resulting composite architecture. ACS Nano 2010, 4:7253–7266.

49. Medintz I. Universal tools for biomolecular attachment to surfaces. Nat Mater 2006, 5:842.

50. Medintz IL. Interfacing biology with nanomaterials. Mater Today 2009, 12:6–7.

51. Prasuhn DE, Blanco-Canosa JB, Vora GJ, Delehanty JB, SusumU K, Mei BC, Dawson PE, Medintz IL. Combining chemoselective ligation with polyhistidine-driven self-assembly for the modular display of biomolecules on quantum dots. ACS Nano 2010, 4:267–278.

52. Biju V. Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. Chem Soc Rev 2014, 13:744–764.
53. Sapsford KE, Tyner KM, Dair BJ, Deschamps JR, Medintz IL. Analyzing nanomaterial biocomjugates: a review of current and emerging purification and characterization techniques. *Anal Chem* 2011, 83:4453–4488.

54. Sorkin A, Zastrow M. Endocytosis and signalling: intertwining molecular networks. *Nat Rev Mol Cell Biol* 2009, 10:609–622.

55. Kastl L, Sasse D, Wulf V, Hartmann R, Mirch J, Iversen TG, Skotland T, Sandvig K. Endocytosis and intracellular transport of nanoparticles: present knowledge and need for future studies. *Nano Today* 2011, 6:176–185.

56. Fonseca SB, Pereira MP, Kelley SO. Recent advances in the use of cell-penetrating peptides for medical and biological applications. *Adv Drug Deliv Rev* 2009, 61:953–964.

57. Delehanty JB, Bradburne CE, Boeneman K, Susumu K, Farrell D, Mei BC, Blanco-Canosa JB, Dawson G, Dawson PE, Mattoussi H, et al. Delivery of quantum-dot-peptide biocomjugates to the cellular cytosol: escaping from the endolysosomal system. *Integr Biol* 2010, 2:265–277.

58. Fonseca SB, Pereira MP, Kelley SO. Recent advances in the use of cell-penetrating peptides for medical and biological applications. *Adv Drug Deliv Rev* 2009, 61:953–964.

59. El-Sayed A, Futaki S, Harashima H. Delivery of macromolecules using arginine-rich cell-penetrating peptides: ways to overcome endosomal entrapment. *AAPS J* 2009, 11:13–22.

60. Nagy A, Hollingsworth JA, Hu B, Steinbrück A, Stark PC, Rios Valdez C, Vuyisich M, Stewart MH, Atha DH, Nelson BC, et al. Functionalization-dependent induction of cellular survival pathways by CdSe quantum dots in primary normal human bronchial epithelial cells. *ACS Nano* 2013, 7:8397–8411.

61. Gosso S, Gavello D, Giachello CNG, Franchino C, Carbone E, Carabelli V. The effect of CdSe-ZnS quantum dots on calcium currents and catecholamine secretion in mouse chromaffin cells. *Biomaterials* 2011, 32:9040–9050.

62. Williams Y, Sukhanova A, Nowostawska M, Davies AM, Mitchell S, Oleinkov V, Gun’ko Y, Nakiev I, Kelleher D, Volkov Y. Probing cell-type-specific intracellular nanoscale barriers using size-tuned quantum dots. *Small* 2009, 5:2581–2588.

63. Gong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W, Popovic Z, Jain RK, Pawendy MG, Fukumura D. Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proc Natl Acad Sci USA* 2011, 108:2426–2431.

64. Gemmill K, Muttenhalter M, Delehanty J, Stewart M, Susumu K, Dawson P, Medintz I. Evaluation of diverse peptidyl motifs for cellular delivery of semiconductor quantum dots. *Anal Bioanal Chem* 2013, 405:6145–6154.

65. Delehanty JB, Boeneman K, Bradburne CE, Robertson K, Bongard JE, Medintz IL. Peptides for specific intracellular delivery and targeting of nanoparticles: implications for developing nanoparticle-mediated drug delivery. *Ther Deliv* 2010, 1:411–433.

66. Marin S, Pujals S, Giralt E, Merkoci A. Electrochemical investigation of cellular uptake of quantum dots decorated with a proline-rich cell penetrating peptide. *Bioconjug Chem* 2011, 22:180–185.

67. Xu P, Li J, Shi L, Selke M, Chen B, Wang X. Synergetic effect of functional cadmium-tellurium quantum dots conjugated with gambogic acid for HepG2 cell-labeling and proliferation inhibition. *Int J Nanomedicine* 2013, 8:3729–3736.

68. Liu BR, Y-w H, Winiarz JG, Chiang H-J, Lee H-J. Intracellular delivery of quantum dots mediated by a histidine- and arginine-rich HR9 cell-penetrating peptide through the direct membrane translocation mechanism. *Biomaterials* 2011, 32:3520–3537.

69. Falanga A, Vitiello MT, Cantisani M, Tarallo R, Guarnieri D, Mignonno E, Netti P, Pedone C, Galdiero M, Galdiiero S. A peptide derived from herpes simplex virus type 1 glycoprotein H: membrane translocation and applications to the delivery of quantum dots. *Nanomaterials* 2011, 7:925–934.

70. Kim C, Lee Y, Kim JS, Jeong JH, Park TG. Thermally triggered cellular uptake of quantum dots immobilized with poly(N-isopropylacrylamide) and cell penetrating peptide. *Langmuir* 2010, 26:14965–14969.

71. Sethuraman VA, Lee MC, Bae YH. A biodegradable pH-sensitive micelle system for targeting acidic solid tumors. *Pharm Res* 2008, 25:657–666.

72. Delehanty JB, Blanco-Canosa JB, Bradburne CE, Susumu K, Stewart MH, Prasuhn DE, Dawson PE, Medintz IL. Site-specific cellular delivery of quantum dots with chemoselectively-assembled modular peptides. *Chem Commun* 2013, 49:7878–7880.

73. Ranjarbavaziri S, Kiani S, Akhlaghi A, Vosough A, Baharvand H, Aghdami N. Quantum dot labeling using positive charged peptides in human hematopoietic and mesenchymal stem cells. *Biomaterials* 2011, 32:5195–5205.

74. Zhao Y, Lo SL, Zheng Y, Lam DH, Wu C, Han MY, Wang S. Quantum dot coating of baculoviral vectors enables visualization of transduced cells and tissues. *Biochem Biophys Res Commun* 2013, 434:110–116.
oxide release and near-infrared fluorescence imaging. *Langmuir* 2013, 29:15032–15042.

77. Geng Y, Lin D, Shao L, Yan F, Ju H. Cellular delivery of quantum dot-bound hybridization probe for detection of intracellular pre-microRNA using chitosan/poly(γ-glutamic acid) complex as a carrier. *PLoS One* 2013, 8:e65540.

78. Wen C-J, Sung CT, Aljuffali IA, Huang Y-J, Fang J-Y. Nanocomposite liposomes containing quantum dots and anticancer drugs for bioimaging and therapeutic delivery: a comparison of cationic, PEGylated and deformable liposomes. *Nanotechnology* 2013, 24:325101.

79. Liu L, Yong KT, Roy I, Law WC, Ye L, Liu J, Kumar R, Zhang X, Prasad PN. Bioconjugated pluronic triblock-copolymer micelle-encapsulated quantum dots for targeted imaging of cancer: in vitro and in vivo studies. *Theranostics* 2012, 2:705–713.

80. Benito-Alifonso D, Tremel S, Hou B, Lockyear H, Mantell J, Fermin DJ, Verkade P, Berry M, Galan MC. Lactose as a “trojan horse” for quantum dot cell transport. *Angew Chem Int Ed Engl* 2013, 52:810–814.

81. Cai X, Li X, Liu Y, Wu G, Zhao Y, Chen F, Gu Z. Galactose decorated acid-labile nanoparticles encapsulating quantum dots for enhanced cellular uptake and subcellular localization. *Pharm Res* 2012, 29:2167–2179.

82. Liu BR, Winiarz JG, Moon JS, Lo SY, Huang YW, Aronstam RS, Lee HJ. Synthesis, characterization and applications of carboxylated and polyethylene-glycolated bifunctionalized InP/ZnS quantum dots in cellular internalization mediated by cell-penetrating peptides. *Colloids Surf B Biointerfaces* 2013, 111:162–170.

83. Cho S, Dawson PE, Dawson G. Antisense palmitoyl thioesterase 1 (PPT1) treatment inhibits PPT1 activity and increases cell death in LA-N-5 neuroblastoma cells. *J Neurosci Res* 2000, 62:234–240.

84. Dawson G, Dawson SA, Marinzi C, Dawson PE. Anti-tumor promoting effects of palmitoyl: protein thioesterase inhibitors against a human neurotumor cell line. *Cancer Lett* 2002, 187:163–168.

85. Bayles AR, ChaHAL HS, ChaHAL DS, Goldbeck CP, Cohen BE, Helms BA. Rapid cytosolic delivery of luminescent nanocrystals in live cells with endosome-disrupting polymer colloids. *Nano Lett* 2010, 10:4086–4092.

86. Medintz IL, Clapp AR, Brunel FM, Tiefenbrunn T, Uyeda HT, Chang EL, Deschamps JR, Dawson PE, Mattoussi H. Proteolytic activity monitored by fluorescence resonance energy transfer through quantum-dot-peptide conjugates. *Nat Mater* 2006, 5:581–589.

87. Derfus AM, Chan WCW, Bhatia SN. Intracellular delivery of quantum dots for live cell labeling and organelle tracking. *Adv Mater* 2004, 16:961–966.

88. Yum K, Wang N, Yu M-F. Electrochemically controlled deconjugation and delivery of single quantum dots into the nucleus of living cells. *Small* 2010, 6:2109–2113.

89. Medepalli K, Alphenaar BW, Keynton RS, Sethu P. A new technique for reversible permeabilization of live cells for intracellular delivery of quantum dots. *Nanotechnology* 2013, 24:205101.

90. Boukany PE, Wu Y, Zhao X, Kwak KJ, Glazer PJ, Leong K, Lee LJ. Nonendocytic delivery of lipoplex nanoparticles into living cells using nanochannel electroporation. *Adv Healthcare Mater* 2013, 3:682–689. doi: 10.1002/adhm.201300213.

91. Xu J, Teslaa T, Wu T-H, Chiou P-Y, Teitell MA, Weiss S. Nanoblade delivery and incorporation of quantum dot conjugates into tubulin networks in live cells. *Nano Lett* 2012, 12:5669–5672.

92. Lee J, Sharei A, Sim WY, Adamo A, Langer R, Jensen KF, Bawendi MG. Nonendocytic delivery of functional engineered nanoparticles into the cytoplasm of live cells using a novel, high-throughput microfluidic device. *Nano Lett* 2012, 12:6322–6327.

93. Zhang M-Z, Yu Y, Yu R-N, Wan M, Zhang R-Y, Zhao Y-D. Tracking the down-regulation of folate receptor-α in cancer cells through target specific delivery of quantum dots coupled with antisense oligonucleotide and targeted peptide. *Small* 2013, 9:4183–4193.

94. Wu YZ, Eisele K, Doroshenko M, Algara-Siller G, Kaiser U, Koyakov K, Weil T. A quantum dot photoswitch for DNA detection, gene transfection, and live-cell imaging. *Small* 2012, 8:3465–3475.

95. Weng KC, Hashizume R, Noble CO, Serwer LP, Drummond DC, Kirpotin DB, Kuwabara AM, Chao LX, Chen FF, James CD, et al. Convection-enhanced delivery of targeted quantum dot-immunoliposome hybrid nanoparticles to intracranial brain tumor models. *Nanomedicine* 2013, 8:1913–1925.

96. Subramaniam P, Lee SJ, Shah S, Patel S, Starovoytov V, Lee KB. Generation of a library of non-toxic quantum dots for cellular imaging and siRNA delivery. *Adv Mater* 2012, 24:4014–4019.

97. Li JM, Zhao MX, Su H, Wang YY, Tan CP, Ji LN, Mao ZW. Multifunctional quantum-dot-based siRNA delivery for HPV18 E6 gene silence and intracellular imaging. *Biomaterials* 2011, 32:7978–7987.

98. Li S, Liu Z, Ji F, Xiao Z, Wang M, Peng Y, Zhang Y, Liu L, Liang Z, Li F. Delivery of quantum dot-siRNA nanoplexes in SK-N-SH cells for BACE1 gene silencing and intracellular imaging. *Mol Ther Nucleic Acids* 2012, 1:e20.

99. Shao D, Zeng QH, Fan Z, Li J, Zhang M, Zhang YL, Li O, Chen L, Kong XG, Zhang H. Monitoring HSV-TK/ganciclovir cancer suicide gene therapy using CdTe/CdS core/shell quantum dots. *Biomaterials* 2012, 33:4336–4344.
100. Li JM, Wang YY, Zhao MX, Tan CP, Li YQ, Le XY, Ji LN, Mao ZW. Multifunctional QD-based co-delivery of siRNA and doxorubicin to HeLa cells for reversal of multidrug resistance and real-time tracking. *Biomaterials* 2012, 33:2780–2790.

101. Ashley CE, Carnes EC, Phillips GK, Padilla D, Durfee PN, Brown PA, Hanna TN, Liu J, Phillips B, Carter MB, et al. The targeted delivery of multi-component cargos to cancer cells by nanoporous particle-supported lipid bilayers. *Nat Mater* 2011, 10:389–397.

102. Winnik FM, Maysinger D. Quantum dot cytotoxicity and ways to reduce it. *Acc Chem Res* 2013, 46:672–680.

103. Yong K-T, Law W-C, Hu R, Ye L, Liu L, Swihart MT, Prasad PN. Nanotoxicity assessment of quantum dots: from cellular to primate studies. *Chem Soc Rev* 2013, 42:1236–1250.

104. Chen N, He Y, Su Y, Li X, Huang Q, Wang H, Zhang X, Tai R, Fan C. The cytotoxicity of cadmium-based quantum dots. *Biomaterials* 2012, 33:1238–1244.

105. Cho SJ, Maysinger D, Jain M, Roder B, Hackbarth S, Winnik FM. Long-term exposure to CdTe quantum dots causes functional impairments in live cells. *Langmuir* 2007, 23:1974–1980.

106. Hoshino A, Hanada S, Yamamoto K. Toxicity of nanocrystal quantum dots: the relevance of surface modifications. *Arch Toxicol* 2011, 85:707–720.

107. Hoshino A, Manabe N, Fujioka K, Suzuki K, Yasuhara M, Yamamoto K. Use of fluorescent quantum dot bioconjugates for cellular imaging of immune cells, cell organelle labeling, and nanomedicine: surface modification regulates biological function, including cytotoxicity. *J Artif Organs* 2007, 10:149–157.

108. Nagy A, Zane A, Cole SL, Severance M, Dutta PK, Waldman WJ. Contrast of the biological activity of negatively and positively charged microwave synthesized CdSe/ZnS quantum dots. *Chem Res Toxicol* 2011, 24:2176–2188.

109. Bradburne CE, Delehanty JB, Boeneman Gemmill K, Mei BC, Mattoussi H, Susumu K, Blanco-Canosa JB, Dawson PE, Medintz IL. Cytotoxicity of quantum dots used for in vitro cellular labeling: role of QD surface ligand, delivery modality, cell type, and direct comparison to organic fluorophores. *Bioconjugate Chem* 2013, 24:1570–1583.

110. Blanco-Canosa JB, Wu M, Susumub K, Petryayeva E, Jennings TL, Dawson PE, Algar WR, Medintz IL. Recent progress in the bioconjugation of quantum dots. *Coord Chem Rev* 2014, 263–264:101–137.

111. Tyrakowski CM, Snee PT. A primer on the synthesis, water-solubilization, and functionalization of quantum dots, their use as biological sensing agents, and present status. *Phys Chem Chem Phys* 2014, 16:837–855.

112. Algar WR, Kim H, Medintz IL, Hildebrandt N. Emerging non-traditional Förster resonance energy transfer configurations with semiconductor quantum dots: investigations and applications. *Coord Chem Rev* 2014, 263–264:65–85.

113. Hötzer B, Medintz IL, Hildebrandt N. Fluorescence in nanobiotechnology – sophisticated fluorophores for novel applications. *Small* 2012, 8:2297–2326.

114. Park J, Nam J, Won N, Jin H, Jun S, Jung S, Cho SH, Kim S. Compact and stable quantum dots with positive, negative, or zwitterionic surface: specific cell interactions and non-specific adsorptions by the surface charges. *Adv Funct Mater* 2011, 21:1558–1566.

115. Jennings TL, Becker-Catania SG, Triulzi RC, Tao G, Scott B, Sapsford KE, Spindel S, Oh E, Jain V, Delehanty JB, et al. Reactive semiconductor nanocrystals for chemoselective biolabeling and multiplexed analysis. *ACS Nano* 2011, 5:5579–5593.