Quantum dots and other nanoparticles: what can they offer to drug discovery?

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Nanocrystals (quantum dots) and other nanoparticles (gold colloids, magnetic bars, nanobars, dendrimers and nanoshells) have been receiving a lot of attention recently with their unique properties for potential use in drug discovery, bioengineering and therapeutics. In this review, structural, optical and biological assets of nanocrystals are summarized and their applications to drug discovery studies are discussed. Unique properties of these nanoparticles can offer new advancements in drug discovery.

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▼ Current drug discovery approaches suffer from biology problems associated with target selection (poorly validated targets fail in discovery or in clinical development) and chemistry problems associated with leads (failure in lead identification or optimization, or the leads prove to be toxic) [1,2]. The establishment of HTS technologies in the 1990s helped to generate new leads in shorter times [3]. However, HTS addresses mainly quantity; needs for improved quality in drug discovery remain unmet. For this, is there a need for a new technology to help to solve some of the central problems related to drug discovery? What can help to identify and validate a disease-specific target or to identify a molecule that can modify this in a therapeutic sense? How about recent developments in nanotechnology? Can we expect help from nanoparticles that are claiming to have advanced properties compared with their counterparts, such as nanocrystals or simple quantum dots?

Quantum dots (QDs) are semiconducting materials that are, in general, synthesized with II-VI or III-V column elements of the periodic table. They are neither atomic nor bulk semiconductors. Their properties originate from their physical size, which ranges from 10–100 Å in radius. Due to their bright fluorescence, narrow emission, broad UV excitation and high photostability [4,5], QDs have been adopted for *in vitro* bioimaging by many researchers as an alternative to organic based fluorophores [4,6–10]. Most recently, *in vivo* applications of these QDs have also been reported [11–13].

In this review, the intrinsic properties of QDs will be summarized and alternative promising nanoparticles will be briefly listed together with their basic principles. However, the million-dollar question still remains: can QDs or other related nanoparticles offer advantages for drug discovery? A realistic discussion together with future opportunities and directions will conclude this review.

Characteristics of quantum dots

Structural properties

Semiconducting QDs are spherical in shape, mostly direct-band-gap materials, and hold hundreds or thousands of atoms depending on their final size. Their radii varies from anywhere between 10–100 Å, which is known to be smaller than the bulk Bohr excitation radius (for CdSe dots, it is 50Å) [14,15]. When the radius of the QDs is smaller than the bulk Bohr excitation radius, it is reasonable to refer to energy levels rather than energy bands under the quantum confinement. QDs reveal unique electrical and optical properties coupled with the atomic structures of the dots cubic (zinc blende) or hexagonal (wurtzite) and due to quantum confinement.

Optical properties

As mentioned earlier, QDs have symmetric and narrow bandwidth of ~30 nm full width at half maximal fluorescence, which enables emission of pure color. By contrast, the bandwidths of organic dyes (e.g. fluorescein) vary between 50–100 nm. This is demonstrated in Figure 1 [16] for CdSe-ZnS QDs at different sizes. In addition, their absorption spectrum reaches into the UV regardless of their size.

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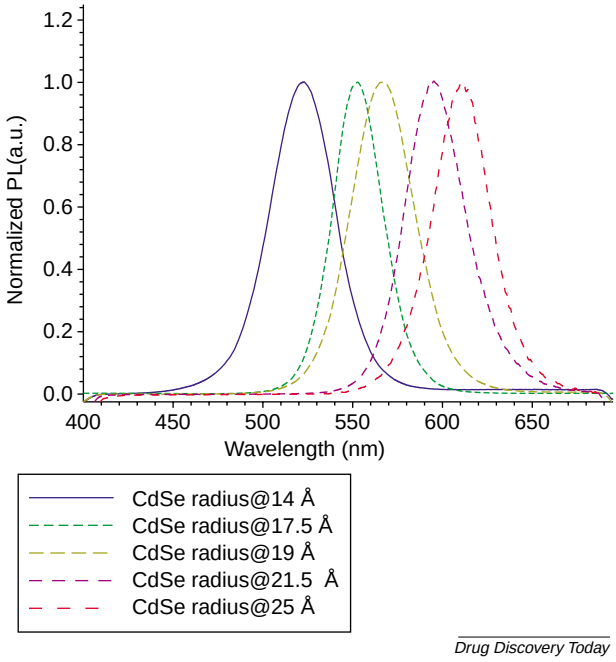

Figure1. Photoluminescence spectra of CdSe-ZnS quantum dots with different core sizes. Quantum dots were excited at 350nm [16]. (re-printed with permission from Elsevier).

This behavior enables multiplexed imaging with a single excitation source and thus prevents overheating of cells or tissue during multi-color imaging with a great promise for both *in vitro* and *in vivo* applications (i.e. several new drug leads can be tested simultaneously) [17]. Similarly, QDs' extinction coefficients are in the order of 10^{-15} cm^2, which is about an order of magnitude greater than organic dyes. With respect to their emission, the quantum yields of CdSe QDs can be anywhere from 40 to 90%. However, one needs to be aware that for biological applications, QDs need to be in a water-soluble form. To address this, various methods have been developed including mercaptoacidic acid, dihydrolipoic acid, dithiothreitol treatments or encapsulation in block copolymers. Mercaptoacid imparts water solubility and provides carboxyl groups for the condensation chemistry necessary for further covalent modification [4]. It has been suggested that the 'bidentate' type of interaction of dihydrolipoic acid (DHLA) [18] or dithiothreitol [19] with the inorganic QDs' surface results in QDs that are more water-stable. The charged surface provided by DHLA-capped CdSe-ZnS QDs drives electrostatic self-assembly of QDs-protein conjugates that, once formed, are surprisingly stable even in high-salt solutions [18]. Both Quantum Dot Corporation and Evident Technology Inc. are selling water soluble QDs using the encapsulation method in block copolymers as demonstrated in [10] and [12].

Although the surface functionalization of QDs improves their solubility, one consequence could be reduced quantum efficiency. In the case of mercaptoacedic acid-treated dots, quantum efficiency can fall from 40–90% to ~6–8% [18,4]. On the other hand, protein-functionalized QDs tend to retain their original quantum efficiency, offer longer shelf life and they can be further functionalized with multiple functional groups such as amines, carboxylic acids, and cysteine residues [18].

Although possessing superb optical characteristics, in 1996 Nirmal *et al.* discovered that QDs underwent an intermittent on–off emission (called 'blinking') under continuous excitation [20], which was attributed to Auger ionization [20,21]. However, even today the principle of this behavior is not well understood. This can simply be a bottleneck during the flow cytometry applications that are commonly used during drug discovery, where the emission from the individual QD might be off due to 'blinking' and the signal from this QD can be missed at the detector. This is a concern when a signal from individual QD is required during the analysis. However, in general, for example in cell-based assays, there are more than one QD involved and, while some are 'blinking', others can be 'on' for the final detection and thus no signal will be missed by the detector.

Photostability

Bawendi and co-workers [22,23] estimated that the molar extinction coefficients of CdSe QDs are ~10^5–10^6 M^{-1} cm^{-1}, depending on the particle size and the excitation wavelength. These values are 10–100 times larger than those of organic dyes. When the emission lifetime for QDs was compared with other commonly used organic dyes, as depicted in Figure 2, superb performance was found [24]. However, in aqueous buffer solutions, QDs with thiol surface functionalized groups can experience photochemical instability. The photochemical instability of the QDs actually includes three distinguishable processes: the photocatalytic oxidation of the thiol ligands on the surface of QDs; the photooxidation of the QDs; and the precipitation of the QDs. As first reported by Aldana [25] at first, the thiol ligands on the surface of a nanocrystal were gradually photocatalytically oxidized using the CdSe nanocrystal core as the photocatalyst. Then, the photogenerated holes in a nanocrystal were trapped onto the thiol ligands bound on the surface of the nanocrystal, which initiated the photooxidation of the ligands and protected the nanocrystal from any photooxidation. After almost all thiol ligands converted to disulfides, (1) the QDs precipitated out of the solution without much variation over their size and size distribution or (2) when the disulfides were insoluble in water, they likely formed a micelle-like structure around

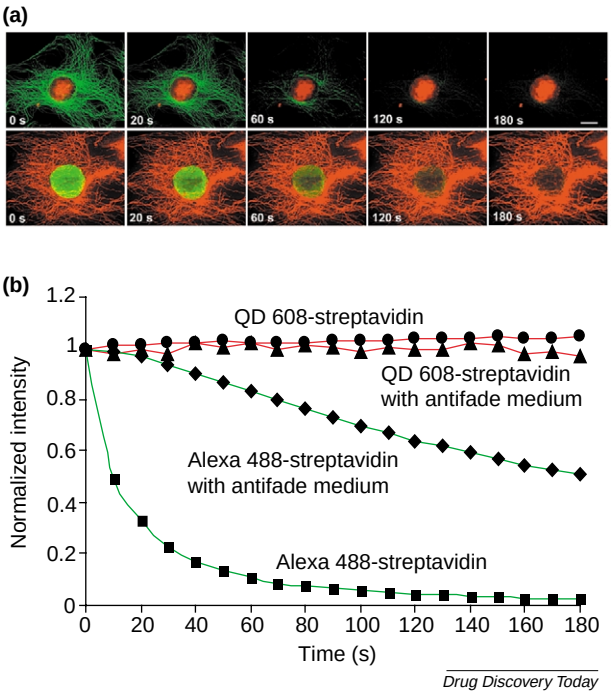

Figure 2. Photostability comparison between QDs and Alexa 488. **(a)** Top row: nuclear antigens were labeled with QD 630-streptavidin (red), and microtubules were labeled with Alexa 488 conjugate to anti-mouse igG (green) simultaneously in a 3T3 cell. Bottom row: microtubules were labeled with QD 630-streptavidin (red), and nuclear antigens were stained green with Alexa 488 conjugated to anti-human IgG. The specimens were continuously illuminated for three minutes with light from a 100 W mercury lamp under a 100x 1.3 oil-imersion objective. An excitation filter (ex 485+ 20 nm) was used to excite both Alexa 88 and QD 630. Emission filters em 535+ 10nm and em 635+10nm on a motorized filter wheel were used to collect Alexa 488 and QD 630 signals, respectively. Images were captured with a cooled CCD camera at ten second intervals for each color automatically. Images at 0, 20, 60, 120 and 180 s are shown. Whereas labelling signals of Alexa 488 faded quickly and became undetectable within two minutes, the signals of QD 630 showed no obvious change for the entire three minute illumination period. Scale bar: 1μm. **(b)** Quantitative analysis of changes in intensities of QD 608-streptavidin (stained microtubules) and Alexa 488-streptavidin (stained nuclear antigens) using specimens mounted with glycerol or antifade mounting medium Vectashield. All experimental conditions were the same as in **(a)** except that the emission filter for QD 608 was 605+ 10nm. Mean fluorescence intensity was automatically measured every ten seconds for three minutes [24]. (reprinted with permission from *Nature Biotechnology*).

the nanocrystal core and kept it soluble in the solution. Finally, the photochemical stability of CdSe QDs was reported to be closely related to the thickness and packing of the respective ligand monolayer.

In other words, QDs have a longer emission lifetime than organic dyes, but their solubility can be affected by the combined results of functionalization and photooxidation. This could cause agglomeration of QDs, which can adversely influence the kinetics of their movement after their agglomeration into larger clusters during *in vitro* and *in vivo* applications. For example, when the drug molecules are attached to the surface of QDs for recording the kinetics of uptake (e.g. size matters under these circumstances), large-size clusters of QDs with drug molecules might never be transported inside the cell or will take longer time to undergo endocytosis. Similarly, attachment of multiple leads to the same QD or QD clusters could also be an issue during the drug uptake.

Recently, many biological applications of QDs have been reported. For instance, using enhanced photostability of QDs, Simon and colleagues reported long-term multiple color imaging of live cells using nanocrystal bioconjugates [26]. Similarly, Mauro and colleagues reported QD-fluorescent resonance energy transfer- (FRET-) based nanoprobes for a biosensing application [27]. QD ligands have been used by Movin and colleagues for erbB/HER receptor-mediated signal transduction applications [28]. Bruchez [24] and colleagues have applied QDs to cancer research. Ruoslahti [13], Webb [11] and Libchaber [12] have also reported *in vivo* applications of QDs.

Biotoxicity

As explained earlier, as a result of their superior optical properties, QDs have become more widely used for *in vivo* applications [11–13]. This raises questions with respect to their biotoxicity. This question has been investigated by number of groups [29–32]. In these studies it has been reported that surface oxidation can occur under combined exposure to the aqueous/ UV-light excitation. This can lead to the release of cadmium ions in the case of CdSe-based QDs. The mechanisms for this were suggested as the tri-n-octylphosphine oxide- (TOPO-) mediated or UV-catalyzed surface oxidation. Toxicity of CdSe QDs in liver culture model is found to be dependent on processing conditions and nanoparticle dose. Under oxidized (30 min exposure to air) or long UV radiation (2–8 h) conditions, even QD concentrations of 0.0625 mg/mL are found to be highly toxic [29].

It is common knowledge that Cd is highly toxic, and this could be a major concern for *in vivo* applications. The surface oxidation of the core QDs can be reduced by using inorganic or organic based shell layers, which could create a barrier for oxygen diffusion; however, a combined aqueous/UV-light excitation environment can still act as a catalyst and enhance the diffusion process. Before QDs are adopted for *in vivo* applications, a comprehensive study of shell type and thickness, as well as the relative diffusion rate of oxygen need to be well understood. As of today, this

concern related to their toxic content being released under the given conditions effectively excludes the choice of QDs as drug delivery vehicles, even though they can offer a lot with additional surface functionalization capability, especially for targeted drug delivery.

Available types

QDs are now studied in various fields including biology, electronics and optoelectronics. For these applications various types of QDs were synthesized, such as InP, InAs, GaAs, and GaN, as well as porous Si and Si/Ge dots. In addition, the family of II-VI compounds such as ZnS and ZnSe QDs has absorption and emission spectra limited to the UV and blue regions. To this end, QDs with heavier atoms such as CdTe or HgSe or hybrids composed of PbSe have been studied due to their extended optical properties into the near-infrared region. This type of dot would be preferred probes for tissue studies, where absorption of the tissue is minimal in the near-infrared region.

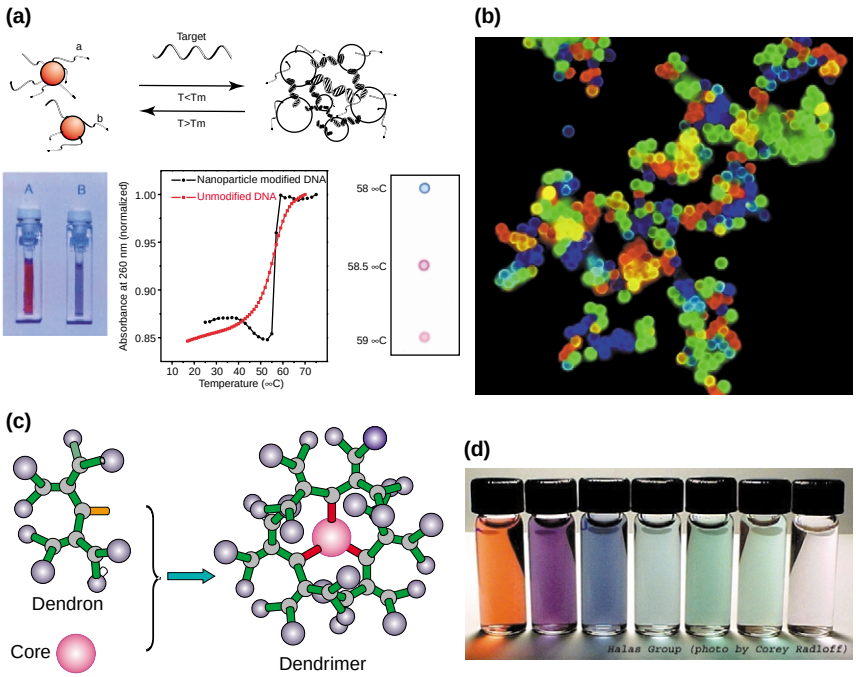

Figure 3. Other nanoparticles: **(a)** gold colloids for detection of DNA sequences (reprinted with permission from Mirkin); **(b)** nanobarcodes (reprinted with permission from Nie), **(c)** dendrimers (reprinted with permission from Frechet), **(d)** nanoshells (reprinted with permission from Halas).

Outline of other nanoparticle technologies

Besides semiconducting QDs, other types of nanoparticles have been developed for biological applications. Below, are selected some of the nanoparticles that can also offer advancements in drug discovery.

Gold colloids

Mirkin [33–34] and colleagues have been using gold colloids for various applications. After surface functionalization with, for example, thiol groups, DNA or other chemical groups can be decorated onto the surface of nanogold. This group recently demonstrated the use of these nanoparticles for detection of genetic sequence in solution (Figure 3a). The addition of two different nanogolds with two different DNA sequences that are complementary for a full complete DNA sequence in a search can cause the hybridization of these nanogold particles into aggregates of gold specks. The color of the test solution then changes from red to blue. In summary, gold nanoparticles can be used for genetic applications and, due to further surface functionalization ability, they can also be used for drug discovery applications as an alternative to QDs. Under these circumstances, instead of the usual fluorescent microscopes, scanning electron or transmission electron microscopes need to be used for visualization. This could limit their function to highly specific applications.

Magnetic tags

Some living microorganisms contain small amounts of ferromagnetic material such as magnetite, which orient the microorganisms in the geomagnetic field. This fact has inspired an approach for detection of the binding effect of substrates on the magnetic nanoparticles that can align under an applied magnetic field. Whereas uniformly aligned particles are detected by magnetic detectors, others that are randomly oriented will be ignored. As a result, this technique does not require a washing step before imaging, because other non-specific moieties inside the same buffer or sample will not bind to these particles and thus will not affect the imaging. During the imaging of magnetic nanoparticles a 'microscope' based on a high-transition temperature superconducting quantum interference device (SQUID) is used. At the present time this technique is not mature enough to be used immediately; however, Clarke and colleagues are working on the enhancement of SQUID microscope [35–36].

Nanobarcodes

Nie [37] *et al*. embedded QDs such as CdSe- and ZnS-capped dots in different colors with highly controlled ratios into polymer microbeads (Figure 3b). This created a large spectrum of beads with different colors and intensities for multiplexed, HTS of DNA or proteins. This technique utilizes the advantages of QDs over organic dyes. The spectrum of QD-embedded microbeads was reported to be 10% narrower than the QDs alone, which further benefits the multiplexed imaging. In addition, the approximate pore separation on the surface of the polymer microbeads was given as ~30 nm within 1.2 μm bead that contains 50,000 QDs. This eliminates the possibility of FRET between the two neighbouring QDs, as the distance between adjacent pores (30nm) greatly exceeds the Förster radius (5–8nm). These promising microbeads were applied for DNA detection and hybridization of target sequences. They can withstand higher temperatures during the hybridization process than QDs. The sensitivity to the low amount of target sequences is currently under investigation.

Dendrimers

Dendrimers are hyperbranched, tree-like structures and have compartmentalized chemical polymers (Figure 3c). Dendrimer chemistry is a rapidly expanding field for basic and applicative reasons. From a topological viewpoint, dendrimers contain three different regions: core, branches, and surface. They can be tailored or modified into biocompatible compounds with low cytotoxicity and high biopermeability. In addition, dendrimers are manufactured in high purities with few structural defects, and are easily analyzed by standard methods as mass spectrometry, infrared spectroscopy and NMR spectroscopy.

Proteins and dendrimers differ in that: proteins consist of folded linear polypeptide chains, whereas dendrimers are mostly formed by covalent bonds, creating a more rigid structure; dendrimers are not as compact as proteins; dendrimers contain far more surface groups capable of being functionalized compared with proteins of similar size. In drug research, dendrimers have been used as drugs for antibacterial and antiviral treatment and have found use as anti-tumor agents, drug- or gene-delivery devices and 'glycocarriers' for the controlled multimeric presentation of biologically relevant carbohydrate moieties, which are useful for targeting modified tissue in malignant diseases for diagnostic and therapeutic purposes [38,39]. One important issue relates to the biocompatibility of these nanoparticles. Dendrimers with cationic surface groups will, in general, cause destabilization of the cell membrane and result in cell lysis [40].Recently, new hydroxy- or methoxy-terminated dendrimers based on a polyester scaffold have shown to be non-toxic *in vitro* and *in vivo* [41,42]. In addition, luminescent dendrimers have been developed by adsorbing, caging or covalently binding fluorescent entities inside or on the surface of the dendrimers. This could give additional advantages in drug research [43].

Nanoshells

Nanoshells are gold-layered dielectric nanoparticles with optical resonances that can be 'tuned' by the control of the relative size of their constituent layers (Figure 3d). In the research groups of Halas and West, these nanoparticles have been applied to number of biological applications such as detection of immunoglobulins in whole blood, and for thermal ablation of cancerous cells both *in vitro* and *in vivo* [44,45]. In the latter case, since nanoshells absorb strongly in the near infrared, where optical transmission through tissue is optimal, cells in the vicinity of these nanoshells can be overheated locally under applied near-infrared light. *In vitro* and *in vivo* studies found that this causes irreversible cellular or tissue damage at these local areas. Combination of this local heating with functionalized drug leads would be an optimum approach to achieving a 'chemo-light' therapy for cancer. The groups mentioned earlier are actively working on the multi-layer and multi-functional nanoshells for applications in biology and optoelectronics.

Conclusion: comparison of technologies

In this review, different types of nanoparticles including QDs, gold colloids, magnetic tags, nanobarcodes, dendrimers and nanoshells are described for their potential use in drug discovery. First, QDs are nanometer-size semiconducting particles that have enhanced optical properties as compared with organic dyes. They have narrow band emission together with large UV absorption spectra, which enables multiplexed imaging under a single light source. Their amenability to further bio-functionalization offers great potential advantages in biology and medicine research. For example, multiple leads can be tested on cell culture simultaneously. Similarly, the absorption of several drug molecules can be studied simultaneously for a longer period of time. Using the surface functionalization properties of QDs, targeting capabilities can be added as well. In addition, due to the inorganic nature of QDs, their interaction with their immediate environment at *in vivo* states can be minimal compared with their organic counterparts. In the case of their biotoxicity, QDs might not be the perfect choice for *in vivo* drug delivery; however, in cell- or tissue-based drug studies QDs offer great imaging results that could not be achieved by organic dyes. In common with

other nanoparticles, QDs have not been totally perfected. For example, size variation during the synthesis [37] of single color dots is ~2-4% (reported by Evident Technologies and Quantum Dot Corporation). At first, this might not seem to be significant; however, for applications such as capillary dielectrophoresis/electrophoresis or gel electrophoresis, it could create false results. Therefore, QD synthesis techniques need to have improved quality control with respect to size distribution before they can be seriously utilized in drug discovery research.

Similarly, one other potential concern for their use in drug discovery would be their total size. For ADME purposes, blue dots (at a diameter of 3.7nm) are the smallest class of the QD family. Even this size can potentially be an issue for such experiments because they are considerably larger than organic dyes. Hence, the use of QDs for this purpose might not be desirable in special cases. Similarly, the number of functional groups attached to an organic dye is usually one, or it can be controlled very precisely. However, in the case of QDs, the functional groups usually decorate the entire surface and thus cause multiple attachments of target molecules. Again if the interest is the absorption of a single drug molecule into a cell, the transport of a large volume (due to multiple attachments of drug molecules to a single QD) across the membrane will be more difficult than a single molecule itself. In addition, to satisfy all the available surface groups, larger numbers of target molecules are needed; this could affect the cost of the experiment. Recently, several methods have been reported to reduce the number of surface groups around a single dot [13]; however, each of these methods adds to the final size of the QDs, which might not be desired in many cases, especially in studies related to kinetics and transport of drug molecules. One other issue with QDs is their 'blinking' characteristics when they are excited with high-intensity light [20]. Would this be an issue for drug discovery research? This could be a limiting factor for fast scan systems such as flow cytometry.

Finally, under combined aqueous-UV excitation conditions, QDs demonstrate oxidation and release of Cd ions into the environment. This is a definite concern for *in vivo* applications. As an alternative, capping the surface of a core dot with a large band-gap-semiconductor or proteins can eliminate or reduce the toxicity. But, it is important to remember that each additional step on the QDs will add to their final size and could even affect their final size distribution during these additional process steps. So, is there a 'magic' nanoparticle that can do better than any of the ones discussed here?

The answer to this question is not trivial. For some applications, using one nanoparticle against the other can be more advantageous, but definitely using one for all is not possible at this stage. For the case of QDs, they can be used for high-throughput cell-based studies with the advantage of multiplexing (i.e. multiple leads can be tested at the same time). However, as discussed earlier there are some limitations yet to be resolved for their use in the drug discovery studies, namely, toxicity, size variation, agglomeration, potential multiple drug attachment to a single QD and blinking. As long as the advantages and disadvantages for each nanoparticle are understood very well, the analysis of the experimental data and some ambiguities in the data can be ruled out in a better way. With the current extra effort being given in the nanotechnology area, some of the shortcomings of these particles should be understood and addressed in the near future. Perhaps the birth of a better nanoparticle can be expected.

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