Quantum dots versus organic dyes as fluorescent labels

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Suitable labels are at the core of luminescence and fluorescence imaging and sensing. One of the most exciting, yet also controversial, advances in label technology is the emerging development of quantum dots (QDs)—inorganic nanocrystals with unique optical and chemical properties but complicated surface chemistry—as *in vitro* and *in vivo* fluorophores. Here we compare and evaluate the differences in physicochemical properties of common fluorescent labels, focusing on traditional organic dyes and QDs. Our aim is to provide a better understanding of the advantages and limitations of both classes of chromophores, to facilitate label choice and to address future challenges in the rational design and manipulation of QD labels.

The investigation of many fundamental processes in the life sciences relies on the fast, sensitive, reliable and reproducible detection of the interplay of biomolecules with one another and with various ionic or molecular species. Fluorescence techniques are very well suited to realize these goals[1–3]. Fluorescence methods encompass several unique experimental parameters (for instance, excitation and emission wavelength, intensity, fluorescence lifetime and emission anisotropy) and offer nanometer-scale resolution and possible sensitivity down to the single-molecule level.

The potential of a detection or imaging method is to a great extent determined by the physicochemical properties of the chromophore used[1,4]. These include its chemical nature and size, its biocompatibility, and the interplay between dye and biological unit. Fluorophore properties affect the detection limit and the dynamic range of the method, the reliability of the readout for a particular target or event, and the suitability for multiplexing, that is, parallel detection of different targets.

There is a variety of chromophores from which to choose: (i) molecular systems with a defined structure, which include small organic dyes[3,4], metal-ligand complexes such as $[Ru(bpy)_3]^{2+}$ (refs. 1,2) and lanthanide chelates[5], and fluorophores of biological origin like phycobiliproteins and genetically encoded fluorescent proteins[6], (ii) nanocrystal chromophores with size-dependent optical and physicochemical properties, which include QDs made from II/VI and III/V semiconductors[7,8], carbon[9] and silicon nanoparticles[10] and self-luminescent organic nanoparticles[11], and (iii) nanometer- to micrometer-sized particles with size-independent optical features[12]. Particle labels have been recently reviewed[12–15], and we do not describe them here.

A suitable label (i) is conveniently excitable, without simultaneous excitation of the biological matrix, and detectable with conventional instrumentation; (ii) is bright, that is, possesses a high molar absorption coefficient at the excitation wavelength and a high fluorescence quantum yield (see **Table 1** for definitions), (iii) is soluble in relevant buffers, cell culture media or body fluids, (iv) is sufficiently stable under relevant conditions, (v) has functional groups for site-specific labeling, (vi) has reported data about its photophysics, and (vi) is available in a reproducible quality. Depending on the application, additional important considerations include (vii) steric and size-related effects of the label, (ix) the possibility to deliver the label into cells, (x) potential toxicity of the label, (xi) suitability of the label

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for multiplexing, and (xii) its compatibility for signal-amplification strategies. Here we consider these properties in a comparison of organic dyes, which are the most versatile molecular labels, with QDs made from II/VI and III/V semiconductors, which are the most frequently used nanocrystal labels in bioanalytics or medical diagnostics. The discussion of many of the properties of the organic dyes, such as their photophysics, also applies to fluorescent proteins. Metal-ligand complexes and lanthanide chelates displaying long lifetimes (several hundred nanoseconds to a few microseconds) as well as phosphorescence emitters and bio- and chemoluminescent systems are beyond the scope of this review[1].

Table 1 | Glossary of terms used

Molar absorption coefficient (ε)	Absorbance divided by the absorption pathlength and the analyte or species concentration.
Photoluminescence (here termed fluorescence) quantum yield (Φ_f)	Number of emitted photons occurring per number of absorbed photons. Φ_f is typically determined relative to a dye of known fluorescence quantum yield[2].
Blinking	Continuously illuminated single QDs or dye molecules emit detectable luminescence for limited times, interrupted by dark periods during which no emission occurs.
Brightness	Product of the molar absorption coefficient at the excitation wavelength and the fluorescence quantum yield; used as measure for the intensity of the fluorescence signal obtainable upon excitation at a specific wavelength or wavelength region.
Emission anisotropy (fluorescence polarization)	Measure for the polarization of the emitted light upon excitation with linearly polarized light. The emission anisotropy reflects the rotational freedom of molecules in the excited state.
Fluorescence lifetime	Average time an excited fluorophore remains in the excited state before it emits a photon and decays to the ground state, measurable in the time or the frequency domain.
Quantum size effect	Alteration of the electronic and thus the optical properties of solids if the dimensions of relevant structural features interfere with the delocalized nature of the electronic states. For semiconductor particles (QDs), this effect occurs typically for sizes in the range of a few to ten nanometers, and results in a blueshift in absorption and in luminescence with decreasing size.
QD-surface passivation	Modification of the surface of bare QD cores to improve properties such as the fluorescence quantum yield or the resistance to chemical reaction. Achieved either by deposition of a layer of inorganic, chemically inert material and organic ligands or of a layer of organic molecules only.
Stokes shift	Difference (usually in frequency units) between the spectral positions of the maxima of the lowest energy (that is, longest wavelength) absorption and the luminescence arising from the same electronic transition. In the case of QDs, this is the difference between the first excitonic absorption band and the emission maximum.
Two-photon action cross-section	Product of the two-photon absorption cross-section and the fluorescence quantum yield, which describes the probability of the simultaneous absorption of two photons and transition of the fluorophore to an excited state that differs energetically from the ground state by the energy of the two photons.

Optical properties

The relevant spectroscopic features of a fluorescent label include the spectral position, width and shape of the fluorophore's absorption and emission bands, the Stokes shift (**Table 1**), the molar absorption coefficient and the fluorescence quantum yield. The fluorescence lifetime and emission anisotropy are other exploitable chromophore properties. The Stokes shift determines spectral overlap and therefore is relevant for the ease of separation of excitation from emission and the efficiency of emission signal collection. It can also affect spectral cross-talk in two- or multi-fluorophore applications such as fluorescence resonance energy transfer (FRET) or spectral multiplexing.

QDs. The optical properties of QDs are controlled by the constituent material, particle size and size distribution (dispersity), and surface chemistry, specifically the number of dangling bonds favoring nonradiative deactivation[16]. QDs have diameters of 1–6 nm. The selection of QD materials has primarily been driven by the ability to prepare particles with the desired optical properties. The most prominent materials for life science applications are CdSe and CdTe, although III/V group or ternary semiconductors such as InP and InGaP, which lack cytotoxic cadmium ions, are possible alternatives (**Fig. 1a–c**). At present, commercial products made of CdSe (from Sigma-Aldrich, Invitrogen, Evident and Plasmachem), CdTe (from Plasmachem) and InP or InGaP (from Evident) are available. To date, the preparation of highly monodisperse (that is, having a narrow size distribution), luminescent and nontoxic QDs made of other materials has not been successful. As the number of dangling bonds at the core particle surface determines fluorescence quantum yields, decay kinetics and stability, inorganic passivation layers and/or organic capping ligands are bound to the particle surface to optimize these features[16]. Thus typical QDs are core-shell (for example, CdSe core with a ZnS shell) or core-only (for example, CdTe) structures functionalized with different coatings. Their properties depend to a considerable degree on particle synthesis and surface modification. Addition of the passivation shell often results in a slight red shift in absorption and emission as compared to the core QD because of tunneling of charge carriers into the shell[16].

Organic dyes. The optical properties of organic dyes (**Table 2** and **Fig. 1d–f**) depend on the electronic transition(s) involved and can be fine-tuned by elaborate design strategies if the structure-property relationship is known for the given class of dye[1,17]. The emission of organic dyes typically originates either from an optical transition delocalized over the whole chromophore (here we refer to these as resonant dyes because of their resonant emission, **Fig. 1d**) or from intramolecular charge transfer transitions (we refer to these as CT dyes)[1]. The majority of common fluorophores—such as fluoresceins, rhodamines, most 4,4′-difluoro-4-bora-3a,4a-diaza-s-indacenes (BODIPY dyes) and most cyanines—are resonant dyes that are characterized by slightly structured, comparatively narrow absorption and emission bands that often mirror each other, a small solvent polarity–insensitive Stokes shift (**Fig. 1d**), high molar absorption coefficients, and moderate-to-high fluorescence quantum yields. The poor separation of the absorption and emission spectrum favors cross-talk between different dye molecules. CT dyes such as coumarins, in contrast, have well-separated, broader and structureless absorption and emission bands in polar solvents, and a larger Stokes shift, the size of which depends on solvent or matrix

polarity (**Fig. 1f**). Their molar absorption coefficients, and in most cases also their fluorescence quantum yields, are generally smaller than those of dyes with a resonant emission. In addition, CT dyes show a strong polarity dependence of their spectroscopic properties and CT dyes absorbing and emitting in the near-infrared (NIR) wavelength typically have low fluorescence quantum yields.

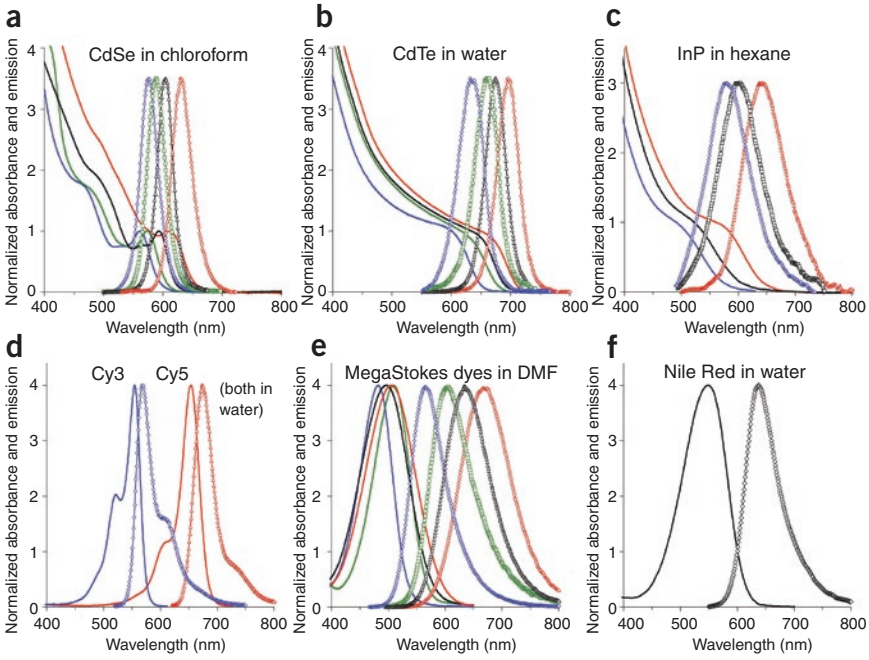

Figure 1 | Spectra of QDs and organic dyes. (**a**–**f**) Absorption (lines) and emission (symbols) spectra of representative QDs (**a**–**c**) and organic dyes (**d**–**f**) color coded by size (blue < green < black < red). MegaStokes dyes were designed for spectral multiplexing in dimethylformamide (DMF).

QDs versus organic dyes. In comparison to organic dyes, QDs have the attractive property of an absorption that gradually increases toward shorter wavelengths (below the first excitonic absorption band) and a narrow emission band of mostly symmetric shape. The spectral position of absorption and emission are tunable by particle size (the so-called quantum size effect; **Fig. 1a**–**c**). The width of the emission peak, in particular, is mainly determined by QD size distribution. The broad absorption allows free selection of the excitation wavelength and thus straightforward separation of excitation and emission. The (size-dependent) molar absorption coefficients at the first absorption band of QDs are generally large as compared to organic dyes[18]. Typical molar absorption coefficients are 100,000–1,000,000 M^{-1} cm^{-1} (refs. 18,19), whereas for dyes, molar absorption coefficients at the main (long-wavelength) absorption maximum are about 25,000–250,000 $M^{-1}cm^{-1}$ (refs. 1,20–25; **Tables 2** and **3**). Fluorescence quantum yields of properly surface-passivated QDs are in most cases high in the visible light range (400–700 nm): 0.65–0.85 for CdSe[26,27], ≤0.6 for CdS[28] and 0.1–0.4 for InP [29,30]; and high for the visible–NIR wavelength (≥700 nm) emitters CdTe and CdHgTe (0.3–0.75)[31,32] as well as for the NIR wavelength (≥800 nm) emitters PbS (0.3–0.7)[33,34] and PbSe (0.1–0.8)[35,36] (**Tables 2** and **3**). In contrast, organic dyes have fluorescence quantum yields that are high in the visible light range but are at best moderate in the NIR wavelength range[17,25,37] (**Tables 2** and **3**). The combined drawbacks of reduced quantum yields at NIR wavelengths and limited photostability of many NIR-wavelength dyes hampers the use of organic dyes for NIR-wavelength fluorescence imaging applications. As compared to organic dyes[38], another favorable feature of QDs is the typically very large two-photon action cross-section[39–42].

With very few exceptions, such as acridone dyes[43], the fluorescence lifetimes of organic dyes are about 5 ns in the visible light and 1 ns in the NIR wavelengths, and are commonly too short for efficient temporal discrimination of short-lived fluorescence interference from scattered excitation light. However, the typically mono-exponential decay kinetics enable straightforward dye identification from measurements of fluorescence lifetimes, making dyes suitable for applications involving lifetime measurements. In the case of QDs, the comparatively long lifetimes (typically five to hundreds of nanoseconds) enable straightforward temporal discrimination of the signal from cellular autofluorescence and scattered excitation light by time-gated measurements, thereby enhancing the sensitivity[44,45]. However, the complicated size-, surface-, wavelength- and time-dependent, bi- or multiexponential QD decay behavior[46,47] (**Fig. 2**) renders species identification from time-resolved fluorescence measurements very difficult. This is an inherent disadvantage of these materials.

Solubilization

Suitable labels should not aggregate or precipitate under relevant conditions—that is, in aqueous solutions *in vitro*, on supports such as microarrays, in cells or *in vivo*. In the case of organic dyes, solubility can be tuned via substituents (such as sulfonic acid groups) as long as the optical properties and other relevant features are not affected by the substitution. There are plenty of organic dyes available that are soluble in relevant media.

QD dispersibility is controlled by the chemical nature of the surface coating. CdTe is inherently dispersible in water, but high-quality CdSe, which is typically synthesized in organic solvents, must be made water-dispersible (that is, aggregation of QDs in aqueous solution must be prevented). This can be accomplished electrostatically, using small charged ligands[48] such as mercaptopropionic acid or cystamine (**Fig. 3**), or with charged surfactants that intercalate with the hydrophobic ligands present from synthesis. Although use of surfactants is the simpler strategy, the intercalated complexes disassemble much more easily compared to QDs with surface ligands. Alternatively, QD dispersal in aqueous solution can be accomplished sterically, via 'bulky' polymeric surface ligands such as polyethylene glycol (**Fig. 3**).

Electrostatically stabilized QDs are typically much smaller than sterically stabilized ones, which is favorable for most biological applications; thus electrostatic stabilization strategies are recommended if small QD labels in low-ionic-strength buffers are required. However, these QDs tend to aggregate in buffers of high ionic strength or in biological matrices[49]. Sterically stabilized QDs

are usually too large to enter cells, but are less sensitive to ionic strength. A compromise can be reached by using smaller, but nevertheless still bulky, charged ligands[50] such as polyethyleneimine or polyelectrolytes[51], or an additional amphiphilic inorganic shell such as silica, which can be further functionalized using standard silica chemistry[50,52,53] (**Fig. 3**). Common examples of ligands and stabilizing shells used for CdSe (the most prominent type of QD), together with typical applications, are summarized in **Supplementary Table 1** online[51,54–57].

It is difficult to predict the effect of surface functionalization on the optical properties of QDs in general terms. Typically, the fluorescence quantum yield and decay behavior respond to surface functionalization and bioconjugation, whereas shape and spectral position of the absorption and emission are barely affected[58].

Attachment to biomolecules

Labeling of biomolecules such as peptides, proteins or oligonucleotides with a fluorophore requires suitable functional groups for covalent binding or for noncovalent attachment of the fluorophore. The advantage of organic dyes in this regard is the commercial availability of a toolbox of functionalized dyes, in conjunction with established labeling protocols, purification and characterization techniques for dye bioconjugates, as well as information on the site-specificity of the labeling procedure[4]. Furthermore, the small size of organic dye labels minimizes possible steric hindrance, which can interfere with biomolecule function, and allows attachment of several fluorophores to a single biomolecule to maximize the fluorescence signal (**Fig. 4**). Nevertheless, site-specificity can be problematic even for organic dyes. Moreover, high label densities

Table 2 | Comparison of properties of organic dyes and QDs

Property	Organic dye	QD[a]
Absorption spectra	Discrete bands, FWHM[b] 35 nm[c] to 80–100 nm[d]	Steady increase toward UV wavelengths starting from absorption onset; enables free selection of excitation wavelength
Molar absorption coefficient	2.5×10^4–2.5×10^5 M^{-1} cm^{-1} (at long-wavelength absorption maximum)	10^5–10^6 M^{-1} cm^{-1} at first exitonic absorption peak, increasing toward UV wavelengths; larger (longer wavelength) QDs generally have higher absorption
Emission spectra	Asymmetric, often tailing to long-wavelength side; FWHM, 35 nm[c] to 70–100 nm[d]	Symmetric, Gaussian profile; FWHM, 30–90 nm
Stokes shift	Normally <50 nm[c], up to >150 nm[d]	Typically <50 nm for visible wavelength–emitting QDs
Quantum yield	0.5–1.0 (visible[e]), 0.05–0.25 (NIR[e])	0.1–0.8 (visible), 0.2–0.7 (NIR)
Fluorescence lifetimes	1–10 ns, mono-exponential decay	10–100 ns, typically multi-exponential decay
Two-photon action cross-section	1×10^{-52}–5×10^{-48} cm^4 s $photon^{-1}$ (typically about 1×10^{-49} cm^4 s $photon^{-1}$)	2×10^{-47}–4.7×10^{-46} cm^4 s $photon^{-1}$
Solubility or dispersibility	Control by substitution pattern	Control via surface chemistry (ligands)
Binding to biomolecules	Via functional groups following established protocols Often several dyes bind to a single biomolecule Labeling-induced effects on spectroscopic properties of reporter studied for many common dyes	Via ligand chemistry; few protocols available Several biomolecules bind to a single QD Very little information available on labeling-induced effects
Size	~0.5 nm; molecule	6–60 nm (hydrodynamic diameter); colloid
Thermal stability	Dependent on dye class; can be critical for NIR-wavelength dyes	High; depends on shell or ligands
Photochemical stability	Sufficient for many applications (visible wavelength), but can be insufficient for high-light flux applications; often problematic for NIR-wavelength dyes	High (visible and NIR wavelengths); orders of magnitude higher than that of organic dyes; can reveal photobrightening
Toxicity	From very low to high; dependent on dye	Little known yet (heavy metal leakage must be prevented, potential nanotoxicity)
Reproducibility of labels (optical, chemical properties)	Good, owing to defined molecular structure and established methods of characterization; available from commercial sources	Limited by complex structure and surface chemistry; limited data available; few commercial systems available
Applicability to single-molecule analysis	Moderate; limited by photobleaching	Good; limited by blinking
FRET	Well-described FRET pairs; mostly single-donor– single-acceptor configurations; enables optimization of reporter properties	Few examples; single-donor–multiple-acceptor configurations possible; limitation of FRET efficiency due to nanometer size of QD coating
Spectral multiplexing	Possible, 3 colors (MegaStokes dyes), 4 colors (energy-transfer cassettes)	Ideal for multi-color experiments; up to 5 colors demonstrated
Lifetime multiplexing	Possible	Lifetime discrimination between QDs not yet shown; possible between QDs and organic dyes
Signal amplification	Established techniques	Unsuitable for many enzyme-based techniques, other techniques remain to be adapted and/or established

Properties of organic dyes are dependent on dye class and are tunable via substitution pattern. Properties of QDs are dependent on material, size, size distribution and surface chemistry.[a]Emission wavelength regions for QD materials (approximate): CdSe, 470–660 nm; CdTe, 520–750 nm; InP, 620–720 nm; PbS, >900 nm; and PbSe, >1,000 nm. [b]FWHM, full width at half height of the maximum. [c]Dyes with resonant emission such as fluoresceins, rhodamines and cyanines. [d]CT dyes. [e]Definition of spectral regions used here: visible, 400–700 nm; and NIR, > 700 nm.

Unless stated otherwise, all values were determined in water for organic dyes and in organic solvents for QDs, and refer to the free dye or QD.

can result in fluorescence quenching, depending on dye structure, charge (that is, owing to electrostatic repulsion between neighboring molecules) and hydrophilicity[17,23,24], and may also influence biomolecule function.

To date, there are no consensus methods for labeling biomolecules with QDs[59]. The general principle for QD biofunctionalization is that first the QDs are made water-dispersible and then are bound to biomolecules (**Fig. 3**). Binding can be done electrostatically, via biotin-avidin interactions, by covalent cross-linking (for instance, carbodiimide-activated coupling between amine and carboxylic groups, maleinimide-catalyzed coupling between amine and sulfhydryl groups, and between aldehyde and hydrazide functions) or by binding to polyhistidine tags[53,59–61]. Alternatively, ligands present during synthesis can be exchanged for biomolecules containing active groups on the surface[55] (**Fig. 3**). The latter strategy works very well for labeling oligonucleotides. Currently, only a few standard protocols for labeling biomolecules with QDs are available, and the choice of suitable coupling chemistries depends on surface functionalization. It is difficult to define general principles because QD surfaces are unique to a large extent, depending on their preparation. Accordingly, for users of commercial QDs, knowledge of surface functionalization is important.

With the exception of fluorescence quenching that results from high label density, most of the challenges in organic dye biofunctionalization also apply to QDs. In addition, QDs can aggregate because of non-optimal surface chemistry. Moreover, in contrast to labeling with small organic fluorophores, several biomolecules are typically attached to a single QD[62] (**Fig. 4**), and it is difficult to control biomolecule orientation. This can affect the spectroscopic properties and colloidal stability of the QD as well as affect biomolecule function. Additionally, the comparatively large size of QDs could sterically hamper access to cellular targets. The function of QD-labeled biomolecules thus needs to be carefully tested in each case.

Extracellular and intracellular targeting

The ability to track biomolecules within their native environment, that is, on the cell surface or inside of cells, is an important property for any fluorescent label and is a prerequisite to assessing molecular function *in vivo*. The challenges include intracellular delivery of the label as well as selective labeling of the target biomolecule within its native setting without affecting its function. As each labeling system has different advantages and liabilities, success of an experiment depends on selecting labels that are matched with the requirements of the biological system, for instance, the location of the target (cell surface, intracellular or vascular compartments), the expression level of the target or whether the target is within a reducing versus an oxidizing environment.

For organic dyes, several strategies for site-specific covalent and noncovalent labeling of proteins in living cells are available. These include enzyme-catalyzed labeling by post-translational modification, as in biotin ligase-catalyzed addition of biotin to biotin acceptor peptides, which may be used to label proteins at the cell surface. Both intracellular and surface labeling have also been achieved by specific chelation of membrane-permeant fluorescent ligands (biarsenical dyes such as FIAsH or ReAsH bind to the tetracysteine motif, and Ni–nitrilotriacetic acid (NTA) conjugates bind to the hexahistidine motif or Zn conjugates) or by self-labeling, in which proteins fused to O^6-alkylguanine–DNA alkyltransferase are combined with enzymatic substrate derivatives (O6-alkylguanine–DNA alkyltransferase (AGT) tag or SNAP tag (Covalys Biosciences))[63–67]. Recently a new protein tag, HaloTag, based on a modified haloalkane dehalogenase designed to covalently bind to synthetic ligands has been introduced[68]. The synthetic ligands for HaloTag consist of a chloroalkane linker attached to molecules such as fluorescent dyes, affinity reagents or solid surfaces, and the system can therefore be used for imaging of fusion proteins in living or fixed cells and for irreversible capture of proteins onto solid supports. Also the use of 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine (trimethoprim) has been reported for protein labeling in cells[69]. In addition, several methods are well established for delivery of organic dye labels into cells. These include acetomethoxymethyl (AM)-ester derivatization as well as simple microinjection, gene guns, cationic liposomes, controlled cell volume or cell membrane manipulation and endocytosis[4,70]. The first strategy in particular, which renders the dyes cell permeant, is a huge advantage for this class of labels.

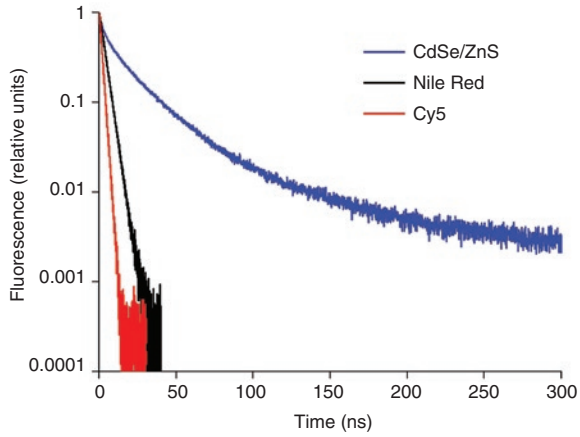

Figure 2 | Fluorescence decay behavior. Fluorescence decay behavior of typical organic fluorophores (mono-exponential, lifetimes of 1.5 ns (Cy5) and 3.6 ns (Nile Red)) in comparison to a typical QD (CdSe/ZnS, multi-exponential, mean lifetime ($\tau_{1/e}$) of 10.3 ns).

Extracellular targeting with QDs is frequently reported. This is typically accomplished through QD functionalization with specific antibodies to image cell-surface receptors[59,60,67] or via biotin ligase–catalyzed biotinylation in conjunction with streptavidin-functionalized QDs[63,71]. Generally, owing to their size, the intracellular delivery of QDs is challenging, and compared to organic dyes, the state of the art of delivery of QDs into cells and internal labeling strategies are far behind[72]. There is no general protocol to achieve this so far, and individual solutions need to be empirically established. However, whole-cell labeling with QDs has been realized through microinjection, electroporation or nonspecific or receptor-mediated endocytosis[56,72–76]. The labeling specificity and efficiency can be improved by using functionalized QDs[72]. Labeling of specific intracellular structures outside endocytosed vesicles or imaging of cellular reactions in the cytoplasm or the nucleus with QDs requires more sophisticated tools, which still need to be worked out in detail. Positively charged peptide-transduction domains such as Tat (peptide from the cationic domain of the HIV-1 Tat protein), polyarginine, polylysine and other specifically designed cell-penetrating peptides, can be coated onto QDs to effect their delivery into cells[77]. It remains to be shown whether other recently developed cell penetrating agents, such as streptaphage, a synthetic ligand based on an

N-alkyl derivative of 3β-cholesterylamine, which was designed for efficient uptake of streptavidin conjugates by mammalian cells[78], or polyproline systems equipped with cationic and hydrophobic moieties[79], can be adapted for QD delivery.

Influence of label microenvironment

The spectroscopic properties of fluorophores are sensitive to temperature and to label microenvironment. In general terms, the microenvironment typically affects the spectral position, the fluorescence lifetime and/or the intensity of the absorption and emission bands of organic dyes, and mainly the fluorescence quantum yield and fluorescence decay behavior of QDs. The relevant features of the label microenvironment include matrix polarity and proticity (hydrogen bonding ability), viscosity, pH and ionic strength as well as the presence of surfactants, of fluorescence quenchers such as oxygen, or of conjugated molecules. The photochemical stability of fluorophores (see also the discussion of stability below) also responds to label microenvironment.

The effect of the microenvironment on the optical properties of organic fluorophores depends on dye class, nature of the emitting

Table 3 | Comparison of the optical properties of selected organic dyes and QDs

Dyes							QDs						
	Absorbance[a] (nm)	Fluorescence[b] (nm)	FWHM[c] (nm)	ε[d] (M^{-1} cm^{-1})	Φ_f	Refs.		Absorbance[e] (nm)	Fluorescence[f] (nm)	FWHM[c] (nm)	ε[d] (M^{-1} cm^{-1})	Φ_f	Refs.
Visible wavelenghts (emission < 700 nm)													
Fluorescein	500 in basic ethanol	541 in basic ethanol	35 in basic ethanol	9.2×10^4 in basic ethanol	0.97 in basic ethanol	23	CdS	350–470	370–500	~ 30	1.0×10^5 (for 350-nm diameter) and 9.5×10^5 (for 450-nm diameter) in methanol	≤ 0.6	19,30
Cy3 (Cy3.18)	550 in PBS, 560 in ethanol	565 in PBS, 575 in ethanol	34 in PBS	1.5×10^5 in ethanol	0.04 in PBS, 0.09 in ethanol	24							
TAMRA	554 in water	573 in water	39 in methanol	~ 1×10^5 in methanol	0.28 in water	22[g]	CdSe	450–640	470–660	~ 30	1.0×10^5 (for 500-nm diameter) and 7.0×10^5 (for 630-nm diameter) in methanol	0.65–0.85	19,28, 29
Texas Red (sulforhodamine 101)	587 in methanol, 576 in ethanol	602 in methanol, 591 in ethanol	35 in methanol	9.6×10^4 in methanol, 1.4×10^5 in ethanol	0.93 in ethanol, 0.35 in water	39[g]							
Nile Red	552 in methanol, 519 in dioxane	636 in methanol, 580 in dioxane	75 in dioxane	4.5×10^4 in methanol, ~ 4.0×10^4 in dioxane	0.7 in dioxane	21[g]	CdTe	500–700	520–750	35–45	1.3×10^5 (for 570-nm diameter) and 6.0×10^5 (for 700-nm diameter) in methanol	0.3–0.75	19,33, 34
Cy5 (Cy5.18)	650 in PBS, 658 in ethanol	667 in PBS, 677 in ethanol	39 in PBS	2.5×10^5 in ethanol	0.27 in PBS, 0.4 in ethanol	24	InP	550–650	620–720	50–90		0.1–0.6	31,32
NIR wavelengths (emission > 700 nm)													
Atto740	740 in PBS	764 in PBS	43 in PBS	1.2×10^5 in PBS	0.10 in PBS	[h]	PbS	800–3,000	>900	80–90		0.26 in HEPES buffer, 0.70 in hexane	35,36
Cy7	747 in water	774 in water	50 in water	2.0×10^5 in water	0.28 in water	[i]							
Alexa 750	749 in phosphate buffer	775 in phosphate buffer	49 in phosphate buffer	2.4×10^5 in phosphate buffer	0.12 in phosphate buffer	[g]	PbSe	900–4,000	>1,000	80–90	1.23×10^5 in $CHCl_3$	0.4–0.5 in $CHCl_3$, 0.12–0.81 in hexane	37,38
IR125 (ICG)	781 in water/methanol 75/25%, 782 in methanol, 786 in ethanol	825 in methanol, 815 in water	58 in methanol	2.1×10^5 in water/methanol 75/25%, 1.95×10^5 in methanol, 1.94×10^5 in ethanol	0.02 in water/methanol 75/25%, 0.04 in methanol, 0.05 in ethanol, 0.01 in water	26							

[a]Longest wavelength absorption maximum. [b]Shortest wavelength emission maximum. [c]Full-with at half height of the emission band. [d]ε values provided for the main (longest-wavelength) absorption band (dyes) and the first excitonic absorption peak (QDs). [e]Size-tunable position of the first excitonic absorption maximum. [f]Size-tunable position of the emission maximum. [g]Manufactured by Invitrogen. [h]Manufactured by ATTOTech. [i]Manufactured by Amersham.

state(s), excited state redox potential, charge and hydrophilicity[24,80–84]. Dyes with a resonant emission such as fluoresceins, rhodamines and cyanines typically show only moderate changes in their spectral characteristics, yet can change considerably in fluorescence quantum yield and lifetime, and can be prone to aggregation-induced fluorescence quenching[23,24,80,82,83]. In contrast, dyes like coumarins, with an emission from an excited state that has a considerable dipole moment, respond with notable spectral changes to changes in microenvironment polarity and can be sensitive to solvent proticity.

For QDs, the microenvironment effect on spectroscopic features is mainly governed by the accessibility of the core surface[60]. This in turn depends on the ligand (and the strength of its binding to the QD surface) and the shell quality[85]. Typically, properly shelled QDs are minimally sensitive to microenvironment polarity provided that no ligand desorption occurs. Also, QD emission is barely responsive to viscosity, contrary to that of many organic dyes, and QDs are less prone to aggregation-induced fluorescence quenching. Nevertheless, QDs are colloids and are thus susceptible to changes in ionic strength; electrostatically stabilized QDs tend to aggregate with increasing ionic strength.

Bioconjugation often leads to a decrease in fluorescence quantum yield for both label types. The parameters that can affect label fluorescence are the chemical nature, the length of the spacer and, at least for organic dyes, the type of neighboring oligonucleotides or amino acids in the bioconjugated form[80,81,84].

The knowledge of such microenvironment effects greatly simplifies label choice. This is generally an advantage of organic dyes. Only a few systematic studies have been performed so far on the effect of the microenvironment on QD spectroscopic properties, the generalization of which is hampered by the broad variety of QD coatings used[60], the fact that ligand adsorption-desorption equilibria are matrix-dependent[85] and the interplay between proper core shielding and microenvironment effects.

Stability

A fluorescent label must be stable under relevant conditions (that is, in the buffer, cell medium or support used), in the presence of typical reagents such as dithiothreithol at common temperatures and under a typical excitation light flux over routinely used detection times. Label stability is of crucial importance for detection sensitivity, especially in single molecule experiments, and for contrast in fluorescence imaging. Blinking (**Table 1**), which is a problem for single molecule applications, is briefly discussed in the section on *Bioanalytical Applications*.

Organic dyes like fluorescein and tetramethyl rhodamine isothiocyanate (TRITC) and the majority of NIR fluorophores have poor photostability[17,61,82,83,86]. In addition, many NIR dyes, such as the clinically approved indocyanine green (ICG), suffer from poor thermal stability in aqueous solution[25], and the presence of

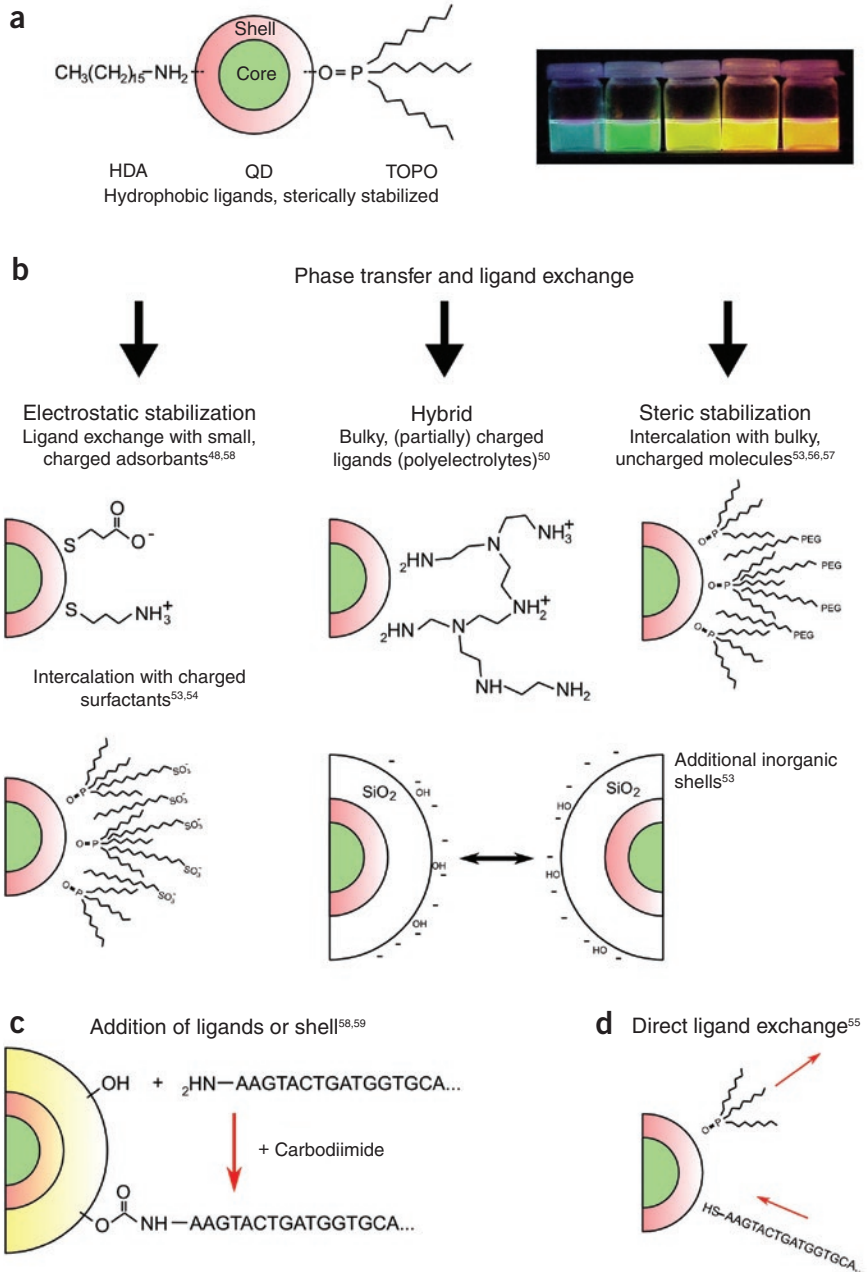

Figure 3 | Overview of strategies to prepare water-dispersible QDs and QD bioconjugates. (**a**) QDs bearing hydrophobic ligands after preparation in organic solvent. HDA, hexadecylamine; TOPO, trioctylphosphineoxide. (**b**) Ligand-exchange strategies to generate water-dispersible QDs. Illustrated are electrostatic colloidal stabilization (left), electrostatic and steric stabilization (middle) and steric stabilization of colloid (right). (**c**) Coupling of water-dispersible QDs to biomolecules; oligonucleotides are shown here as an example. (**d**) Alternatively, the QDs bearing hydrophobic ligands can be subjected to direct ligand exchange.

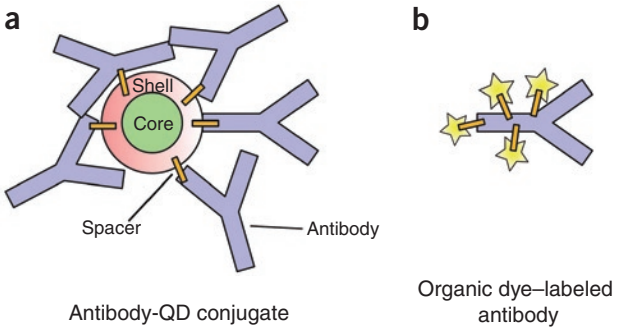

Figure 4 | Schematics of a QD-antibody conjugate and a dye-labeled antibody, reflecting the proportions of the components.

ozone can result in dye decomposition, as observed for Cy5[87]. In the last years, many organic dyes such as the Alexa dyes[82,83] have been designed that display enhanced photostability in comparison to first-generation fluorophores such as fluorescein, and, owing to technical improvements, readout times for many fluorescence techniques have decreased. Despite these improvements, limited dye photostability can still hamper microscopic applications requiring high excitation light intensities in the UV-visible light region or requiring long-term imaging.

In contrast, adequately surface-passivated QDs display excellent thermal and photochemical stability and photo-oxidation is almost completely suppressed for relevant time intervals as a consequence of their additional inorganic surface layers and shielding of the core material[61,76,88,89]. This is a considerable advantage over organic fluorophores for imaging applications that use intense laser excitation sources or for long-term imaging[61], as has been demonstrated in comparisons of CdSe-labeled and rhodamine-labeled tubulin[90], of CdSe and Texas Red[91], as well as of antibodies labeled with CdSe, fluorescein isothiocyanate (FITC), R-phycoerythrin and AlexaFluor 488 (ref. 92). However, photooxidation of QDs has been observed[93] as has the QD-specific phenomenon of photobrightening[94], and undesired aggregation of QDs can contribute to reduced stability[95].

For more details on organic dye stability, especially for imaging and single-molecule applications, see references 4,81–83. We note that, because of the extremely broad variety of conditions that need to be considered when evaluating label stability—excitation wavelength and intensity, matrix or microenvironment, label concentration and, in the case of QDs, surface chemistry—stability data assembled from the literature cannot replace targeted stability studies. To evaluate the bioanalytical potential of QDs more realistically, systematic stability studies under relevant conditions are needed[96,97].

Quantification

Target quantification using fluorescence is affected to a non-negligible extent by both the stability of the fluorescent label and the sensitivity of its spectroscopic properties to the environment. Organic dyes have been successfully applied for quantification in a broad variety of *in vitro* fluorescence applications, but reports of analyte quantification with QD labels are still rare. QD photobrightening can hamper direct quantification and may render the use of reference standards necessary[94]. For single-molecule spectroscopic applications, blinking of QDs and of organic dyes can be a considerable disadvantage[93,98]. For example, QD blinking has been reported to affect the results from bioaffinity studies[99]. Another aspect that may influence the usability of QDs for quantification is the fact that not all QDs in a preparation are luminescent; some exist in permanently nonfluorescent states[100].

Toxicity

Any substance, elemental or molecular, can be cytotoxic. Although this property is not relevant for *ex vivo* applications such as immunoassays, it is critical for imaging in cells or *in vivo*. Cytotoxicity data for many traditional organic dyes are available (**Table 2**). In general, with the exception of DNA intercalators, toxicity of organic dyes is not a major problem. In the case of QDs, the cytotoxicity of elements such as cadmium, which is present in many of these nanocrystals, is well known. Thus it is critical to know whether these cytotoxic substances can leak out of the QD particles over time, upon illumination or oxidation[93,95,97], in addition to whether ligands or coatings are cytotoxic[101]. There are reports in the literature in which cytotoxicity of QDs was observed[72,102,103], and others in which it was not[74,75,102,104]. In cases where cytotoxicity was observed, it was usually attributed to leaking of Cd^{2+}, cytotoxic surface ligands and/or nanoparticle aggregation[101–103].

The question of QD cytotoxicity is often directly connected with particle preparation, as well as with the preparation of protective inorganic surface layers. The clear assignment of cytotoxicity requires verified data using two or more independent test systems[105]. In addition, standardization in the experimental set up, such as choice of model (cell line, animal species) and exposure conditions is necessary[101]. It is common belief that in the case of CdSe, a properly prepared, close ZnS shell or multiple shells, such as a ZnS/SiO_2 shell, render leakage of cadmium ions and thus cytotoxicity unlikely. To avoid cytotoxic materials in general, possible alternatives to classical QD labels could be III/V group (binary or ternary) semiconductors such as InP or InGaP[29,106]. However, these are much more difficult to synthesize and do not as yet display photoluminescence intensities comparable with CdSe. Another alternative may be Mn^{2+}-doped ZnSe[107]. Additionally, nanotoxicity of QDs may also pose a problem[72]. Nanotoxicity refers to the ability of a substance to be cytotoxic owing to its size and independent of its constituent materials. Even though there are no systematic studies on the nanotoxicity of QDs, the results from the cytotoxicity studies suggest that nanotoxicity is not a substantial factor in cytotoxicity[101].

Comparability

Reliable and comparable fluorescence measurements require fluorescent labels with reproducible physicochemical properties and established tools to evaluate this. Organic dyes can be synthesized on a large scale and characterized according to their structure and purity. This is more difficult for dye-biomolecule conjugates, such as fluorophore-labeled antibodies, owing to batch-to-batch variations in label density and label density distribution, but is still manageable in principle. The colloidal nature of QDs, in conjunction with the broad variety of synthetic strategies and surface functionalities, renders the characterization of QD-bioconjugates more challenging compared to that of organic dyes. This is further complicated by the fact that commercial distributors usually refrain from providing any information about the ligands.

FRET

There exists an ever-increasing toolbox of commercial functionalized organic fluorophores with extensively described FRET

properties for use as donors and acceptors in spectroscopic 'rulers', or to sense conformational changes or other processes involving a change in distance[1,108]. For many FRET applications that do not require very small molecules, organic dyes have been increasingly replaced by fluorescent proteins[2,4,6,108]. Generally, the disadvantages of organic dyes and fluorescent proteins for FRET applications have their basis in cross-talk, which results from direct acceptor excitation due to the relatively broad absorption bands of these fluorophores. Further difficulties can be encountered in spectral discrimination of the fluorescence emission, owing to their relatively broad emission bands, their small Stokes shifts, and the 'red tails' of the emission spectra in the case of dyes like fluoresceins, rhodamines, BODIPY and cyanines (**Fig. 1a**). This can render tedious correction of measured signals necessary.

Recently QDs have been successfully exploited as FRET donors with organic dyes as acceptors, with the QD emission size-tuned to match the absorption band of the acceptor dye[45,108]. Owing to the free choice of the QD excitation wavelength, cross-talk can be circumvented in such FRET pairs. However, the distance-dependence of FRET means that both the size of the QD itself and that of the surface coating affect the FRET efficiency[108]; this typically renders FRET less efficient as compared to FRET with organic dyes. Owing to the considerable size of QDs, this limitation can be partly overcome by increasing the number of neighboring small organic acceptor dyes[108]. The application of QDs as FRET acceptors is not recommended because of their broad absorption bands, which favor excitation cross-talk. Generally, FRET applications of QDs should only be considered if there is another QD-specific advantage for the system in question, such as the possibility to avoid excitation cross-talk, their longer fluorescence lifetimes or their very large two-photon action cross-sections[42]. In most cases, fluorescent proteins or organic dyes are to be favored for FRET.

Multiplexing

For parallel analysis of different analytes, multiplexing detection schemes are required.

Spectral multiplexing. Spectral multiplexing or multicolor detection is typically performed at a single excitation wavelength, and discriminates between different fluorescent labels based on their emission wavelength. A tunable Stokes shift and very narrow, preferably well-separated emission bands of simple shape are the desirable optical properties of a suitable fluorophore for this application.

Owing to the optical properties of organic dyes (**Fig. 1d,f** and **Table 2**), their suitability for multicolor signaling at single wavelength excitation is limited, with the rare exception of the recently introduced MegaStokes dyes (Dyomics GmbH), for which the Stokes shift can be controlled, but where spectral unmixing of the emission signals is nevertheless required because of their comparatively broad emission bands (**Fig. 1e**). An increasingly common multiplexing approach uses donor-acceptor dye combinations (so-called tandem dyes or energy-transfer cassettes) that make use of FRET from the donor to the acceptor fluorophore to increase the spectral separation of absorption and emission[108]. A typical example for a four-color label system consists of a 5-carboxyfluorescein donor attached to four different fluorescein- and rhodamine-type acceptors (for example, 6-carboxy 4', 5'-dichloro-2', 7'-dimethoxy fluorescein, 5-carboxytetramethylrhodamine and 5-(and 6-)carboxy-X-rhodamine) via a spacer such as an oligonucleotide. FRET dye-labeled primers and FRET-based multiplexing strategies are the backbone of modern DNA analysis, for instance, enabling automated DNA sequencing and robust multiplex diagnostic methods for the detection of PCR products[108]. The limitations of organic dyes for FRET applications discussed above also limit the efficiency of these FRET-based multiplexing systems. This can be overcome by multi-wavelength excitation using different lasers[109], an approach that is becoming affordable because of progress in laser technology and is already used in flow cytometry[110]. For state-of-the-art cytometers, the independent detection of 12 different analytes has been reported using organic labels[110].

QDs are the ideal candidates for spectral multiplexing at a single excitation wavelength because of their unique flexibility in excitation and their very narrow and symmetric emission bands, which simplify color discrimination[60,75,111]. Depending on QD choice, simultaneous detection and quantification of several different analytes with QD labels can also require spectral deconvolution of measured signals, as has been recently demonstrated for a multiplexed fluoroimmunoassay for four different toxins[62].

Lifetime multiplexing. Multiplexing can also be performed by making use of fluorophore-specific decay behavior, measured at a single excitation and a single emission wavelength, to discriminate between different fluorophores. This approach is less sensitive to cross-talk, but requires sufficiently different lifetimes and, ideally, mono-exponential decay kinetics. So far, lifetime multiplexing, as well as combined spectral and lifetime discrimination, have only been realized with organic dyes[43,112,113]. In the case of QDs, lifetime multiplexing is most likely only reasonable for the discrimination of long-lived QDs (showing multi-exponential decay) from short-lived fluorophores (with mono-exponential decay) and requires fitting routines that consider the multi-exponential decay behavior of QDs.

Signal amplification

Fluorescence signal can be amplified using several techniques, including enzymatic amplification, avidin-biotin or antibody-hapten secondary detection techniques, nucleic acid amplification, controlled aggregation, chromophore-metal interactions (metal-enhanced fluorescence), and multiple-fluorophore labels (for example, phycobiliproteins or particle labels, including systems with releasable fluorophores)[1,12,108,114–118]. These approaches have been established for traditional dyes and can often be used only for certain applications, such as fluoroimmunoassays. They can be transferred to QDs to varying degrees. Strategies involving the use of a fluorogenic enzyme substrate cannot be transferred to QD technology, whereas controlled aggregation approaches and the construction of chromophore-doped particle labels are suitable for both organic dyes and QDs[12,111,116]. Chromophore-metal (silver or gold) interactions have been exploited to improve the spectroscopic features of organic dyes, yielding a sizeable fluorescence enhancement in conjunction with a reduction in fluorescence lifetime and an increased photostability[1,117]. These effects, which are caused by dipole-dipole coupling of the excited fluorophores to metal plasmons and are dependent on the type, shape and size of the metal, on the type of chromophore, and on geometrical parameters (for example, metal-fluorophore distance), have led to sophisticated dye-metal nanoparticle systems and (dye-doped) core/shell nanostructures with emission enhancement factors of 10 up to a few hundred, depending on the quantum yield of the fluorophore[119]. Analogous hybrid

materials composed of QDs and metal nanoparticles reveal only moderate amplification effects (for example, fivefold fluorescence enhancement for CdTe-Au system)[120]. The potential of this and other signal amplification approaches to optimize QD properties and to enable new sensor applications still needs to be thoroughly investigated.

Applications of QDs: status and future trends

Organic molecules are well established as fluorescent labels for *in vitro* assays and *in vivo* imaging, despite their non-optimum spectroscopic features and photochemical instability. They present a simple, safe and comparatively inexpensive option, owing to their availability from many commercial sources, established functionalization protocols and extensively studied properties. There also exist many different instances in which QDs have been applied to biological systems. Although most of these studies are proof-of-principle, they underline the growing potential of these reagents. QDs are very attractive candidates for bioanalytical applications that can either exploit their potential for spectral multiplexing and do not require strong signal amplification, or that rely on NIR fluorescence.

Aside from their unique potential for all bioanalytical applications requiring or benefiting from multiplexing, QDs could have a bright future in NIR fluorescence *in vivo* imaging, which requires labels that exhibit high fluorescence quantum yields in the 650–900 nm window, have adequate stability, good water solubility and low cytotoxicity in conjunction with large two-photon action cross-sections as desired for deep-tissue imaging. The only clinically approved organic fluorophore ICG (**Table 3**) suffers from a very low fluorescence quantum yield[25], limited stability and binding to plasma proteins. Improved organic substitutes with pending approval still possess small quantum yields compared to QDs emitting at NIR wavelengths, such as CdTe (**Table 3**). Moreover, QDs are attractive candidates for the development of multifunctional composite materials for the combination of two or more biomedical imaging modalities, like NIR fluorescence–magnetic resonance imaging[121].

And yet, the routine use of QDs at present is strongly limited by the very small number of commercial systems and the limited amount of data on their reproducibility and comparability as well as on their potential for quantification. To the best of our knowledge, no attempt has yet been reported comparing differently functionalized QDs from various sources (companies as well as research groups) in a Round Robin test, to evaluate achievable fluorescence quantum yields, and batch-to-batch variations for different materials and surface chemistries (including typical ligands and bioconjugates). Such data would be very helpful for QD users and would be the first step to derive and establish quality criteria for these materials. At the present state of QD technology, as best practice for their use, it is advisable to choose a supplier who provides as much information on the preparation method as possible, to use a single nanoparticle batch within a series of experiments, and to compare the spectroscopic features of QDs from different batches before use.

Other issues that remain to be addressed relating to QD technology are the use of these labels for lifetime multiplexing and the development of suitable algorithms for data analysis and for time-resolved FRET. Strategies for large-scale synthesis, especially for as-yet noncommercialized NIR QDs, more systematic studies on the influence and control of QD surface chemistry and the establishment of functionalization protocols are necessary to pave the way for QD technologies. A first straightforward step in this direction would be to design a reliable test for the quality of the surface coating, that is, the degree of perfection of the surface shell, as this is the most crucial parameter affecting fluorescence quantum yield, stability and cytotoxicity[88]. In addition, the cytotoxicity of differently functionalized QDs (including typical ligands) should be systematically assessed using previously standardized procedures.

As long as the drawbacks of QDs detailed in this review are not solved, for ensemble measurements, well-established organic labels should be favored for routine applications and for applications requiring very accurate quantification. For single-molecule or single-particle imaging and tracking applications, QDs are, in principle, indisputably superior to most organic fluorescent dyes owing to their photostability, which should allow single-fluorophore tracking for much longer times than with organic fluorophores. However, the intermittence in emission or 'blinking' that is universally observed for QDs, the causes and mechanism of which are as yet not completely understood, needs to be overcome for single-molecule applications[98,99]. There is some hope that blinking can be suppressed by improved surface chemistries and addition of reducing agents like β-mercaptoethanol or oligo(phenylene vinylene)[122], making QDs eventually the ideal labels for all applications that require exceptional photostability. In contrast, blinking may be exploited for superresolution microscopy by analyzing the intermittent fluorescence to allow identification of the light emitted by each individual label and to localize it accurately with a resolution of a few tens of nanometers[123].

Conclusion

QDs have been applied for detection and imaging in several areas in the life sciences, ranging from microarray technology to fluorescence *in situ* hybridization to *in vivo* imaging. Despite many superior optical properties, such as size-tunable absorption and emission, extremely broad and intense absorption enabling a unique flexibility in excitation, high fluorescence quantum yields even in the NIR wavelengths and large two-photon action cross-sections as compared to established organic dyes, the solutions for using QDs have so far been individual ones. The fact that QDs behave not as molecules but as nanocolloids complicates their application in biological environments. At present, users of QDs must weigh the costs of finding a solution to the challenges of their particular experimental system against the benefits of the advanced spectroscopic features of QDs. We anticipate future improvements in QDs or QD-doped particles will provide increased benefit in particular for areas in which long-term luminescence stability, high brightness or multi-colour detection are crucial.

Note: Supplementary information is available on the Nature Methods website.

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Quantum dots versus organic dyes as fluorescent labels

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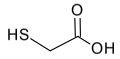
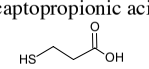
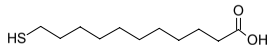
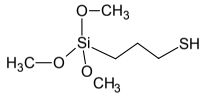
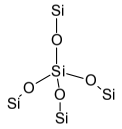
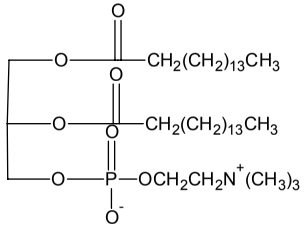
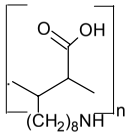
Supplementary Table 1 Most prominent examples for ligands and stabilizing shells for CdSe/ZnS and typical applications.

	LIGAND	ABBR.	STABILIZ.	APPLICATIONS	ADVANTAGES	LIMITATIONS	REF.
Thiols	Mercaptoacetic acid [54] 	MAA	electrostatic	Coupled to transferrin, QDs underwent receptor-mediated endocytosis in cultured HeLa cells. Labeled with immunomolecules, QDs recognized specific antigens/antibodies [56]. DNA immobilization to QDs surfaces and possibility of hybrid assemblies [56].	More stable than rhodamine 6G against photobleaching [56]. Stable and strongly fluorescent QDs [56]	Decrease in fluorescence intensity of QDs after hybridization [56]	[56], [56]
	3-Mercaptopropionic acid [55] 	MPA					
	Mercaptoundecanoic acid 	MUA					
	Mercaptopropyltrimethoxysilane 	MPS					
Polyethylene imine	$H(NH\text{-}CH_2\text{-}CH_2)_nNH_2$	PEI	hybrid	Proteins can be directly coupled to PEI amine groups.	PEI enhance photooxidation of QDs.		[52]
Silica		SiO_2	hybrid	Silica can be easily functionalized and then bioconjugated.	Silica is inert and protects QDs from oxidation.	Sometimes the size of the encapsulated QDs is too big for bio-applications.	[53]
Amphiphilic polymers	Polyethyleneglycol [58] also with functionalizations $-(CH_2\text{-}CH_2)_n\text{-}OH$	PEG,	steric	Conjugation with DNA and in vivo imaging (embryogenesis) [56], in vivo cancer targeting and imaging [55], encoding of cells [58], noninvasive in vivo imaging with localization depending on surface coating [59].	Encapsulation without any surface modification [56]. QDs stable, fluorescent (20 times more then organic dyes [55]) and not photobleaching in vivo [56, 58, 59]. In contrast to organic dyes, the emission spectra of QDs can be shifted away from autofluorescence [55]. QDs can create a larger number of cell codes than organic dyes [58]. Increase in circulating lifetimes and good targeting to specific sites in tissues [59].	Little toxicity at high injection concentrations [56, 58]. After 4 months, distribution of fluorescence changed and in some tissue was very weak [59]	[56], [55], [58], [59]
	Phospholipid-block copolymer micelle (n-polyethylenephosphatidyl ethanolamine + phosphatidylcholine) [82] 	PEG-PE + DPPC					
	Octyl-amine-modified polyacrylic acid [83], 	PAA					
	Triblock copolymer + PEG [56]