Bioconjugate Chemistry

Review

pubs.acs.org/bc

Quantum Dot Surface Chemistry and Functionalization for Cell Targeting and Imaging

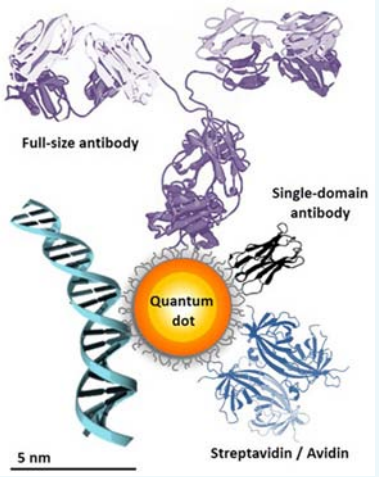
Regina Bilan,[†] Fabrice Fleury,[‡] Igor Nabiev,*[,†,§] and Alyona Sukhanova*[,†,§]

[†]Laboratory of Nano-Bioengineering, National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), 31 Kashirskoe sh., 115409 Moscow, Russian Federation

[‡]DNA repair group, UFIP, CNRS UMR6286, Univertité de Nantes, 2 rue de la Houssinière, 44322 Nantes Cedex 3, France

[§]Laboratoire de Recherche en Nanosciences, EA4682-LRN, 51 rue Cognacq Jay, UFR de Pharmacie, Université de Reims Champagne-Ardenne, 51100 Reims, France

ABSTRACT: Quantum dots (QDs) are highly fluorescent nanoscale crystals with size-dependent emission spectra. Due to their excellent photophysical properties, QDs are a promising alternative to organic fluorescent dyes and fluorescent proteins for cell targeting, imaging, and drug delivery. For biomedical applications, QDs should be chemically modified to be stable in aqueous solutions and tagged with the recognition molecules or drugs. Here, we review surface modification approaches to, and strategies for, conjugation of bioactive molecules with QDs. There are a variety of methods of QD surface modification and QD incorporation into larger delivery systems that yield fluorescent nanocarriers from 10 nm to several micrometers. Conjugates of QDs with peptides, proteins, antibodies, oligonucleotides, and small molecules have been used for fluorescent targeting, tracking, and imaging both *in vitro* and *in vivo*. Due to an extremely high stability to photobleaching, QDs were used for long-term visualization. QD applications pave the way for new generations of ultrasensitive detection, diagnostic systems, as well as drug delivery approaches, combining accurate targeting, delivery, and imaging in a single assay.



1. INTRODUCTION

Quantum dots (QDs) are highly fluorescent inorganic semiconductor nanocrystals with a diameter ranging from 2 to 10 nm.[1] QDs may consist of a semiconductor core (CdSe, CdS, CdTe, InP, InAs, or PbSe) alone or a core and a shell. The shell (e.g., ZnS) protects the core from oxidation and enhances the quantum yield.[2] The photoluminescent parameters of QDs are tunable, and their emission wavelengths depend (nonlinearly) on their size (Figure 1), which is controlled by the duration of nanocrystal growth in solution.

In recent years, QDs have been increasingly widely used in cell targeting, imaging, and drug delivery due to their unique optical and physicochemical properties. Although organic fluorescent dyes and fluorescent proteins are more traditionally used for bioimaging applications, they are substantially inferior to QDs in terms of their photophysical properties: QDs have broad excitation spectra; narrow, sharp emission spectra with an approximately Gaussian shape (Figure 1a,b); and large Stocks shifts (>100 nm). These spectral properties offer great opportunities for multiplexed analysis and multicolor imaging:[3] a single light source can excite many colors of QDs simultaneously, and the emission peaks can be distinguished with high resolution. QDs are nearly 20 times brighter and thousands of times more stable against photobleaching than organic dyes are.[4,5] The high brightness of QD fluorescence is a result of high molar adsorption coefficients (several times higher than for fluorescent dyes and proteins) combined with a high quantum yield (Table 1). Another advantage of QDs is their extremely long luminescence lifetimes, which allows QDs to be distinguished from other fluorophores in FLIM analyses. In our previous study, we demonstrated an extremely high resistance of QDs to photobleaching in a model of cell labeling with QD−antibody conjugates. QD bioconjugates have been found to be 4200-, 2600-, and 420-fold more resistant to photobleaching than fluorescein isothiocyanate, phycoerythrin, and AlexaFluor488 bioconjugates, respectively (Figure 1c).[5]

QDs can be prepared by means of high-temperature inorganic or aqueous synthesis. In addition, biosynthesis of semiconductor QDs by living systems, including yeast,[6] fungi,[7] bacteria,[8,9] and even earthworms,[10] has been reported. Although it is already possible to control the QD size by choosing the biosynthesis conditions and synthesize QDs with quantum yields up to 33%,[6] the method has not been adjusted for preparative production. Inorganic synthesis, which is more widely used, results in hydrophobic water-insoluble QDs. However, biological and medical applications require QDs that are highly water-soluble, biocompatible, and functionalized with biomolecules and/or drugs. The development of efficient

Received: June 23, 2014
Revised: February 21, 2015
Published: February 24, 2015

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

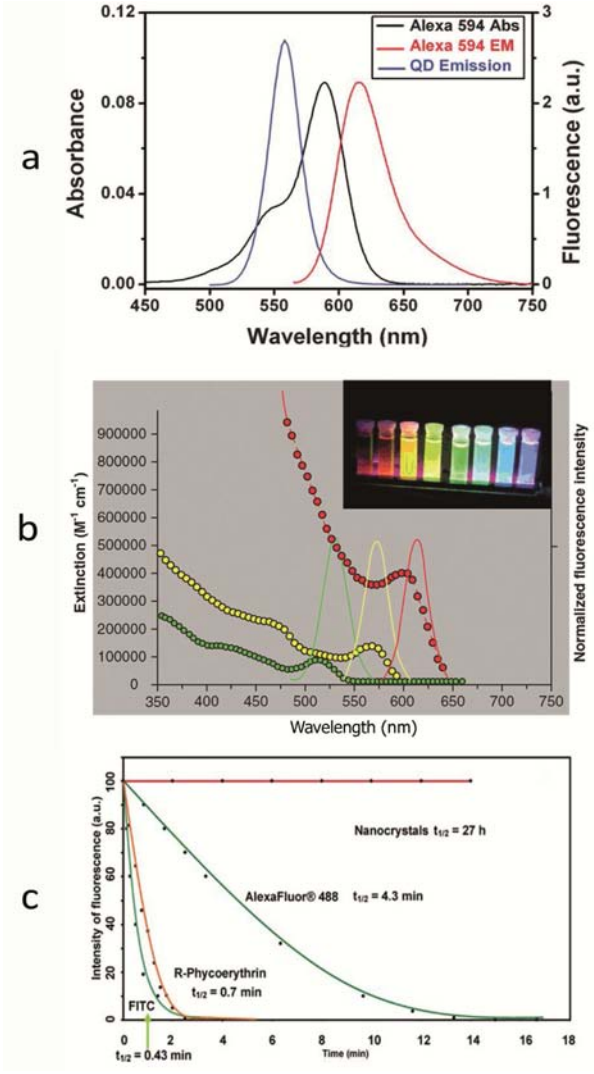


Figure 1. Photophysical properties of quantum dots (QDs). (a) Absorption spectrum (black line, left scale) and fluorescence spectrum (red line, right scale) of Alexa 594 labeled DNA (1 mM), and fluorescence spectrum of the MPA-capped CdSe/ZnS core/shell QD (blue line, right scale). Reprinted from Zhou, D., Piper, J. D., Abell, C., Klenerman, D., Kang, D. J., and Ying, L. (2005) Fluorescence resonance energy transfer between a quantum dot donor and a dye acceptor attached to DNA. *Chem. Commun. (Camb.) 14*, 4807−4809, Copyright 2005 with permission from Elsevier. (b) Size-tunable fluorescence spectra of CdSe QDs. Adapted from Sukhanova, A., and Nabiev, I. Fluorescent nanocrystal-encoded microbeads for multiplexed cancer imaging and diagnosis. *Crit. Rev. Oncol. Hemat. 68*, 39−59, Copyright 2008, with permission from Elsevier. (c) Time-dependent photobleaching curves of antibody conjugates with fluorescent nanocrystals, AlexaFluor488, R-Phycoerythrin, and FITC. Adapted from Sukhanova, A., Devy, J., Venteo, L., Kaplan, H., Artemyev, M., Oleinikov, V., Klinov, D., Pluot, M., Cohen, J. H., and Nabiev, I. (2004) Biocompatible fluorescent nanocrystals for immunolabeling of membrane proteins and cells. *Anal. Biochem. 324*, 60−67. Copyright 2004, with permission from Elsevier.

strategies for surface functionalization and bioconjugation remains an important challenge. A number of solubilization and functionalization strategies have been proposed. For example, QDs are incorporated into a liposome phospholipid bilayer,[11,12] loaded into liposomes,[13] or bound to the liposome surface[14] to obtain fluorescent labels for *in vivo* and *in vitro* imaging.[15] Another approach to QD solubilization is coating of QDs with hydrophilic ligands, amphiphilic polymers, or silica. Organic polymer shells are formed out of homo- and copolymers, and hyperbranched polymers.[16] Usually, encapsulation of QDs into polymers or lipids considerably increases the particle size (from 20 nm to several micrometers), which limits their biological applications. However, other solubilization approaches, such as the ligand exchange strategy, use small molecules instead of polymers or lipids to obtain stable QDs with a hydrodynamic diameter <9 nm.[17] Compact biocompatible QDs with a high fluorescence intensity are excellent candidates for a number of *in vivo* and *in vitro* studies. Current and prospective biological and medical applications of QD conjugates with biomolecules include their use for cell labeling,[18] single-molecule tracking,[19] deep-tissue and tumor imaging,[20] biosensing,[21] flow cytometry,[22] photodynamic therapy,[23] and targeted drug delivery.[24] QDs fluorescing in the NIR and IR regions can be synthesized (Table 1); therefore, they are suitable for NIR imaging. Large two-photon action cross sections (Table 1), substantially exceeding those of fluorescent dyes, make QDs very promising probes for two-photon imaging. In our view, QDs two-photon-excited and emitting in the NIR region are of great interest for *in vivo* imaging.

In this Review, we describe recent achievements in QD research and applications. The first part deals with the strategies of solubilization and stabilization of QDs in aqueous solutions under physiological conditions. The second part considers approaches to the functionalization of QDs with biological molecules. The third part describes a wide variety of QD bioconjugates and their implications for bioimaging, biosensing, and drug delivery.

2. QUANTUM DOT SURFACE MODIFICATION STRATEGIES

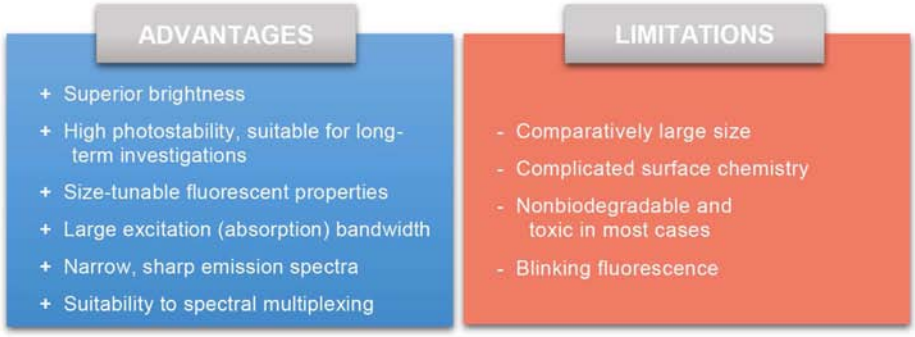
For current biological applications of QDs, it is important to choose an appropriate strategy for solubilization and stabilization of QDs in aqueous solutions under physiological conditions. There are two main approaches to the surface modification of QDs based on (i) hydrophobic interaction of amphiphilic molecules with QDs and (ii) interaction of polar groups of the coating molecules with the QD surface (the ligand exchange strategy). The applicability of a particular strategy is determined not only by the task at hand, but also by the chemical properties of QDs, which depend on the method of their synthesis.

2.1. Coating of Quantum Dots with Amphiphilic Molecules. Widely used high-temperature methods of QD synthesis employing surfactants, such as tri-*n*-octylphosphine (TOP), tri-*n*-octylphosphine oxide (TOPO),[25] and octadecylamine,[26] as stabilizing agents yield water-insoluble QDs with a hydrophobic surfactant layer on the surface protecting nanocrystals from oxidization. To make these QDs suitable for biomedical applications, their surface should be modified with polar surface-active compounds, which both makes them water-soluble and stable in aqueous solutions and provides functional groups for subsequent conjugation with biomolecules. It is important to choose the appropriate protocol for QD-surface modification that would not impair the photophysical properties of the nanocrystals. For example, some solubilization techniques may lead to a decrease in the QD quantum yield.[27]

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

Table 1. Specific Characteristics of Quantum Dots As Advanced Nanophotonic Probes: (a) Advantages and Limitations of Quantum Dots As Fluorescent Probes in Bioimaging Applications; (b) Optical Properties of Quantum Dots in Comparison with Fluorescent Dyes and Fluorescent Proteins[a]

(a)



(b)

Property	QDs	Organic dyes	Fluorescent proteins
Size	2–50 nm (depending on the capping agent and solubilization strategy)	0.5–1 nm	2.4 × 4.2 nm
Molar adsorption coefficient, M^{-1} cm^{-1}	100,000–1000,000 (at the first excitonic absorption peak, increasing towards the UV region)	25,000–250,000	20,000–160,000
Excitation spectra	Broad, from UV to the first size-dependent excitonic peak	Slightly structured, asymmetric	Asymmetric, tailing to the short-wavelength side
Emission spectra; full width at half-maximum (FWHM)	Symmetric, Gaussian shape; 20–40 nm (visible) >80 nm (NIR)	Asymmetric, often tailing to the long-wavelength side; 30–100 nm	Asymmetric, tailing to the long-wavelength side 75 nm
Stocks shifts	100–500 nm	10–150 nm	8–180 nm
Optical range	350–4000 nm	386–804 nm	445–690 nm
Quantum yield	0.1–0.9 (visible) 0.2–0.7 (NIR)	0.5–1.0 (visible) 0.05–0.25 (NIR)	0.1–0.9 (visible to far red) 0.06–0.1 (NIR)
Fluorescence life time	20 ns to hundreds of ns; bi- or multiexponential decay behavior	About 5 ns in the visible light; 1 ns in the NIR; mono-exponential decay behavior	1–4 ns
Two-photon action cross-section, Goeppert-Mayer units (GM)	2000–47000	1–200	10–645

[a]Some parts of (b) are adapted by permission from Macmillan Publishers Ltd.: Resch-Genger, U., Grabolle, M., Cavaliere-Jaricot, S., Nitschke, R., and Nann, T. Quantum dots versus organic dyes as fluorescent labels. (2008) *Nat. Methods* 5, 763−775, copyright 2008.

QDs may be coated with the amphiphilic graft and block copolymers.[28−30] When the polymer is added to the solution of QDs coated with hydrophobic surfactants (TOP, TOPO), the hydrophobic chains of the polymer intercalate between the surfactant molecules, while the hydrophilic groups are exposed on the surface, thereby stabilizing QDs in the aqueous environment (Figure 2a). Polymers on the surface of QDs may be additionally cross-linked to increase the nanoparticle stability. Generally, the procedure of solubilization consists of dissolving the amphiphilic polymer and QDs in a nonpolar solvent, such as chloroform, evaporating the organic solvent from the mixture, and adding an aqueous buffer solution.[31] The following parameters are known to influence the colloidal stability of polymer-coated QDs: (i) the number of exposed

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

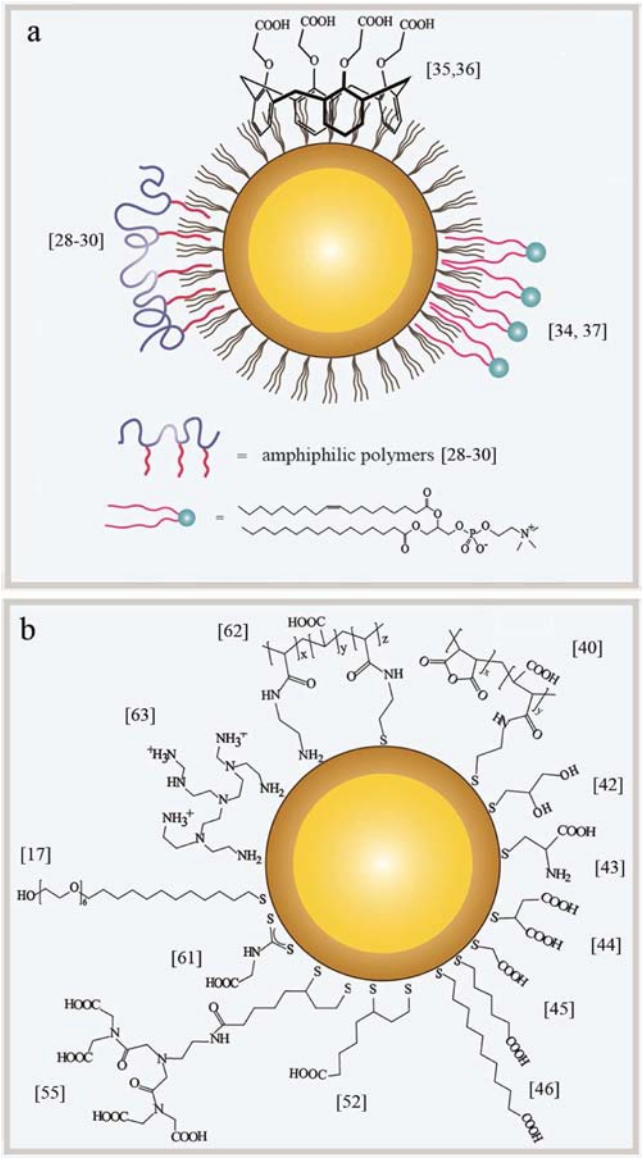


Figure 2. Quantum dot surface modification strategies. (a) Coating of quantum dots (QDs) with amphiphilic molecules (amphiphilic polymers, small molecules, and lipids). The aliphatic moieties of amphiphilic molecules intercalate between TOPO molecules on the surface of QDs, and hydrophilic moieties solubilize QDs in the aqueous solution. (b) Capping compounds for surface modification through ligand exchange. Thiol- or amine-containing compounds replace TOPO and chelate the QD surface, providing QD solubility in water.

charged groups in the QD-coating polymer; (ii) the ratio between the hydrophobic and hydrophilic moieties in the polymer and the length and the number of hydrophobic chains per polymer molecule; (iii) the molecular weight of the polymer, and (iv) the polymer to QD ratio. In each particular experiment, a number of systematic studies on QD−polymer interactions should be performed in order to determine the optimal conditions for QD solubilization.[32,33]

QDs have been successfully coated with small amphiphilic molecules, such as phospholipids[34] and calixarenes,[35,36] using the approach described above. Introduction of poly(ethylene glycol) (PEG) increases the circulation time of phospholipid micelles, which is particularly important in biodistribution studies using QDs as contrast agents[37] or drug delivery vehicles.

2.2. Water-Solubilization of QDs Using Thiol- or Amine-Containing Ligands. The coating of QDs with amphiphilic molecules and the so-called ligand exchange strategy differ from each other in the nature of the interactions involved. In the former case, the coating occurs through hydrophobic interactions of the nonpolar parts of amphiphilic molecules with TOPO on the surface of QDs. In the latter case, QDs are directly bound with coating molecules, usually thiol- or amine-containing compounds. Both strategies have advantages and disadvantages. The direct attachment of thiol-containing molecules to QDs, instead of the bulky TOPO/polymer coating, yields much smaller nanoparticles. This is essential for many applications: e.g., the particle size may dramatically affect the biodistribution and pharmacokinetics *in vivo*,[38,39] as well as the efficiency of FRET-based assays, which are highly sensitive to the donor−acceptor distance.[40] However, replacement of hydrophobic ligands with hydrophilic molecules and subsequent transfer of QDs from a nonpolar environment to a polar one often leads to a decrease in the quantum yield.[41]

Small thiol-containing molecules[42−46] are most frequently used for solubilization of QDs (Figure 2). In order to modify QDs with thiol-containing compounds, the surface surfactant layer (e.g., TOPO for CdSe/ZnS QDs) should be removed. Then, QDs are capped with thiol-containing molecules in such a way that the thiol groups, which have a very high affinity for the ZnS shell of QDs, replace surfactant molecules on the QD surface. This approach is known as the ligand exchange strategy.[5,47] The second method for binding thiol-containing compounds to the surface of QDs is direct synthesis of QDs in water in the presence of thiol capping ligands as stabilizers.[48,49] The synthesis occurs at room temperature and provides water-soluble, bright nanoparticles.[45] Microwave-assisted synthesis in water in the presence of thioacids is also a promising method yielding QDs with a high quantum yield.[50,51]

The main disadvantage of this method is that thiol-capped QDs are unstable because of photoinduced dimerization of thiol groups on the particle surface. An increase in the number of chelating groups per molecule can improve the stability of QDs in water. Dihydrolipoic acid, which contains two thiol groups, has appeared to be a more stable alternative because of a higher affinity of dithiol-containing molecules for the QD surface compared to monothiol-containing compounds.[52] Recently, tridentate ligands[53] and multidentate thiolated ligands[54] were suggested as more effective stabilizing agents, ensuring a higher stability of the resultant QDs. Introduction of additional hydrophilic moieties, e.g., carboxylic acid groups, into a chelating molecule[55] may also enhance the stability of the resultant water-soluble QDs. Thus, one of the ways to improve the stability of QDs could be to increase both the number of thiol groups and the hydrophilicity of the moieties exposed on the QD surface. For example, QDs capped with multivalent thiol polymer ligands based on poly(maleic anhydride) homopolymer grafted with PEG chains are characterized by long-term stability, high quantum yield, resistance to photobleaching, and small hydrodynamic size. The use of such a compact coating of the QD surface instead of the bulky amphiphilic polymer ensured a significantly higher FRET efficiency.[40] In our previous study,[17] we engineered compact and very stable QDs with a hydrodynamic diameter of <9 nm covered with PEG-based low-molecular-weight polymers

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

containing a thiol group, an aliphatic segment, and a hydrophilic PEG segment. These molecules formed a self-assembled inner monolayer on the QD surface solubilized with PEG chains containing an OH, NH_2, or COOH terminal group. By varying the ratio between these groups on the surface of capped QDs, it was possible to obtain QDs with a desired surface charge that were capable of conjugating with antibodies, thus providing ultrasmall diagnostic nanoprobes with the minimum possible nonspecific binding to cells and tissues. PEG derivatives not only are used for nanoparticle solubilization,[56,57] but also may serve as linkers for conjugation of biomolecules, drugs, or fluorophores with nanoparticles of different types, including QDs.[58,59]

Charged groups on the nanoparticle surface coating can bind oppositely charged molecules of biological fluids, including blood, if nanoparticles are administered *in vivo*. This may result in an undesirable immune response and rapid elimination of QDs from the circulation.[60] In addition, charged groups on the surface may cause nonspecific binding in the course of immunofluorescent labeling of cells and tissues.[17] Hydrophilic and uncharged PEG chains attached to the surface of QDs ensure colloidal stability and biocompatibility and prevent undesirable interactions. Modification of the QD surface with PEG chains with terminal functional groups, such as amino groups, allows subsequent immobilization of biomolecules. PEG chains functionalized at both ends may also serve as cross-linkers: e.g., amine-PEG-amine or carboxyl-PEG-carboxyl added, in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), to glutathione-coated QDs (with both carboxyl and amino groups on the surface) cross-link the coating and improve the colloidal stability of QDs.[26]

An alternative to using thiols as capping agents is the attachment of bidentate dithiocarbamate moieties[61] and amine-containing molecules, which, like thiols, also form quite strong bonds with the nanoparticle surface (Figure 2). Smith et al.[62] proposed an approach to the fabrication of stable polymer-coated QDs using multifunctional multidentate polymer ligands containing both thiol and amino groups for attachment to the QD surface and an additional carboxyl group for nanoparticle solubilization. The multidentate polymer ligands introduced by replacing thioglycerol on the QD surface have been found to provide a more compact coating compared to the traditional amphiphilic polymers. Branched amino-functionalized polymers are also of interest. For example, coating with hyperbranched polyethylenimine (PEI) efficiently transfers QDs from nonpolar solvents to water. Due to a high positive charge, PEI-coated QDs easily penetrate through the cell membrane. However, PEI has been found to enhance photooxidation of QDs[63] and to be highly cytotoxic. Grafted PEG segments reduce the cytotoxicity of PEI and have a strong effect on the intracellular pathways of internalized QDs.[64]

Silica shells around QDs have been shown to be extremely effective for stabilization of QDs and their bioconjugation. For example, a silica shell prevents the leakage of toxic ions and the flocculation of particles and helps to maintain the photoluminescence. Introduction of additional thiol, amine, phosphate, carboxylate, or PEG functional groups into the silica shell provides a better control of the conjugation process.[65] In comparison with other solubilization methods, silica encapsulation results in more stable but significantly larger particles, at least tens of nanometers to several micrometers in diameter.

In addition to the above approaches to solubilization and functionalization of the QD surface, a number of combined approaches involving silica and polymer capping have been suggested.[66] For example, there is a three-step procedure based on silanization of QDs followed by grafting the hydrophilic silica surface with long-chain hydrocarbons. The hydrophobic surface of QDs can also be made hydrophilic by attaching amphiphilic lipid−PEG molecules. This synergistic combination yields QDs with a significantly increased resistance to aggressive chemicals, including strong acids, fluorescing in a wide range of pH.[58]

3. PRINCIPLES OF COVALENT AND NONCOVALENT BINDING OF BIOMOLECULES TO THE SURFACE OF FUNCTIONALIZED QUANTUM DOTS

Exceptional brightness and photostability of QDs have made them excellent fluorescent labels for cell targeting and bioimaging. For biological applications, QDs are often conjugated with specific ligands, such as peptides, proteins, nucleic acids, and low-molecular-weight biorecognition ligands. Primarily, the surface of QDs should be modified by introduction of the functional groups necessary for bioconjugation. Although there are a wide variety of strategies for conjugating QDs with biomolecules, all of them should meet some general requirements:

(i) Degradation of the natural activity of biomolecules in the process of conjugation should be minimized.
(ii) The obtained conjugates should be stable under physiological conditions and have a long shelf life.
(iii) Conjugation of biomolecules should be highly oriented: the binding sites of the recognition molecules should face outward so that they could bind their respective antigens contained in the solution. The quantity of the recognition molecules on the surface of a nanoparticle should be controlled.[67]

There are two main approaches to immobilization of biomolecules on the QD surface: covalent linkage and noncovalent binding. Noncovalent binding is determined by hydrophobic, electrostatic, or affinity interactions between biomolecules and the QD surface; covalent linkage is obtained via different bioconjugation techniques using activated functional groups at the surface of QDs. In addition, molecular adapters, such as avidin and biotin, covalently attached to biomolecules and/or to the QD surface are widely used for conjugation.

3.1. Noncovalent Coupling of Quantum Dots and Biomolecules. Water-soluble QDs containing charged groups on the surface readily interact with oppositely charged biomolecules. However, these interactions are difficult to control; the coupling is not oriented and often leads to a decrease in the activity of the coupled biomolecules. For example, association of proteins with a charged surface of nanoparticles influences the protein secondary structure, which may alter their enzymatic activity.[68] Many factors, such as pH, ionic strength, and the presence of competing molecules or chelating agents, have been found to affect the electrostatic coupling between QDs and biomolecules.

A well-known method of electrostatic coupling of proteins with QDs is self-assembly on negatively charged QDs due to the natural positive surface charge of the protein[69,70] or high positive charges of some protein domains, such as the leucine zipper at the C-terminus of recombinant proteins.[71] Interaction of positively charged histidine residues (in polyhistidine-tag motifs of proteins) with dihydrolipoic acid-capped QDs is also

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

an efficient technique for QD−protein coupling.[72] This method significantly reduces the overall size of the bioconjugates. The size of peptide−QD conjugates can also be reduced by using only the relevant fragments of antibodies (Abs) or short peptides with attached His-tags that adequately mimic the behavior of a full-size protein.[73] Another approach to QD−protein coupling is based on metal-affinity coordination of His-tag motifs to zinc ions on the shell of the CdSe/ZnS core/shell nanocrystals.[74−76] Almost any protein engineered to express an oligoHis or leucine zipper sequence at its C-terminus could be used to form high-affinity complexes with QDs. Unlike covalent linkage, this method usually results in protein−QD dispersions with little or no particle aggregation. An alternative conjugation approach using an amphiphilic peptide has been proposed by Pinaud et al.[77] The peptide was composed of a hydrophobic adhesive domain consisting of multiple cysteines and hydrophobic 3-cyclohexylalanine residues and a negatively charged hydrophilic domain. Cys repeats in the adhesive domain acted as surface ligands binding to the ZnS shell of the CdSe/ZnS nanoparticles. The hydrophobic 3-cyclohexylalanine residues around the cysteines facilitated the replacement of TOPO on the QD surface. The hydrophilic domain, which ensures water solubility of the QDs, can be modified to include binding sequences, e.g., biotin and other peptide sequences. Not only peptides, but also oligonucleotides with a thiol functionality at the 5′- or 3′-end, have been successfully immobilized on QDs with the use of the same types of interactions. The complexes have been prepared through replacement of 3-mercaptopropionic acid capping QDs with thiol-functionalized oligonucleotides.[78]

3.2. Use of Molecular Adaptors for Coupling Biomolecules with Quantum Dots. Self-assembling molecular adaptors are molecules capable of specific noncovalent interactions with each other. A high specificity and strong binding capacity of molecular adaptors have made them efficient and widely used tools for bioconjugation.

The approach that uses avidin−biotin systems for coupling of biomolecules with QDs is based on the molecular recognition mechanism. The small molecule of biotin is specifically recognized by the protein receptor avidin with a dissociation constant of about 10^{-15} M. This system is widely used in biological applications; a large variety of biomolecules and synthetic polymers modified with biotin and avidin (as well as streptavidin and neutravidin) are commercially available. QD−Ab complexes of this type may be designed in the form of a ternary affine system (a streptavidin-conjugated QD, biotinylated secondary antibodies, and primary antibodies) or a double system (biotinylated antibodies and a streptavidin-conjugated QD).[79] Avidin has been covalently linked to fluorescent nanoparticles containing carboxyl groups on the surface with the use of EDC.[80] Alternatively, streptavidin with a polyhistidine tag has been bound to QDs via metal-affinity interactions.[81] It should be noted that, since endogenous biotin (also known as vitamin H) is common in mammalian tissues and may interfere with biotinylated conjugates, streptavidin−biotin systems may be unsuitable for *in vivo* applications.

The barnase−barstar system, initially developed for Ab multimerization,[82] is used for bioconjugation along with the streptavidin−biotin system. The bacterial ribonuclease barnase and its natural inhibitor barstar are small proteins (12 and 10 kDa, respectively) with an extremely high mutual affinity comparable with the affinity of streptavidin−biotin interaction. Unlike biotin, which is not a peptide, barnase and barstar may be fused with proteins and antibodies by genetic engineering methods. Complexes of QDs with recombinant antitumor antibodies have been constructed using the barnase−barstar system.[82,83]

Molecular adaptor proteins (proteins A, G, and A/G) attached to the QD surface through a leucine zipper or histidine tag[84,85] may specifically bind immunoglobulins of different types. This is the easiest way to construct oriented functional QD−Ab complexes for imaging applications. The approach does not require any modification of antibodies.[86] QD complexes with antibodies may be prepared using a more complex linker consisting of a molecular adaptor protein and a second protein used as a purification tool.[87] Protein G modified with the leucine zipper acts as a molecular adaptor to connect a QD with immunoglobulines (Ig), whereas maltose-binding protein, also connected with QD via the leucine zipper, serves as a purification tool for separating the QD−IgG conjugate from excess IgG by means of affinity chromatography.

3.3. Covalent Linkage of Biomolecules with Quantum Dots. Covalently linked QD bioconjugates are usually obtained using the standard protein conjugation techniques. The most common method of covalent linkage involves the binding of primary amines of the biomolecules to EDC-activated carboxylic acid groups on the surface of QDs. The reactive intermediate *o*-acylisourea, formed through the activation of carboxylic acids, tends to rapidly hydrolyze in aqueous solutions; therefore, a large excess of EDC is usually added to the reaction mixture. This drawback may be overcome by conversion of the unstable *o*-acylisourea intermediate into a more stable N-hydroxysuccinimide (NHS) ester, sulfonated NHS ester, or (sulfo)fluorophenyl ester intermediate.[88] The advantage of this method for protein conjugation is that proteins contain at least one primary amine and do not need additional chemical modifications before conjugation. On the other hand, polypeptides usually have more than one primary amine or carboxylic group; hence, undesirable cross-linking and aggregation may occur. Carbodiimide techniques have been used for conjugation of QDs with proteins, peptides, antibodies, and oligonucleotides. For example, for preparation of an oligonucleotide−QD conjugate, an amine-modified oligonucleotide was added to QDs capped with EDC-activated mercaptoacetic acid.[89] It is important that a modified oligonucleotide, unlike most proteins, contains a single amino group; therefore, this technique allows the orientation of the attached biomolecules on the QD surface to be controlled and excludes the formation of aggregates. Attempts at conjugation of antibodies via primary amino groups usually result in the loss of antigen-binding capacity due to engaging the amino groups of the lysine residues that are close to the antigen recognition site.[86] Conjugation of QD via thiol groups of biomolecules is more selective and prevents cross-linking and other side reactions. Due to homogeneous orientation of antibodies on the QD surface, this approach results in nanoprobes with a higher specificity than EDC-mediated random conjugation does. In this technique, disulfide bonds of antibodies are reduced using dithiothreitol or 2-mercaptoethylamine. The resultant active fragments of full-size Abs readily interact with maleimide-, pyridyl disulfide-, or iodacetyl-activated QDs to form a covalent bond.[90] It has been shown that conjugation through thiol groups of reduced Abs may preserve the integrity of the Ab active site in the case of mild reduction only. Reducing the disulfide bonds of antibodies by dithiothrietol at standard conditions (20 mM) results in light chain (25 kDa),

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

heavy chain (50 kDa), and partially cleaved heavy−light chain (75 kDa) fragments (Figure 3), with only the 75 kDa heavy−light chain fragments retaining the functional activity. Heterobifunctional NHS−maleimide cross-linker-based conjugation of all these fragments to QDs strongly limits the number of functional capture molecules in a nanoprobe and their capacity for binding the target. To solve this problem, Mahmoud et al. have developed an advanced procedure for partial reduction of antibodies using 2-mercaptoethanol-amine-HCl or low concentrations of dithiothreitol (0.075 mM). Under these reaction conditions, the disulfide bonds between the heavy chains are reduced, with the disulfide bonds between the heavy and light chains remaining intact, which results in a high yield of functionally active 75 kDa fragments.[91] These active fragments of the Ab molecule are conjugated to the QD surface using an NHS−maleimide cross-linker (such as SMCC) in an oriented manner to ensure functional activity of the conjugate. The resultant probe has been found to be as much as 26 times more sensitive than the QD−Ab probes prepared by the standard method, where the number of functionally active heavy−light chain fragments attached to a QD is much smaller due to nonselective reduction.

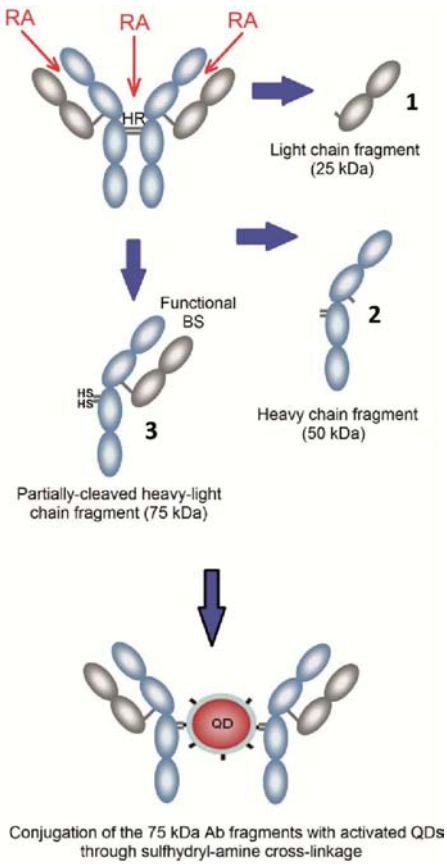


Figure 3. Strategy of reduction antibody (RA) with reducing agent and conjugation of reduced antibodies with QDs. After the disulfide bond cleavage according to different reduction protocols, the fragments that can result include light chain fragment (1), heavy chain fragment (2), and partially cleaved heavy−light chain fragment (3). For structure 3, only the disulfide bonds of the antibody's hinge region (HR) are cleaved. Of all fragments only heavy−light chain fragment retains its functional activity. The conjugation of the SMCC-activated QDs with fragments (3) generates nanoprobes with highly improved functionality of ligand-specific recognition and binding. Reprinted from Mahmoud, W., Rousserie, G., Reveil, B., Tabary, T., Millot, J. M., Artemyev, M., Oleinikov, V. A., Cohen, J. H., Nabiev, I., and Sukhanova, A. (2011) Advanced procedures for labeling of antibodies with quantum dots. *Anal. Biochem. 416*, 180−185, Copyright 2011, with permission from Elsevier.

A new approach of bioorthogonal click-chemistry has been intensively developed in recent years. The main ideology of bioorthogonal reactions is that they do not affect the functional groups that are common for biomolecules, such as amino, carboxyl, thiol, and hydroxyl groups, and do not normally occur in living organisms. The chemical groups involved in bioorthogonal reactions rapidly and selectively react with one another. The main stages of these reactions are Staudiger ligation and cycloaddition reactions. Huisgen 1,3-dipolar cycloaddition (Figure 4a), a click chemistry reaction also known as copper-catalyzed azide alkyne cycloaddition (CuAAC), is a reaction between an alkyne and an azide resulting in triazole linkage. In the presence of Cu(I) salts,

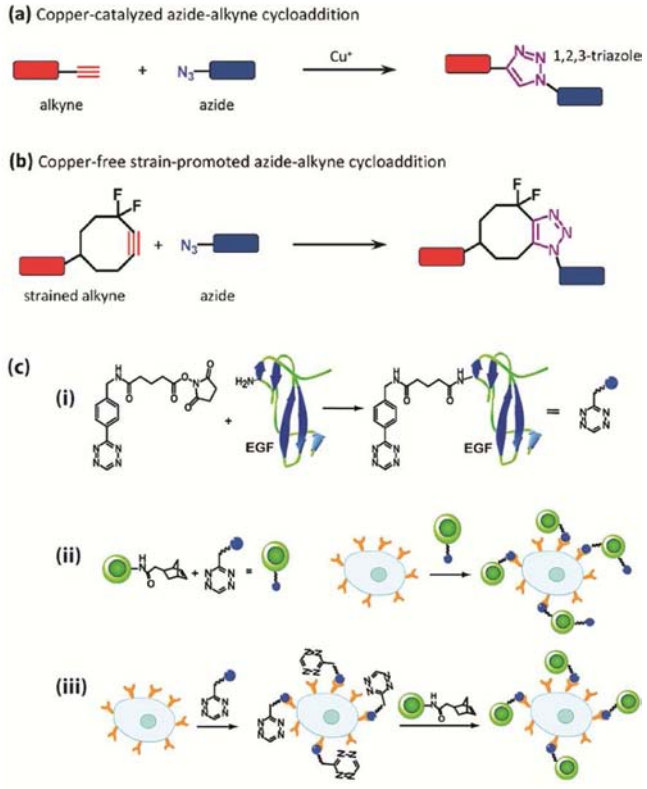


Figure 4. Bioorthogonal chemistry approach. Adapted with permission from Algar, W. R., Prasuhn, D. E., Stewart, M. H., Jennings, T. L., Blanco-Canosa, J. B., Dawson, P. E., and Medintz, I. L. (2011) The controlled display of biomolecules on nanoparticles: a challenge suited to bioorthogonal chemistry. *Bioconjugate Chem. 22*, 825−858. Copyright 2011 American Chemical Society. (a) Copper-catalyzed azide alkyne cycloaddition, the reaction between an alkyne and azide that results in a triazole linkage. (b) Copper-free azide−alkyne cycloaddition, which is promoted by the cycle's strain of cyclooctyne instead of using Cu(I) as a catalyst. (c) (i) Modification of epidermal growth factor (EGF) with NHS-activated tetrazine derivative. (ii) Direct labeling of cells with preformed nonbornene-coated QDs (green circles). For direct labeling, the norbornene coated QDs were previously coupled with modified EGF and the resulting conjugates were added to cells. (iii) In situ conjugation of norbornene-coated QDs to tetrazine-functionalized EGF, overexpressed on the surface of human skin cancer cells.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

Table 2. Conjugates of QDs with Peptides and Antibodies, and Their Composition and Applications

type of conjugate	conjugated molecule	technique for preparation of QD conjugate	application of QD conjugate	ref
QD−IgG	anti-HER2	covalent conjugation	targeting of fixed and live cancer cells	120,
			in vivo delivery of QD−anti-HER2 conjugates into tumors of mice	141, 142
	anti-EGFR	covalent conjugation	*in vivo* imaging and immunohistochemical analysis of tumors	143
	anti-lamin A IgG anti-HSP90 IgG anti-K_i-67 IgG anti-Cox-4 IgG anti-*b*-tubulin IgG	conjugation via an adaptor molecule (protein A)	imaging platform for multicolor, multicycle fluorescent cell staining using five different QD−Ab conjugates in a cycle	123
QD−scFv	anti-HER1/EGFR scFv anti-HER2/neu scFv	conjugation via adaptor molecules (the barnase−barstar system)	targeting of cultured cancer cells	126
	anti-GRP78 scFv	covalent conjugation	detection of GRP78 protein in both live and fixed cancer cells	128
			treatment of breast tumor in an *in vivo* xenograft model	
QD−sdAb	anti-HER2 sdAb	covalent conjugation	detection of HER2 biomarker in lung and breast cancer cells	121
	anti-CEA sdAb	covalent conjugation	flow cytometry quantitative discrimination of CEA-positive and CEA-negative tumor cells	17, 131
			immunofluorescence staining of CEA in histological samples of human appendix and colon carcinoma	
QD−peptide	arginine-rich cell-penetrating peptide SR9 histidine- and arginine-rich cell-penetrating peptide HR9	noncovalent interactions	SR9- and HR9-mediated intracellular delivery of QDs	139, 140
	organelle-targeting peptides	electrostatic adsorption	nuclear and mitochondrial targeting with QDs	14
	RGD and RAD peptides	covalent conjugation	*in vivo* tumor-targeted fluorescence imaging using NIR-emitting QDs	144

which catalyze the reaction, the cycloaddition proceeds just within minutes at room temperature.[92] However, in the case of QD functionalization, strong inhibition of QD luminescence has been observed in the presence of copper ions.[93,94] For this reason, copper-catalyzed reactions cannot be used for QD bioconjugation. The research group headed by Bertozzi was the first to suggest copper-free modification of 1,3-dipolar cycloaddition (Figure 4b), known as copper-free click chemistry.[92] Instead of copper, the reaction of azide alkyne cycloaddition is promoted by the cycle strain in the cyclooctyne molecule (strain-promoted alkyne−azide cycloaddition), and the reaction proceeds very quickly without a catalyst.

The advantages of bioorthogonal reactions over traditional conjugation methods are their rapid kinetics and tolerance to functional groups abundant in cells. Therefore, it is possible to perform the conjugation directly in living systems with high specificity. Over the past few years, there has been growing interest in bioorthogonal chemistry, and a number of studies have used this approach for bioconjugation applications, such as development of drug delivery systems,[95] nanoparticle bioconjugation,[96] and conjugation of QDs with DNA[97] and monoclonal Abs.[98] Cyclooctyne-functionalized QDs have been used as labeling agents for *in vivo* imaging of the metabolic incorporation of azide-modified sialic acids into cell membrane glycoconjugates,[99] and vice versa, cyclooctyne-modified transferrin has been attached to azide-modified water-soluble CdSe/ZnS QDs.[100] The method preserves the protein function intact and maintains the high fluorescence quantum yields and colloidal stability of the QDs. The conjugates have been successfully used for labeling HeLa cells overexpressing transferrin receptors on their surface.[100] Another cycloaddition reaction is inverse-electron-demand Diels−Alder cycloaddition involving tetrazine and strained alkenes. This method has been applied to targeting norbornene-coated QDs to live cancer cells labeled with tetrazine-modified epidermal growth factor (Figure 4c).[101]

It should be noted that, although a number of techniques have been developed for QD functionalization and bioconjugation, most of them are more complicated than the corresponding techniques for organic fluorescent dyes. Since QDs are colloidal nanoparticles, whose properties differ from those of small molecules, the stability of QDs under the same conditions depends on their composition and size, the coating material, and the success of bioconjugation. Unlike organic dyes containing only a single functional group for labeling, QDs usually contain more than one such group, which may result in multivalent targeting. To solve this problem, several strategies for controlling the number of functional ligands on the nanoparticle surface have been proposed. For example, a large polymer chain modified with a controlled number of biotin moieties was used for nanoparticle coating, so that, for geometric reasons, only one polymer is bound to each nanoparticle.[102] Alternatively, free-radical polymerization was performed on the nanoparticle surface, resulting in a single moiety of interest per nanoparticle.[103] Another strategy is to perform fractionation in order to separate conjugates with desired numbers of ligands per nanoparticle. Metal ion affinity chromatography[104] and gel electrophoretic fractionation[105] have been used to produce conjugates containing specific numbers of DNA,[106] PEG,[105] and streptavidin[107] molecules.

Thus, although bioconjugation of QDs still remains a nontrivial task, a significant progress has been made in developing various methods of biomolecule labeling with QDs.[86] To date, conjugates of QDs with monoclonal Abs and streptavidin and kits for labeling monoclonal Abs by means of both traditional EDC-mediated coupling and the novel technique of site-specific click-coupling are commercially available.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

Table 3. Conjugates of QDs with Nucleic Acids and Specific Ligands, and Their Compositions and Applications

type of conjugate	conjugated molecule	technique for preparation of QD conjugate	application of QD conjugate	ref
QD–nucleic acid	anti-insulin receptor aptamer	biotin–streptavidin binding	monitoring of the dynamics of individual insulin receptors in the plasma membranes of living cells	159
	anti-angiogenin aptamer	covalent conjugation	labeling and investigation of the intracellular localization of angiogenin	160
	anti-mucin 1 aptamer	DNA is directly attached to the surface of QDs during their synthesis	active tumor-targeted imaging of mucin 1 *in vitro* and *in vivo*	156
	anti-nucleolin aptamer	EDC-mediated covalent conjugation	triple cellular imaging using three different QD-conjugated aptamers	158
	anti-tenascin-C aptamer			
	anti-mucin 1 aptemer			
	Plasmin DNA	covalent conjugation	long-term intracellular and intranuclear tracking of plasmid DNA	157
	siRNA	electrostatic complexation	gene silencing and real-time tracking of siRNA during delivery and transfection into cells	164–166
	siRNA, RGD peptide and HIV-Tat peptide	reversible disulfide linkage for fast release of siRNA. robust linkage for evaluation of cellular uptake and localization of siRNA	targeted peptide-mediated delivery and intracellular tracking of siRNA	162
	antisense oligonucleotide and peptide p160 (with specificity for MCF-7 breast cancer cells)	biotin–streptavidin binding	targeted cell-type-specific delivery; real-time tracking; and selective down-regulating of the expression of folate receptor α	169
QD–specific ligand	folic acid	covalent conjugation	*in vitro* cancer cell labeling and *in vivo* tumor targeting	146
	(1) coenzyme A; (2) cholera toxin subunit B; (3) streptavidin	(1) SMCC-mediared covalent conjugation; (2) covalent conjugation; (3) biotin–streptavidinbinding	(1) transmembrane epidermal growth factor receptor; (2) glucosylphospatidylinositolanchored protein CD59; (3) ganglioside GM1–cholera toxin subunit B clusters	135
	brain-derived neurotrophic factor	biotin–streptavidin binding	intracellular trafficking of tyrosine kinase receptors	137

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609–624

4. QUANTUM DOT BIOCONJUGATES FOR DIAGNOSIS AND IMAGING

Fluorescent proteins and organic fluorophores are extensively used in many bioimaging and biosensing investigations. However, their fluorescence is relatively weak and unstable, which precludes the use of intense photon beams for excitation and limits the possibility of long-term studies. QDs have several advantages over fluorescent proteins and other organic fluorophores (Table 1): their fluorescence lifetimes, brightness, and photostability are much higher, allowing long-term observation; unique spectral properties of QDs (broad excitation spectra and large Stockes shifts) make it easy to perform multicolor imaging using a single excitation light source. However, the main problem still limiting QD applications in living systems is their toxicity.[108−110] In brief, QD toxicity may be related to both their composition, the chemistry of the capping material, and their size. However, along with an ever-increasing number of reports on QDs toxicity,[111−115] there is extensive research aimed at obtaining nontoxic QDs,[116−118] which can be safely used for biomedical applications. For example, silicon QDs represent a promising biocompatible alternative to toxic heavy-metal-based QDs. Recent studies in animal models have demonstrated no signs of toxicity as judged by the animal behavior, body mass, or blood composition, even at high intravenous doses of silicon QDs. Though biocompatible, silicon is not biodegradable and tends to accumulate in organs and cause adverse effects in livers of mice several months after treatment.[110] However, no adverse effects have been found in monkeys in long-term investigations, which gives grounds to presume that such QDs could be safe for humans and approved for clinical use in the near future.

Using covalent and noncovalent linkage techniques described above, QDs can be conjugated to a wide variety of biomolecules, including peptides, proteins, antibodies, oligonucleotides, and polysaccharides, as well as low-molecular-weight drugs. Here, we describe some examples and prospects of the use of such bioconjugates for diagnosis, imaging, and drug delivery.

4.1. Conjugates of QDs with Peptides, Proteins, and Antibodies. QD−Ab conjugates are of growing interest today, especially in terms of immunofluorescent staining of cells and tissues. Since the first application of QDs to specific staining of fixed cells in 1998,[119] plenty of tumor-associated antigens, such as HER2, EGFR, and CEA, have been detected on cultured cancer cells,[120] as well as fixed cells,[121] and tissue specimens,[122] by using QD−Ab conjugates (Table 2). Generally, QD−Ab conjugates or a combination of QD−streptavidin (QD−avidin) with biotinylated antibodies are used for labeling antigens expressed on the cell membrane. Most authors report that QD-based immunostaining has a higher detection sensitivity than conventional clinical techniques. Moreover, due to their unique spectral properties (the broad excitation spectrum allows simultaneous excitation of QDs with different emission colors), QDs can be excellent probes for multiplexed analysis[123] in immunohisto- and immunocytochemistry performed by means of epifluorescence or confocal microscopy. For example, simultaneous identification of several subtypes of breast cancer cells[124] and low-abundance (about 1%) types of cells[125] in biopsy specimens has been reported. The size of the probe used for targeting may affect the accessibility of epitopes and the depth of the probe penetration into tissue. Ideally, a diagnostic nanoprobe should not exceed 15 nm in size and should contain high-affinity homogeneously oriented capture molecules on its surface. The use of full-size monoclonal Abs is limited by complicated techniques of their conjugation with nanoparticles, as well as their poor penetration into tissues and slow blood clearance when they are used for targeting and imaging. Various forms of smaller Ab fragments have been suggested recently as good alternatives. For example, single-chain variable fragments (scFv) have been conjugated to QDs to obtain compact nanoprobes for cell targeting and imaging[126−128] (Table 2). Single-domain antibodies (sdAbs, so-called nanobodies) with a molecular weight of about 13 kDa, which is 12 times smaller than conventional IgG antibodies, are the smallest possible naturally occurring antigen-binding fragments known. They are derived from the unique heavy-chain antibodies of camelids (llamas and camels) and sharks with the use of molecular engineering techniques. Conjugation of sdAbs with QDs results in a compact probe that can be used for targeting, imaging, and flow cytometry.[17,129,130]

In our previous studies,[17,131] we developed compact conjugates of QDs and single-domain antibodies with a cysteine residue specifically integrated into the C-terminal region of antibodies using genetic engineering. Covalent linkage through the SH group of the cysteine residue resulted in highly oriented conjugates, with all antigen-binding sites facing the outside. We found that immunostaining with these compact oriented probes had a higher detection sensitivity compared to conventional clinical techniques. The conjugates turned out to be promising probes for immunolabeling of CEA in tissue specimens.

Due to their high brightness and photostability, QDs appear to be a promising tool for imaging and tracking of individual membrane receptors[132] and intracellular proteins[133] at the single-particle level. Conjugates of QDs with antibodies and other proteins make it possible to track the dynamics of integrins[134] and receptors[135] (such as EGFR,[136] and tyrosine kinase receptor[137]) on the surface of living cells (Tables 2, 3).

QD-mediated intracellular labeling and tracking represents a more complicated task than labeling of plasma membrane proteins. Attachment of guide molecules, such as guide antibodies and translocation peptides,[138] can mediate the intracellular delivery of QDs. Most of these molecules contain positively charged motifs, which facilitate interaction with the negatively charged cell membrane. For example, cell-penetrating peptides containing a positively charged histidine- and arginine-rich motifs have been used to facilitate the cellular uptake of QDs.[139,140] Furthermore, QDs conjugated with organelle-targeting peptides have been used for labeling mitochondria and nuclei.[14] Due to the high photostability of QDs, it is possible to observe individual QDs for minutes or even hours and, hence, to track them through all stages of their delivery. For example, *in vivo* delivery of QD−anti-HER2 antibody conjugates has been tracked from their circulation in blood to their binding to HER2, internalization, and movement from the cell membrane to the perinuclear region.[141]

QDs conjugated with tissue-specific biomarkers are also used for *in vivo* imaging and targeted delivery. Nonconjugated QDs have been proven to passively accumulate in tumors due to an enhanced permeability and the retention (EPR) effect. For targeted tumor delivery, a number of guide molecules, such as antitumor antibodies (anti-HER2[142] and anti-EGFR[143]) and tumor targeting peptides (arginine−glycine−aspartic acid (RGD), arginine−alanine−aspartic acid (RAD),[144,145] and folic acid[146]), are commonly used (Tables 2, 3). The efficiency

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

of deep-tissue *in vivo* imaging may be considerably improved using QDs emitting in the near-infrared region (NIR). Specifically, NIR imaging is usually performed in "transparent optical windows" in the ranges of 700−900 and 1000−1350 nm, where tissue absorbance is low. Another benefit of QDs is large two-photon absorption cross sections and, hence, the capacity for simultaneous absorption of two photons and subsequent transition to an excited state. The QD absorption cross-section may exceed 10^4 Goeppert-Mayer units (GM), which is several orders of magnitude larger than those of conventional fluorescent dyes and fluorescent proteins.[147] This makes QDs the best fluorescent label to be used for multiphoton imaging.[131]

4.2. Conjugates of QDs with RNA and DNA. DNA and short interfering RNA (siRNA) represent an important laboratory tool for probing gene functions and hold great potential for treatment of many human diseases. However, the major trouble for clinical applications of gene-based therapeutics is related to the difficulty of safely and effectively introducing them into cells of interest. Therefore, development of effective nonviral gene delivery methods preserving the stability of nucleic acids, delivering them specifically to the desired tissue, and facilitating their cellular uptake are of current interest.

Gene delivery vehicles, such as liposomes, cationic polymers, and nanoparticles, are under investigation as nonviral vectors for gene delivery. For intracellular plasmid trafficking and better understanding of the critical steps of the transfection process, DNA or RNA are commonly labeled with fluorescent dyes. QDs may be delivered to cells by various methods, such as mechanical transfection techniques (electroporation and microinjection), receptor-mediated endocytosis using guide molecules (antibodies and cell-penetrating peptides), and nonspecific endocytosis. Therefore, QDs themselves may serve as effective nonviral gene vectors that combine two functionalities, delivery and real-time visualization.

Endosomal escape remains the main challenge for QD intracellular delivery and restricts quantitate intracellular labeling. Most techniques for QD delivery involve some form of endocytosis as the critical step yielding QDs encapsulated in vesicles. As a result, QDs and biorecognition molecules are screened from the intracellular environment and eventually aggregate in the acidic lysosomal environment. Some mechanical techniques that do not involve endocytosis or vesicle encapsulation, such as electroporation,[148] microinjection,[149] and mechanical disruption of the cell membrane with glass beads or by needle scratching,[150] have been used to overcome endosomal escape upon QD delivery. Other strategies are aimed at ensuring fast escape from endocytic organelles after cell internalization.[151] Some types of QD coatings containing multivalent amine groups, the so-called "proton sponges", have been found to destabilize endosome membrane and facilitate the release from endosomal vesicles.[152] A number of peptides, such as pH-sensitive peptides disrupting membranes at acidic pH and cell-penetrating peptides, have also been shown to stimulate endosomal escape.[153,154]

Like polypeptides, DNA and siRNA may be attached to the surface of QDs through covalent or electrostatic interactions. Alternatively, DNA/siRNA and QDs can be coloaded into larger drug-delivery systems, such as micelles and polymer particles. The resulting nanoprobes are used both for *in vitro* intracellular delivery and tracking[155] and for *in vivo* gene delivery[156] (Table 3).

QDs have been used for long-term intracellular and intranuclear tracking of plasmid DNA, since they produce a highly stable fluorescent signal suitable for 24 h time-lapse confocal imaging. For this purpose, plasmid DNA was covalently conjugated to QDs. The tagged DNA remained functional, serving as a template for gene transcription upon entering the nucleus: after transfection into cells, these QD−DNA conjugates were capable of expressing the reporter protein, namely, enhanced green fluorescent protein.[157] Aptamers are single-stranded oligonucleotides that can bind to various targets similarly to antibodies, but they have several advantages, such as a small size and low immunogenicity; in addition, they are easy to synthesize. QD−aptamer conjugates have been used for labeling and tracking of individual targets: nucleolin, mucin, the extracellular matrix protein tenascin-C,[158] and insulin receptors in the plasma membrane,[159] as well as angeogenin tracking in living cell during the angiogenesis, from bonding to the cell membrane and internalization to localization in lysosomes.[160]

Delivery of QD−RNAs into cells has also been described. Chen et al. were the first to codeliver QDs and siRNA using cationic liposomes in order to trace siRNA *in vitro*.[161] Later, a number of approaches without using liposomes were proposed; for this purpose, QD−siRNA particles were formed through covalent conjugation of siRNA to the surface of QDs[162] or formation of polyelectrolyte complexes (so-called nanoplexes[163]) of siRNA with positively charged QDs coated with polyethylenimine,[164] amine-functionalized PEG,[165] and L-arginine.[166] Yezhelyev et al. developed QDs coated with a proton-absorbing polymer containing both carboxylic and tertiary amino groups (proton sponges) for efficient siRNA delivery into the cell and subsequent release from intracellular vesicles.[167] In addition to a significant improvement in gene silencing efficiency, the cellular toxicity of QD−siRNA particles turned out to be five to six times lower compared to the existing transfection agents.

Of special importance is that coattachment of two or more different biorecognition molecules to a single QD results in a multifunctional particle with synergetic efficiency. For example, Derfus et al. proposed a multifunctional nanoparticle based on QD targeted with both tumor-homing peptides and siRNA for improved delivery.[168] Jung et al. demonstrated that modification of QD−siRNA with two functional peptides, RGD and HIV-Tat, improved selective cell internalization as compared to the modification with RGD alone.[162] In that study, two different specific linkers were used for the attachment of siRNA to QDs, a linker with reversible disulfide linkage ensuring fast release of siRNA upon entering the cell and a more robust linker enabling the evaluation of the cellular uptake and localization of the siRNA within the cellular compartments.

Introduction of cell-specific biorecognition molecules ensures cell-type-specific delivery. This approach is aimed at inhibiting genes in a cell-specific manner, which is a prerequisite for development of chemotherapies. For example, multifunctional conjugate of a QD, an antisense oligonucleotide, and peptide p160 (with specificity for MCF-7 breast cancer cells) designed for real-time tracking of cellular delivery in the target MCF-7 cell line decreases the expression rate of folate receptor α.[169]

5. CONCLUSION

QDs have a number of advantages over fluorescent dyes and proteins due to their superior photophysical and spectral properties. Their high photostability and the tunability of their

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

optical characteristics up to the far-infrared region make QDs an attractive tool for deep-tissue, long-term imaging. Recent progress in QD surface chemistry and bioconjugation techniques has made it possible to attach almost any biomolecule of interest to the QD surface. To summarize, we consider the following issues to be particularly important for the development and applications of QD bioconjugates:

- Due to their unique spectral properties, QD-based bioconjugates are promising fluorescent probes for fluorescent labeling, especially for multiplexed analysis. Despite a number of breakthrough studies on QDs, most commercial kits and fluorescent probes are still based on conventional fluorophores. It may be expected that QD-based systems for multiplexed analysis of panels of biomarkers will be widely used for routine clinical ex vivo diagnostics in the near future.
- QD toxicity is a complex issue; it depends on a number of factors, including the QD chemical composition, size, charge, and surface chemistry. It is obvious that QDs cannot be safely used *in vivo* until the problem of their toxicity is solved. An ideal solubilization strategy should reduce QD toxicity and undesirable nonspecific QD uptake by living tissues and scavenging by the reticuloendothelial system. Nontoxic fluorescent nanocrystals that do not contain heavy metals are being developed as a credible alternative.
- Being colloidal fluorescent nanoparticles, QDs may serve as dual-function tools for simultaneous delivery of biomolecules and visualization. Attachment of multiple specific biofunctionalities (such as antibodies, peptides, and nucleic acids) to the surface of QDs allows a "magic tool" to be created for site-specific delivery and simultaneous tracking.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: igor.nabiev@gmail.com.
*E-mail: alyona.sukhanova@univ-reims.fr.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by the Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014−2020, contract no. RFMEFI57814X0054.

ABBREVIATIONS

Ab(s), antibody(ies); CEA, carcinoembryonic antigen; EDC, 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide; EGFR, epidermal growth factor receptor; FRET, Förster resonance energy transfer; HER2, human epidermal growth factor receptor 2; NIR, near-infrared; NHS, *N*-hydroxysuccinimide; PEG, polyethylene glycol; PEI, polyethylenimine; RGD, arginine−glycine−aspartic acid; scFv, single-chain variable fragment; sdAb(s), single-domain antibody(ies); SMCC, succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate cross-linker; TOP, tri-*n*-octylphosphine; TOPO, tri-*n*-octylphosphine oxide; QD(s), quantum dot(s)

REFERENCES

(1) Medintz, I. L., Uyeda, H. T., Goldman, E. R., and Mattoussi, H. (2005) Quantum dot bioconjugates for imaging, labelling and sensing. *Nat. Mater. 4*, 435−446.
(2) Hines, M. A., and Guyot-Sionnest, P. (1996) Synthesis and characterization of strongly luminescing ZnS-capped CdSe nanocrystals. *J. Phys. Chem. 100*, 468−471.
(3) Samir, T. M., Mansour, M. M., Kazmierczak, S. C., and Azzazy, H. M. (2012) Quantum dots: heralding a brighter future for clinical diagnostics. *Nanomedicine (London, U. K.) 7*, 1755−1769.
(4) Chan, W. C., and Nie, S. (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science 281*, 2016−2018.
(5) Sukhanova, A., Devy, J., Venteo, L., Kaplan, H., Artemyev, M., Oleinikov, V., Klinov, D., Pluot, M., Cohen, J. H., and Nabiev, I. (2004) Biocompatible fluorescent nanocrystals for immunolabeling of membrane proteins and cells. *Anal. Biochem. 324*, 60−67.
(6) Bao, H. F., Hao, N., Yang, Y. X., and Zhao, D. Y. (2010) Biosynthesis of biocompatible cadmium telluride quantum dots using yeast cells. *Nano Res. 3*, 481−489.
(7) Chen, G., Yi, B., Zeng, G., Niu, Q., Yan, M., Chen, A., Du, J., Huang, J., and Zhang, Q. (2014) Facile green extracellular biosynthesis of CdS quantum dots by white rot fungus Phanerochaete chrysosporium. *Colloids Surf., B 117*, 199−205.
(8) Gallardo, C., Monrás, J. P., Plaza, D. O., Collao, B., Saona, L. A., Durán-Toro, V., Venegas, F. A., Soto, C., Ulloa, G., Vásquez, C. C., et al. (2014) Low-temperature biosynthesis of fluorescent semiconductor nanoparticles (CdS) by oxidative stress resistant Antarctic bacteria. *J. Biotechnol. 187*, 108−115.
(9) Pawar, V., Kumar, A. R., Zinjarde, S., and Gosavi, S. (2013) Bioinspired inimitable cadmium telluride quantum dots for bioimaging purposes. *J. Nanosci. Nanotechnol. 13*, 3826−3831.
(10) Stürzenbaum, S. R., Höckner, M., Panneerselvam, A., Levitt, J., Bouillard, J. S., Taniguchi, S., Dailey, L. A., Ahmad Khanbeigi, R., Rosca, E. V., Thanou, M., et al. (2013) Biosynthesis of luminescent quantum dots in an earthworm. *Nat. Nanotechnol. 8*, 57−60.
(11) Muthu, M. S., Kulkarni, S. A., Raju, A., and Feng, S. S. (2012) Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots. *Biomaterials 33*, 3494−3501.
(12) Tian, B., Al-Jamal, W. T., Al-Jamal, K. T., and Kostarelos, K. (2011) Doxorubicin-loaded lipid-quantum dot hybrids: surface topography and release properties. *Int. J. Pharm. 416*, 443−447.
(13) Wen, C. J., Zhang, L. W., Al-Suwayeh, S.A., Yen, T. C., and Fang, J. Y. (2012) Theranostic liposomes loaded with quantum dots and apomorphine for brain targeting and bioimaging. *Int. J. Nanomed. 7*, 1599−1611.
(14) Derfus, A. M., Chan, W. C. W., and Bhatia, S. N. (2004) Intracellular delivery of quantum dots for live cell labeling and organelle tracking. *Adv. Mater. 16*, 961−966.
(15) Al-Jamal, W. T., and Kostarelos, K. (2007) Liposome-nanoparticle hybrids for multimodal diagnostic and therapeutic applications. *Nanomedicine (London, U. K.) 2*, 85−98.
(16) Tomczak, N., Janczewski, D., Han, M., and Vancso, G. J. (2009) Designer polymer−quantum dot architectures. *Prog. Polym. Sci. 34*, 393−430.
(17) Sukhanova, A., Even-Desrumeaux, K., Kisserli, A., Tabary, T., Reveil, B., Millot, J. M., Chames, P., Baty, D., Artemyev, M., Oleinikov, V., et al. (2012) Oriented conjugates of single-domain antibodies and quantum dots: toward a new generation of ultrasmall diagnostic nanoprobes. *Nanomed. Nanotechnol. 8*, 516−525.
(18) Parak, W. J., Pellegrino, T., and Plank, C. (2005) Labelling of cells with quantum dots. *Nanotechnology 16*, R9.
(19) Baba, K., and Nishida, K. (2012) Single-molecule tracking in living cells using single quantum dot applications. *Theranostics 2*, 655−667.
(20) Cassette, E., Helle, M., Bezdetnaya, L., Marchal, F., Dubertret, B., and Pons, T. (2013) Design of new quantum dot materials for deep tissue infrared imaging. *Adv. Drug Delivery Rev. 65*, 719−31.
(21) Li, J., and Zhu, J. J. (2013) Quantum dots for fluorescent biosensing and bio-imaging applications. *Analyst 138*, 2506−2515.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

(22) Akinfieva, O., Nabiev, I., and Sukhanova, A. (2013) New directions in quantum dot-based cytometry detection of cancer serum markers and tumor cells. *Crit. Rev. Oncol. Hematol. 86*, 1−14.
(23) Qi, Z. D., Li, D. W., Jiang, P., Jiang, F. L., Li, Y. S., Liu, Y., Wong, W. K., and Cheah, K. W. (2011) Biocompatible CdSe quantum dot-based photosensitizer under two-photon excitation for photodynamic therapy. *J. Mater. Chem. 21*, 2455−2458.
(24) Probst, C. E., Zrazhevskiy, P., Bagalkot, V., and Gao, X. (2013) Quantum dots as a platform for nanoparticle drug delivery vehicle design. *Adv. Drug Delivery Rev. 65*, 703−718.
(25) Samokhvalov, P., Artemyev, M., and Nabiev, I. (2013) Basic principles and current trends in colloidal synthesis of highly luminescent semiconductor nanocrystals. *Chemistry 19*, 1534−1546.
(26) Zhang, P., and Han, H. (2012) Compact PEGylated polymer-caged quantum dots with improved stability. *Colloids Surf., A 402*, 72−79.
(27) Liu, W., Choi, H. S., Zimmer, J. P., Tanaka, E., Frangioni, J. V., and Bawendi, M. (2007) Compact cysteine-coated CdSe(ZnCdS) quantum dots for *in vivo* applications. *J. Am. Chem. Soc. 129*, 14530−14531.
(28) Lin, C. A., Sperling, R. A., Li, J. K., Yang, T. Y., Li, P. Y., Zanella, M., Chang, W. H., and Parak, W. J. (2008) Design of an amphiphilic polymer for nanoparticle coating and functionalization. *Small 4*, 334−341.
(29) Qian, J., and Gao, X. (2013) Triblock copolymer-encapsulated nanoparticles with outstanding colloidal stability for siRNA delivery. *ACS Appl. Mater. Interfaces 5*, 2845−2852.
(30) Schmidtke, C., Pöselt, E., Ostermann, J., Pietsch, A., Kloust, H., Tran, H., Schotten, T., Bastús, N. G., Eggers, R., and Weller, H. (2013) Amphiphilic, cross-linkable diblock copolymers for multifunctionalized nanoparticles as biological probes. *Nanoscale 5*, 7433−7444.
(31) Anderson, R. E., and Chan, W. C. (2008) Systematic investigation of preparing biocompatible, single, and small ZnS-capped CdSe quantum dots with amphiphilic polymers. *ACS Nano 2*, 1341−1352.
(32) Geidel, C., Schmachtel, S., Riedinger, A., Pfeiffer, C., Müllen, K., Klapper, M., and Parak, W. J. (2011) A general synthetic approach for obtaining cationic and anionic inorganic nanoparticles via encapsulation in amphiphilic copolymers. *Small 7*, 2929−2934.
(33) Luccardini, C., Tribet, C., Vial, F., Marchi-Artzner, V., and Dahan, M. (2006) Size, charge, and interactions with giant lipid vesicles of quantum dots coated with an amphiphilic macromolecule. *Langmuir 22*, 2304−2310.
(34) Galloway, J. F., Winter, A., Lee, K. H., Park, J. H., Dvoracek, C. M., Devreotes, P., and Searson, P. C. (2012) Quantitative characterization of the lipid encapsulation of quantum dots for biomedical applications. *Nanomed. Nanotechnol. 8*, 1190−1199.
(35) Jin, T., Fujii, F., Sakata, H., Tamura, M., and Kinjo, M. (2005) Calixarene-coated water-soluble CdSe−ZnS semiconductor quantum dots that are highly fluorescent and stable in aqueous solution. *Chem. Commun. 22*, 2829−2831.
(36) Joshi, K. V., Joshi, B. K., Pandya, A., Sutariya, P. G., and Menon, S. K. (2012) Calixarene capped ZnS quantum dots as an optical nanoprobe for detection and determination of menadione. *Analyst 137*, 4647−4650.
(37) Papagiannaros, A., Levchenko, T., Hartner, W., Mongayt, D., and Torchilin, V. (2009) Quantum dots encapsulated in phospholipid micelles for imaging and quantification of tumors in the near-infrared region. *Nanomed. Nanotechnol. 5*, 216−224.
(38) Balasubramanian, S. K., Poh, K. W., Ong, C. N., Kreyling, W. G., Ong, W. Y., and Yu, L. E. (2013) The effect of primary particle size on biodistribution of inhaled gold nano-agglomerates. *Biomaterials 34*, 5439−5452.
(39) Kulkarni, S. A., and Feng, S. S. (2013) Effects of particle size and surface modification on cellular uptake and biodistribution of polymeric nanoparticles for drug delivery. *Pharm. Res. 30*, 2512−2522.
(40) Duan, H., Kuang, M., and Wang, Y. A. (2010) Quantum dots with multivalent and compact polymer coatings for efficient fluorescence resonance energy transfer and self-assembled biotagging. *Chem. Mater. 22*, 4372−4378.
(41) Smith, A. M., Duan, H., Rhyner, M. N., Ruan, G., and Nie, S. (2006) A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. *Phys. Chem. Chem. Phys. 8*, 3895−3903.
(42) Aboulaich, A., Balan, L., Ghanbaja, J., Medjahdi, J., Merlin, C., and Schneider, R. (2011) Aqueous route to biocompatible ZnSe:Mn/ZnO core/shell quantum dots using 1-thioglycerol as stabilizer. *Chem. Mater. 23*, 3706−3713.
(43) Sukhanova, A., Venteo, L., Devy, J., Artemyev, M., Oleinikov, V., Pluot, M., and Nabiev, I. (2002) Highly stable fluorescent nanocrystals as a novel class of labels for immunohistochemical analysis of paraffin-embedded tissue sections. *Lab. Invest. 82*, 1259−1261.
(44) Chen, S., Zhang, X., Zhang, Q., Hou, X., Zhou, Q., Yan, J., and Tan, W. (2011) CdSe quantum dots decorated by mercaptosuccinic acid as fluorescence probe for Cu^{2+}. *J. Lumin. 131*, 947−951.
(45) Zhao, S. M., and Han, S. Q. (2010) Thioglycolic acid capped CdS quantum dots as fluorescence probe for ultrasensitive determination of tetracycline and oxytetracycline. *J. Chin. Chem. Soc. 57*, 1353−1360.
(46) Aldeek, F., Balan, L., Lambert, J., and Schneider, R. (2008) The influence of capping thioalkyl acid on the growth and photoluminescence efficiency of CdTe and CdSe quantum dots. *Nanotechnology 19*, 475401.
(47) Hezinger, A. F., Tessmar, J., and Göpferich, A. (2008) Polymer coating of quantum dots - a powerful tool toward diagnostics and sensorics. *Eur. J. Pharm. Biopharm. 68*, 138−152.
(48) Lesnyak, V., Gaponik, N., and Eychmüller, A. (2013) Colloidal semiconductor nanocrystals: the aqueous approach. *Chem. Soc. Rev. 42*, 2905−2929.
(49) Gaponik, N., Talapin, D. V., Rogach, A. L., Hoppe, K., Shevchenko, E. V., Kornowski, A., Eychmüller, A., and Weller, H. (2002) Thiol-capping of CdTe nanocrystals: an alternative to organometallic synthetic routes. *J. Phys. Chem. B 106*, 7177−7185.
(50) He, Y., Lu, H. T., Sai, L. M., Lai, W. Y., Fan, Q. L., Wang, L. H., and Huang, W. (2007) Microwave-assisted synthesis of water-dispersed CdTe nanocrystals with high luminescent efficiency and narrow size distribution. *Chem. Mater. 19*, 359−365.
(51) Duan, J., Song, L., and Zhan, J. (2010) One-pot synthesis of highly luminescent CdTe quantum dots by microwave irradiation reduction and their Hg^{2+}-sensitive properties. *Nano Res. 2*, 61−68.
(52) Nagaraja, A. T., Sooresh, A., Meissner, K. E., and McShane, M. J. (2013) Processing and characterization of stable, pH-sensitive layer-by-layer modified colloidal quantum dots. *ACS Nano 7*, 6194−6202.
(53) Gravel, E., Tanguy, C., Cassette, E., Pons, T., Knittel, F., Bernards, N., Garofalakis, A., Ducongé, F., Dubertret, B., and Doris, E. (2013) Compact tridentate ligands for enhanced aqueous stability of quantum dots and *in vivo* imaging. *Chem. Sci. 4*, 411−417.
(54) Liu, L., Guo, X., Li, Y., and Zhong, X. (2010) Bifunctional multidentate ligand modified highly stable water-soluble quantum dots. *Inorg. Chem. 49*, 3768−3775.
(55) Zhou, D., Li, Y., Hall, E. A., Abell, C., and Klenerman, D. (2011) A chelating dendritic ligand capped quantum dot: preparation, surface passivation, bioconjugation and specific DNA detection. *Nanoscale 3*, 201−211.
(56) Boulmedais, F., Bauchat, P., Brienne, M. J., Arnal, I., Artzner, F., Gacoin, T., Dahan, M., and Marchi-Artzner, V. (2006) Water-soluble pegylated quantum dots: from a composite hexagonal phase to isolated micelles. *Langmuir 22*, 9797−9803.
(57) Mei, B. C., Susumu, K., Medintz, I. L., and Mattoussi, H. (2009) Polyethylene glycol-based bidentate ligands to enhance quantum dot and gold nanoparticle stability in biological media. *Nat. Protoc. 4*, 412−423.
(58) Hu, X., and Gao, X. (2010) Silica-polymer dual layer-encapsulated quantum dots with remarkable stability. *ACS Nano 4*, 6080−6086.
(59) Dif, A., Boulmedais, F., Pinot, M., Roullier, V., Baudy-Floc'h, M., Coquelle, F. M., Clarke, S., Neveu, P., Vignaux, F., Le Borgne, R., et al.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

(2009) Small and stable peptidic PEGylated quantum dots to target polyhistidine-tagged proteins with controlled stoichiometry. *J. Am. Chem. Soc. 131*, 14738−14746.
(60) Shemetov, A. A., Nabiev, I., and Sukhanova, A. (2012) Molecular interaction of proteins and peptides with nanoparticles. *ACS Nano 6*, 4585−4602.
(61) Dubois, F., Mahler, B., Dubertret, B., Doris, E., and Mioskowski, C. (2007) A versatile strategy for quantum dot ligand exchange. *J. Am. Chem. Soc. 129*, 482−483.
(62) Smith, A. M., and Nie, S. (2008) Minimizing the hydrodynamic size of quantum dots with multifunctional multidentate polymer ligands. *J. Am. Chem. Soc. 130*, 11278−11279.
(63) Nann, T. (2005) Phase-transfer of CdSe@ZnS quantum dots using amphiphilic hyperbranched polyethylenimine. *Chem. Commun. 13*, 1735−1736.
(64) Duan, H., and Nie, S. (2007) Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings. *J. Am. Chem. Soc. 129*, 3333−3338.
(65) Wolcott, A., Gerion, D., Visconte, M., Sun, J., Schwartzberg, A., Chen, S., and Zhang, J. Z. (2006) Silica-coated CdTe quantum dots functionalized with thiols for bioconjugation to IgG proteins. *J. Phys. Chem. B 110*, 5779−5789.
(66) Liu, J. Q., Song, T., Yang, Q. H., Tan, J., Huang, D. H., and Chang, J. (2013) Highly stable quantum dots with silica-poly-(EGDMA-co-MAA) synergistic protection and the preliminary application in immunoassay. *J. Mater. Chem. B 1*, 1156−1163.
(67) Huo, Q. (2007) A perspective on bioconjugated nanoparticles and quantum dots. *Colloids Surf., B 59*, 1−10.
(68) Lin, Z., Cui, S., Zhang, H., Chen, Q., Yang, B., Su, X., Zhang, J., and Jin, Q. (2003) Studies on quantum dots synthesized in aqueous solution for biological labeling via electrostatic interaction. *Anal. Biochem. 319*, 239−243.
(69) Ji, X., Zheng, J., Xu, J., Rastogi, V. K., Cheng, T. C., DeFrank, J. J., and Leblanc, R. M. (2005) (CdSe)ZnS quantum dots and organophosphorus hydrolase bioconjugate as biosensors for detection of paraoxon. *J. Phys. Chem. B 109*, 3793−3799.
(70) Goldman, E. R., Balighian, E. D., Mattoussi, H., Kuno, M. K., Mauro, J. M., Tran, P. T., and Anderson, G. P. (2002) Avidin: a natural bridge for quantum dot-antibody conjugates. *J. Am. Chem. Soc. 124*, 6378−6382.
(71) Mattoussi, H., Mauro, J. M., Goldman, E. R., Anderson, G. P., Sundar, V. C., Mikulec, F. V., and Bawendi, M. G. (2000) Self-assembly of CdSe - ZnS quantum dot bioconjugates using an engineered recombinant protein. *J. Am. Chem. Soc. 122*, 12142−12150.
(72) Goldman, E. R., Medintz, I. L., Hayhurst, A., Anderson, G. P., Mauro, J. M., Iverson, B. L., Georgiou, G., and Mattoussi, H. (2005) Self-assembled luminescent CdSe−ZnS quantum dot bioconjugates prepared using engineered poly-histidine terminated proteins. *Anal. Chim. Acta 534*, 63−67.
(73) Clapp, A. R., Goldman, E. R., and Mattoussi, H. (2006) Capping of CdSe-ZnS quantum dots with DHLA and subsequent conjugation with proteins. *Nat. Protoc. 1*, 1258−1266.
(74) Goldman, E. R., Medintz, I. L., Whitley, J. L., Hayhurst, A., Clapp, A. R., Uyeda, H. T., Deschamps, G. R., Lassman, M. E., and Mattoussi, H. (2005) A hybrid quantum dot-antibody fragment fluorescence resonance energy transfer-based TNT sensor. *J. Am. Chem. Soc. 127*, 6744−6751.
(75) Lao, U. L., Mulchandani, A., and Chen, W. (2006) Simple conjugation and purification of quantum dot - antibody complexes using a thermally responsive elastin-protein L scaffold as immuno-fluorescent agents. *J. Am. Chem. Soc. 128*, 14756−14757.
(76) Medintz, I. L., Berti, L., Pons, T., Grimes, A. F., English, D. S., Alessandrini, A., Facci, P., and Mattoussi, H. (2007) A reactive peptidic linker for self-assembling hybrid quantum dot−DNA bioconjugates. *Nano Lett. 7*, 1741−1748.
(77) Pinaud, F., King, D., Moore, H. P., and Weiss, S. (2004) Bioactivation and cell targeting of semiconductor CdSe/ZnS nanocrystals with phytochelatin-related peptides. *J. Am. Chem. Soc. 126*, 6115−6123.
(78) Mitchell, G. P., Mirkin, C. A., and Letsinger, R. L. (1999) Programmed assembly of DNA functionalized quantum dots. *J. Am. Chem. Soc. 121*, 8122−8123.
(79) Pinaud, F., King, D., Moore, H. P., and Weiss, S. (2012) Development of a quantum-dot-labelled magnetic immunoassay method for circulating colorectal cancer cell detection. *World J. Gastroenterol. 18*, 4419−4426.
(80) Meiser, F., Cortez, C., and Caruso, F. (2004) Biofunctionalization of fluorescent rare-earth-doped lanthanum phosphate colloidal nanoparticles. *Angew. Chem., Int. Ed. 43*, 5954−5957.
(81) Liu, W., Howarth, M., Greytak, A. B., Zheng, Y., Nocera, D. G., Ting, A. Y., and Bawendi, M. G. (2008) Compact biocompatible quantum dots functionalized for cellular imaging. *J. Am. Chem. Soc. 130*, 1274−1284.
(82) Deyev, S. M., Waibel, R., Lebedenko, E. N., Schubiger, A. P., and Plückthun, A. (2003) Design of multivalent complexes using the barnase-barstar module. *Nat. Biotechnol. 21*, 1486−1492.
(83) Zdobnova, T. A., Lebedenko, E. N., and Deyev, S. M. (2011) Quantum dots for molecular diagnostics of tumors. *Acta Naturae 3*, 29−47.
(84) Goldman, E. R., Balighian, E. D., Kuno, M. K., Labrenz, S., Anderson, G. P., Mauro, J. M., and Mattoussi, H. (2002) Luminescent quantum dot-adaptor protein-antibody conjugates for use in fluoroimmunoassays. *Phys. Status Solidi B 229*, 407−414.
(85) Tran, P. T., Goldman, E. R., Anderson, G. P., and Mauro, J. M. (2002) Use of luminescent CdSe − ZnS nanocrystal bioconjugates in quantum dot-based nanosensors. *Phys. Status Solidi 432*, 427−432.
(86) Montenegro, J. M., Grazu, V., Sukhanova, A., Agarwal, S., Fuente, J. M., Nabiev, I., Greiner, A., and Parak, W. J. (2013) Controlled antibody/(bio-) conjugation of inorganic nanoparticles for targeted delivery. *Adv. Drug Delivery Rev. 65*, 677−688.
(87) Goldman, E. R., Anderson, G. P., Tran, P. T., Mattoussi, H., Charles, P. T., and Mauro, J. M. (2002) Conjugation of luminescent quantum dots with antibodies using an engineered adaptor protein to provide new reagents for fluoroimmunoassays. *Anal. Chem. 74*, 841−847.
(88) Hermanson, G. T. (2008) *Bioconjugate Techniques*, 2nd ed., Elsevier Inc.
(89) Algar, W. R., and Krull, U. J. (2006) Adsorption and hybridization of oligonucleotides on mercaptoacetic acid-capped CdSe/ZnS quantum dots and quantum dot-oligonucleotide conjugates. *Langmuir 22*, 11346−11352.
(90) Pathak, S., Davidson, M. C., and Silva, G. (2007) Characterization of the functional binding properties of antibody conjugated quantum dots. *Nano Lett. 7*, 1839−1845.
(91) Mahmoud, W., Rousserie, G., Reveil, B., Tabary, T., Millot, J. M., Artemyev, M., Oleinikov, V., Cohen, J. H. M., Nabiev, I., and Sukhanova, A. (2011) Advanced procedures for labeling of antibodies with quantum dots. *Anal. Biochem. 416*, 180−185.
(92) Byoce, M., and Bertozzi, C. R. (2011) Bringing chemistry to life. *Nat. Methods 8*, 638−642.
(93) Son, D. H., Hughes, S. M., Yin, Y., and Alivisatos, P. (2004) Cation exchange reactions in ionic nanocrystals. *Science 306*, 1009−1012.
(94) Binder, W. H., Sachsenhofer, R., Straif, C. J., and Zirbs, R. (2007) Surface-modified nanoparticles via thermal and Cu(I)-mediated "click" chemistry: generation of luminescent CdSe nanoparticles with polar ligands guiding supramolecular recognition. *J. Mater. Chem. 17*, 2125−2132.
(95) Lallana, E., Sousa-Herves, A., Fernandez-Trillo, F., Riguera, R., and Fernandez-Megia, E. (2012) Click chemistry for drug delivery nanosystems. *Pharmacol. Res. 29*, 1−34.
(96) Algar, W. R., Prasuhn, D. E., Stewart, M. H., Jennings, T. L., Blanco-Canosa, J. B., Dawson, P. E., and Medintz, I. L. (2011) The controlled display of biomolecules on nanoparticles: a challenge suited to bioorthogonal chemistry. *Bioconjugate Chem. 22*, 825−858.
(97) Zhang, H., Feng, G., Guo, Y., and Zhou, D. (2013) Robust and specific ratiometric biosensing using a copper-free clicked quantum dot-DNA aptamer sensor. *Nanoscale 5*, 10307−10315.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

(98) Kotagiri, N., Li, Z., Xu, X., Mondal, S., Nehorai, A., and Achilefu, S. (2014) Antibody quantum dot conjugates developed via copper-free click chemistry for rapid analysis of biological samples using a microfluidic microsphere array system. *Bioconjugate Chem. 25*, 1272−1281.
(99) Bernardin, A., Cazet, A., Guyon, L., Delannoy, P., Vinet, F., Bonnaffé, D., and Texier, I. (2010) Copper-free click chemistry for highly luminescent quantum dot conjugates: application to *in vivo* metabolic imaging. *Bioconjugate Chem. 21*, 583−588.
(100) Schieber, C., Bestetti, A., Lim, J. P., Ryan, A. D., Nguyen, T. L., Eldridge, R., White, A. R., Gleeson, P. A., Donnelly, P. S., Williams, S. J., et al. (2012) Conjugation of transferrin to azide-modified CdSe/ZnS core-shell quantum dots using cyclooctyne click chemistry. *Angew. Chem., Int. Ed. 51*, 10523−10527.
(101) Han, H. S., Devaraj, N. K., Lee, J., Hilderbrand, S., Weissleder, R., and Bawendi, M. G. (2010) Development of a bioorthogonal and highly efficient conjugation method for quantum dots using tetrazine-norbornene cycloaddition. *J. Am. Chem. Soc. 132*, 7838−1839.
(102) Wilson, R., Chen, Y., and Aveyard, J. (2004) One molecule per particle method for functionalising nanoparticles. *Chem. Commun. (Cambridge) 10*, 1156−1157.
(103) Bokern, S., Gries, K., Agarwal, S., Görtz, H.-H., Warzelhan, V., and Greiner, A. (2011) Precisely designed gold nanoparticles by surface polymerization — artificial molecules as building blocks for novel materials. *Adv. Funct. Mater. 21*, 3753−3759.
(104) Levy, R., Wang, Z. X., Duchesne, L., Doty, R. C., Cooper, A. I., Brust, M., and Fernig, D. G. (2006) A generic approach to monofunctionalized protein-like gold nanoparticles based on immobilized metal ion affinity chromatography. *ChemBioChem 7*, 592−594.
(105) Sperling, R. A., Pellegrino, T., Li, J. K., Chang, W. H., and Parak, W. J. (2006) Electrophoretic separation of nanoparticles with a discrete number of functional groups. *Adv. Funct. Mater. 16*, 943−948.
(106) Pellegrino, T., Sperling, R. A., Alivisatos, A. P., and Parak, W. J. (2007) Gelelectrophoresis of Gold-DNA Nanoconjugates. *J. Biomed. Biotechnol. 2007*, 26796.
(107) Lin, C. A., Sperling, R. A., Li, J. K., Yang, T. Y., Li, P. Y., Zanella, M., Chang, W. H., and Parak, W. J. (2008) Design of an amphiphilic polymer for nanoparticle coating and functionalization. *Small 4*, 334−341.
(108) Ye, L., Yong, K. T., Liu, L., Roy, I., Hu, R., Zhu, J., Cai, H., Law, W. C., Liu, J., Wang, K., et al. (2012) A pilot study in non-human primates shows no adverse response to intravenous injection of quantum dots. *Nat. Nanotechnol. 7*, 453−458.
(109) Erogbogbo, F., Yong, K. T., Roy, I., Hu, R., Law, W. C., Zhao, W., Ding, H., Wu, F., Kumar, R., Swihart, M. T., et al. (2011) In vivo targeted cancer imaging, sentinel lymph node mapping and multi-channel imaging with biocompatible silicon nanocrystals. *ACS Nano 5*, 413−423.
(110) Liu, J., Erogbogbo, F., Yong, K. T., Ye, L., Liu, J., Hu, R., Chen, H., Hu, Y., Yang, Y., Yang, J., et al. (2013) Assessing clinical prospects of silicon quantum dots: studies in mice and monkeys. *ACS Nano 7*, 7303−7310.
(111) Hardman, R. (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect. 114*, 165−172.
(112) Wu, X., Tian, F., Zhao, J. X., and Wu, M. (2013) Evaluating pharmacokinetics and toxicity of luminescent quantum dots. *Expert Opin. Drug Metab. Toxicol. 9*, 1265−1277.
(113) Yong, K. T., Law, W. C., Hu, R., Ye, L., Liu, L., Swihart, M. T., and Prasad, P. N. (2013) Nanotoxicity assessment of quantum dots: from cellular to primate studies. *Chem. Soc. Rev. 42*, 1236−1250.
(114) Hoshino, A., Hanada, S., and Yamamoto, K. (2011) Toxicity of nanocrystal quantum dots: the relevance of surface modifications. *Arch. Toxicol. 85*, 707−720.
(115) Ghaderi, S., Ramesh, B., and Seifalian, A. M. (2011) Fluorescence nanoparticles "quantum dots" as drug delivery system and their toxicity: a review. *J. Drug Targeting 19*, 475−486.
(116) Yang, S. T., Wang, X., Wang, H., Lu, F., Luo, P. G., Cao, L., Meziani, M. J., Liu, J. H., Liu, Y., Chen, M., et al. (2009) Carbon dots as nontoxic and high-performance fluorescence imaging agents. *J. Phys. Chem. C 113*, 18110−18114.
(117) Pons, T., Pic, E., Lequeux, N., Cassette, E., Bezdetnaya, L., Guillemin, F., Marchal, F., and Dubertret, B. (2010) Cadmium-free $CuInS_2$/ZnS quantum dots for sentinel lymph node imaging with reduced toxicity. *ACS Nano 4*, 2531−2538.
(118) Maity, A. R., Palmal, S., Basiruddin, S. K., Karan, N. S., Sarkar, S., Pradhan, N., and Jana, N. R. (2013) Doped semiconductor nanocrystal based fluorescent cellular imaging probes. *Nanoscale 5*, 5506−5513.
(119) Bruchez, M., Jr., Moronne, M., Gin, P., Weiss, S., and Alivisatos, A. P. (1998) Semiconductor nanocrystals as fluorescent biological labels. *Science 281*, 2013−2016.
(120) Ag, D., Bongartz, R., Dogan, L. E., Seleci, M., Walter, J. G., Demirkol, D. O., Stahl, F., Ozcelik, S., Timur, S., and Scheper, T. (2014) Biofunctional quantum dots as fluorescence probe for cell-specific targeting. *Colloids Surf., B 114*, 96−103.
(121) Rakovich, T. Y., Mahfoud, O. K., Mohamed, B. M., Prina-Mello, A., Crosbie-Staunton, K., Van Den Broeck, T., De Kimpe, L., Sukhanova, A., Baty, D., Rakovich, A., et al. (2014) Highly sensitive single domain antibody-quantum dot conjugates for detection of HER2 biomarker in lung and breast cancer cells. *ACS Nano 8*, 5682−5695.
(122) Liu, X. L., Peng, C. W., Chen, C., Yang, X. Q., Hu, M. B., Xia, H. S., Liu, S. P., Pang, D. W., and Li, Y. (2011) Quantum dots-based double-color imaging of HER2 positive breast cancer invasion. *Biochem. Biophys. Res. Commun. 409*, 577−582.
(123) Zrazhevskiy, P., and Gao, X. (2013) Quantum dot imaging platform for single-cell molecular profiling. *Nat. Commun. 4*, 1619.
(124) Liu, J., Lau, S. K., Varma, V. A., Moffitt, R. A., Caldwell, M., Liu, T., Young, A. N., Petros, J. A., Osunkoya, A. O., Krogstad, T., et al. (2010) Molecular mapping of tumor heterogeneity on clinical tissue specimens with multiplexed quantum dots. *ACS Nano 4*, 2755−2765.
(125) Liu, J., Lau, S. K., Varma, V. A., Kairdolf, B. A., and Nie, S. (2010) Multiplexed detection and characterization of rare tumor cells in Hodgkin's lymphoma with multicolor quantum dots. *Anal. Chem. 82*, 6237−6243.
(126) Zdobnova, T. A., Stremovskiy, O. A., Lebedenko, E. N., and Deyev, S. M. (2012) Self-assembling complexes of quantum dots and scFv antibodies for cancer cell targeting and imaging. *PLoS One 7*, e48248.
(127) Nikitin, M. P., Zdobnova, T. A., Lukash, S. V., Stremovskiy, O. A., and Deyev, S. M. (2010) Protein-assisted self-assembly of multifunctional nanoparticles. *Proc. Natl. Acad. Sci. U.S.A. 107*, 5827−5832.
(128) Xu, W., Liu, L., Brown, N. J., Christian, S., and Hornby, D. (2012) Quantum dot-conjugated anti-GRP78 scFv inhibits cancer growth in mice. *Molecules 17*, 796−808.
(129) Zaman, M. B., Baral, T. N., Jakubek, Z. J., Zhang, J., Wu, X., Lai, E., Whitfield, D., and Yu, K. (2011) Single-domain Ab bioconjugated near-IR quantum dots for targeted cellular imaging of pancreatic cancer. *J. Nanosci. Nanotechnol. 11*, 3757−3763.
(130) Anderson, G. P., Glaven, R. H., Algar, W. R., Susumu, K., Stewart, M. H., Medintz, I. L., and Goldman, E. R. (2013) Single domain Ab-quantum dot conjugates for ricin detection by both fluoroimmunoassay and surface plasmon resonance. *Anal. Chim. Acta 786*, 132−138.
(131) Hafian, H., Sukhanova, A., Turini, M., Chames, P., Baty, D., Pluot, M., Cohen, J. H., Nabiev, I., and Millot, J. M. (2014) Multiphoton imaging of tumor biomarkers with conjugates of single-domain antibodies and quantum dots. *Nanomed. Nanotechnol. 10*, 1701−1709.
(132) Courty, S., and Dahan, M. (2013) Tracking individual membrane proteins using quantum dots. *Cold Spring Harbor Protocols 2013* (10), 925−927.
(133) Courty, S., and Dahan, M. (2013) Tracking individual intracellular proteins using quantum dots. *Cold Spring Harbor Protocols 2013* (11) DOI: 10.1101/pdb.prot078238.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624

(134) Chen, H., Titushkin, I., Stroscio, M., and Cho, M. (2007) Altered membrane dynamics of quantum dot-conjugated integrins during osteogenic differentiation of human bone marrow derived progenitor cells. *Biophys. J. 92*, 1399−1408.
(135) Bruchez, M. P. (2011) Quantum dots find their stride in single molecule tracking. *Curr. Opin. Chem. Biol. 15*, 775−780.
(136) Clausen, M. P., Arnspang, E. C., Ballou, B., Bear, J. E., and Lagerholm, B. C. (2014) Simultaneous multi-species tracking in live cells with quantum dot conjugates. *PLoS One 9*, e97671.
(137) Vermehren-Schmaedick, A., Krueger, W., Jacob, T., Ramunno-Johnson, D., Balkowiec, A., Lidke, K. A., and Vu, T. Q. (2014) Heterogeneous intracellular trafficking dynamics of brain-derived neurotrophic factor complexes in the neuronal soma revealed by single quantum dot tracking. *PLoS One 9*, e95113.
(138) Copolovici, D. M., Langel, K., Eriste, E., and Langel, Ü. (2014) Cell-penetrating peptides: design, synthesis, and applications. *ACS Nano 8*, 1972−1994.
(139) Liu, B. R., Li, J. F., Lu, S. W., Leel, H. J., Huang, Y. W., Shannon, K. B., and Aronstam, R. S. (2010) Cellular internalization of quantum dots noncovalently conjugated with arginine-rich cell-penetrating peptides. *J. Nanosci. Nanotechnol. 10*, 6534−6543.
(140) Liu, B. R., Huang, Y. W., Winiarz, J. G., Chiang, H. J., and Lee, H. J. (2011) Intracellular delivery of quantum dots mediated by a histidine- and arginine-rich HR9 cell-penetrating peptide through the direct membrane translocation mechanism. *Biomaterials 32*, 3520−3537.
(141) Tada, H., Higuchi, H., Wanatabe, T. M., and Ohuchi, N. (2007) In vivo real-time tracking of single quantum dots conjugated with monoclonal anti-HER2 antibody in tumors of mice. *Cancer Res. 67*, 1138−1144.
(142) Rizvi, S. B., Rouhi, S., Taniguchi, S., Yang, S. Y., Green, M., Keshtgar, M., and Seifalian, A. M. (2014) Near-infrared quantum dots for HER2 localization and imaging of cancer cells. *Int. J. Nanomed. 9*, 1323−1337.
(143) Yang, K., Zhang, F. J., Tang, H., Zhao, C., Cao, Y. A., Lv, X. Q., Chen, D., and Li, Y. D. (2011) In-vivo imaging of oral squamous cell carcinoma by EGFR monoclonal antibody conjugated near-infrared quantum dots in mice. *Int. J. Nanomed. 6*, 1739−1745.
(144) Gao, J., Chen, K., Xie, R., Xie, J., Yan, Y., Cheng, Z., Peng, X., and Chen, X. (2010) In vivo tumor-targeted fluorescence imaging using near-infrared non-cadmium quantum dots. *Bioconjugate Chem. 21*, 604−609.
(145) Li, Y., Li, Z., Wang, X., Liu, F., Cheng, Y., Zhang, B., and Shi, D. (2012) In vivo cancer targeting and imaging-guided surgery with near infrared-emitting quantum dot bioconjugates. *Theranostics 2*, 769−776.
(146) Chen, H., Li, L., Cui, S., Mahounga, D., Zhang, J., and Gu, Y. (2011) Folate conjugated CdHgTe quantum dots with high targeting affinity and sensitivity for *in vivo* early tumor diagnosis. *J. Fluoresc. 2*, 793−801.
(147) Larson, D. R., Warren, R. Z., William, R. M., Clark, S. W., Bruchez, M. P., Wise, F. W., and Webb, W. W. (2003) Water soluble quantum dots for multiphoton fluorescence imaging *in vivo*. *Science 300*, 1434−1436.
(148) Sun, C., Cao, Z., Wu, M., and Lu, C. (2014) Intracellular tracking of single native molecules with electroporation-delivered quantum dots. *Anal. Chem. 86*, 11403−11409.
(149) Delehanty, J. B., Bradburne, C. E., Susumu, K., Boeneman, K., Mei, B. C., Farrell, D., Blanco-Canosa, J. B., Dawson, P. E., Mattoussi, H., and Medintz, I. L. (2011) Spatiotemporal multicolor labeling of individual cells using peptide-functionalized quantum dots and mixed delivery techniques. *J. Am. Chem. Soc. 133*, 10482.
(150) Emerson, N. T., Hsia, C.-H., Rafalska-Metcalf, I. U., and Yang, H. (2014) Mechanodelivery of nanoparticles to the cytoplasm of living cells. *Nanoscale 6*, 4538−4543.
(151) Breger, J., Delehanty, J. B., and Medintz, I. L. (2015) Continuing progress toward controlled intracellular delivery of semiconductor quantum dots. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 7*, 131−151.
(152) Duan, H., and Nie, S. (2007) Cell-penetrating quantum dots based on multivalent and endosome-disrupting surface coatings. *J. Am. Chem. Soc. 129*, 3333−3338.
(153) Koshman, Y. E., Waters, S. B., Walker, L. A., Los, T., de Tombe, P., Goldspink, P. H., and Russell, B. (2008) Delivery and visualization of proteins conjugated to quantum dots in cardiac myocytes. *J. Mol. Cell Cardiol. 45*, 853−856.
(154) Erazo-Oliveras, A., Muthukrishnan, N., Baker, R., Wang, T. Y., and Pellois, J. P. (2012) Improving the endosomal escape of cell-penetrating peptides and their cargos: strategies and challenges. *Pharmaceuticals (Basel) 5*, 1177−1209.
(155) Ishihama, Y., and Funatsu, T. (2009) Single molecule tracking of quantum dot-labeled mRNAs in a cell nucleus. *Biochem. Biophys. Res. Commun. 381*, 33−8.
(156) Zhang, C., Ji, X., Zhang, Y., Zhou, G., Ke, X., Wang, H., Tinnefeld, P., and He, Z. (2013) One-pot synthesized aptamer-functionalized CdTe:Zn^{2+} quantum dots for tumor-targeted fluorescence imaging *in vitro* and *in vivo*. *Anal. Chem. 85*, 5843−5849.
(157) Srinivasan, C., Lee, J., Papadimitrakopoulos, F., Silbart, L. K., Zhao, M., and Burgess, D. J. (2006) Labeling and intracellular tracking of functionally active plasmid DNA with semiconductor quantum dots. *Mol. Ther. 14*, 192−201.
(158) Kang, W. J., Chae, J. R., Cho, Y. L., Lee, J. D., and Kim, S. (2009) Multiplex imaging of single tumor cells using quantum-dot-conjugated aptamers. *Small 5*, 2519−2522.
(159) Chang, M., Kwon, M., Kim, S., Yunn, N. O., Kim, D., Ryu, S. H., and Lee, J. B. (2014) Aptamer-based single-molecule imaging of insulin receptors in living cells. *J. Biomed. Opt. 19*, 051204.
(160) Li, J., Zhang, P., Yang, X., Wang, K., Guo, Q., Huang, J., and Li, W. (2014) Aptamer-mediated indirect quantum dot labeling and fluorescent imaging of target proteins in living cells. *Nanotechnology 25*, 505502.
(161) Chen, A. A., Derfus, A. M., Khetani, S. R., and Bhatia, S. N. (2005) Quantum dots to monitor RNAi delivery and improve gene silencing. *Nucleic Acids Res. 2005* (33), e190.
(162) Jung, J., Solanki, A., Memoli, K. A., Kamei, K., Kim, H., Drahl, M. A., Williams, L. J., Tseng, H. R., and Lee, K. (2010) Selective inhibition of human brain tumor cells through multifunctional quantum-dot-based siRNA delivery. *Angew. Chem., Int. Ed. 49*, 103−107.
(163) Bonoiu, A., Mahajan, S. D., Ye, L., Kumar, R., Ding, H., Yong, K. T., Roy, I., Aalinkeel, R., Nair, B., Reynolds, J. L., et al. (2009) MMP-9 gene silencing by a quantum dot-siRNA nanoplex delivery to maintain the integrity of the blood brain barrier. *Brain Res. 1282*, 142−155.
(164) Lee, H., Kim, I. K., and Park, T. G. (2010) Intracellular trafficking and unpacking of siRNA/quantum dot-PEI complexes modified with and without cell penetrating peptide: confocal and flow cytometric FRET analysis. *Bioconjugate Chem. 21*, 289−295.
(165) Zhao, J., Qiu, X., Wang, Z., Pan, J., Chen, J., and Han, J. (2013) Application of quantum dots as vectors in targeted survivin gene siRNA delivery. *OncoTargets and Therapy 6*, 303−309.
(166) Li, J. M., Zhao, M. X., Su, H., Wang, Y. Y., Tan, C. P., Ji, L. N., and Mao, Z. W. (2011) Multifunctional quantum-dot-based siRNA delivery for HPV18 E6 gene silence and intracellular imaging. *Biomaterials 32*, 7978−7987.
(167) Yezhelyev, M. V., Qi, L., O'Regan, R. M., Nie, S., and Gao, X. (2008) Proton-sponge coated quantum dots for siRNA delivery and intracellular imaging. *J. Am. Chem. Soc. 130*, 9006−9012.
(168) Derfus, A. M., Chen, A. A., Min, D. H., Ruoslahti, E., and Bhatia, S. N. (2007) Targeted quantum dot conjugates for siRNA delivery. *Bioconjugate Chem. 18*, 1391−1396.
(169) Zhang, M. Z., Yu, Y., Yu, R. N., Wan, M., Zhang, R. Y., and Zhao, Y. D. (2013) 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 9*, 4183−4193.

DOI: 10.1021/acs.bioconjchem.5b00069
Bioconjugate Chem. 2015, 26, 609−624