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Modification of quantum dots with nucleic acids

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Contents

I.	Introduction	1209
II.	Synthesis of quantum dots	1210
III.	Conjugates of quantum dots and nucleic acids	1215
IV.	Conclusion	1220

Abstract. The key principles and modern approaches to targeted modification of semiconductor colloidal nanoparticles, quantum dots, which exhibit unique photophysical properties and are a promising class of luminescent markers, are discussed. Attention is given to the preparation of their bioconjugates with nucleic acids, promising tools for biological microchips and resonance energy transfer sensors. The bibliography includes 80 references.

I. Introduction

The persistent interest in the development of new methods for the synthesis of nanomaterials and structures based on them, which has continuously increased during the last 10 years, is caused by the unique prospects for the use of these objects in various fields of modern science such as photonics, catalysis, electronics, information technology and medicine.[1] Biological applications[2] of nanomaterials seem to be most promising, because this could soon lead to improvement of the existing approaches to the diagnosis and treatment of various diseases.

Luminescent markers are widely used in nanobiotechnology and nanodiagnostics to visualize cells and tissues, to qualitatively and quantitatively determine biomacromolecules and to track the course of biological processes.[1–4] Traditionally, organic dyes, fluorescent proteins and polymers serve as luminophores.[1] However, in some cases, these systems have relatively low chemical and photochemical stability, which results in inevitable limitations and decreases the efficiency of diagnostic procedures.[1–8]

Rather recently,[4] it has been proposed to use luminescent semiconductor nanocrystals, quantum dots (QD), as luminescent labels for bioanalysis. These isolated nanoobjects the properties of which differ considerably from the properties of a bulk material of the same composition have unique optical characteristics. The perfection of biological analysis techniques is associated first of all with the following advantages of quantum dots[3,7] over organic luminophores:

— high photostability,
— broad excitation and absorption bands,
— narrow peak and high brightness of luminescence,
— large surface area,
— change in the optical properties (positions of the absorption and emission maxima) upon the variation of the nanoparticle composition, size and shape,
— the possibility of the chemical modification of their surface in order to prepare nanoparticles capable of binding to various biomolecules.

These factors[1–6] make quantum dots promising objects for the use in multiplex analysis,[4,7,8] in particular, in matrix biochips, which is a key line of development of nanobiodiagnostics.

Currently, approaches to the synthesis of quantum dots and heterostructures based on them,[9–24] which allow the control of key characteristics of QD such as the size, the shape, the composition, surface functionality, the solubility of polar or non-polar media, chemical and photochemical stability, biocompatibility, are extensively developed. The typical hydrodynamic radius of the QD usually varies from 3 to 50 nm depending on the size of the inorganic core and the shell (for 'core – shell'-type structures), the nanoparticle shape and the stabilizer type. Unlike fluorescent dyes, which can 'mark' only one biomolecule, one quantum dot can bear up to several tens of biological macromolecules on the surface due to the large surface area to volume ratio.[2]

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However, a major drawback of QD, which prevents their active use in biological analysis, is high toxicity. As a rule, quantum dots contain heavy elements such as Cd, Hg, Pb and so on. In addition, changes in the functional properties of biomolecules after they had been added to QD were detected. Conjugation may, for example, increase the hybridization time of nucleic acids or decrease the activity of antibodies.[3]

In recent years, some methods for surface functionalization of quantum dots to increase their biocompatibility have been developed.[25] In addition, partial or full replacement and/or modification of the surface stabilizers in order to form multifunctional nanoparticles suitable for bioconjugation are possible.[1,2] These multifuctional QD may react with various sorts of biomolecules with high specificity. Theoretically, one quantum dot can be functionalized with various oligonucleotides that represent specific analytical probes for various nucleotide sequences.[2] The conjugates of luminescent QD and nucleic acids are promising materials for DNA diagnostics using optical spectroscopy. Despite the large number of publications[1–4,6–8] on the use of quantum dots and other nanoparticles in biological analysis, the knowledge of QD conjugates with nucleic acids has not been described systematically as yet.

This review is devoted to important up-to-date approaches to immobilization of nucleic acids on quantum dots. Here we present methods for surface modification of semiconductor nanoparticles for biological applications,[25] methods for the preparation of conjugates (using specific linkers, proteins,[26,27] chemisorption[26,28] or electrostatic interactions[29]). Examples of application of these complexes as DNA sensors are given.[2–5,7,8,30–32] The attention is focused on the aspects of conjugate stability and stoichiometry (molar ratio of the biomolecules added and nanoparticles), new approaches to the use of polydentate addition of nucleic acids to QD and its influence on the stability of the resulting complexes and, hence, on their potential attraction for biodiagnostics.

II. Synthesis of quantum dots

1. Preparation of quantum dots by colloid chemistry techniques

The synthesis of nanoparticles of cadmium chalcogenides by traditional approaches of colloid chemistry have been considered in the literature rather comprehensively.[10,13–24] Decomposition of organometallic precursors in a hot coordinating solvent (120–360 °C) is among the most efficient methods as regards the QD yield, final optical characteristics and monodispersity.[10,13–21] This method has been successfully used for the preparation of semiconductor nanoparticles of a specified size (in the 1–10 nm range) coated by surfactants. The latter ensure the electronic and chemical passivation of the surface dangling bonds and prevent the uncontrolled growth and agglomeration of nanoparticles. According to the basic views on the nucleation and particle growth described by La Mer and Dinegar,[33] this approach includes several stages separated in time. The first stage is nucleation at high supersaturations in the system; this is followed by termination of nanoparticle growth, which is the second stage, and the final (slowest) stage is the Ostwald ripening.

Apart from the approaches based on high-temperature decomposition of the precursors in a coordinating solvent, various preparation routes of quantum dots at lower temperature were also reported. These routes include syntheses with bifunctional short-chain compounds (for example, thiols) used as stabilizing agents; this gives colloids stable in aqueous medium.[22–24] The low-temperature methods are applicable only to the synthesis of materials with ionic bonds, for example, $A^{II}B^{IV}$-type semiconductors, while $A^{III}B^{V}$-type semiconductors cannot be obtained in this way.[10]

Precursors of water-soluble QD are usually formed upon the reaction of metal ions (Zn^{2+}, Cd^{2+}, Hg^{2+}) with gaseous H_2X (X = Te, Se, S) in aqueous solution under nitrogen in the presence of thiol as a stabilizer.[23] The presence of the chalcogenide initiates the nucleation with initially broad particle size distribution. The synthesis of QD from the precursor obtained in the first stage is most often performed by gentle heat treatment at the solvent boiling point[22,23] or by hydrothermal method,[23,34] in particular, using microwave radiation.[24] The growth and ageing of such nanoparticles occurs over several days, which is reflected as the red shift of the luminescence spectra. In the case of mercury telluride, the formation of nanoparticles is observed even at room temperature.[23] The nanoparticles thus formed are composed of an inorganic core surrounded by the shell of thiol molecules. The latter ensure the stability of the colloidal solution owing to the presence of a sulfur atom, which passivates the surface metal atoms, and a chemical function owing to the presence of functional groups, which are usually located in the aliphatic chain of the thiol.

Currently, thioglycolic acid ($HSCH_2CO_2H$, TGA) or 3-mercaptopropionic acid ($HSCH_2CH_2CO_2H$, MPA) are used most often as stabilizers in the synthesis of CdTe nanoparticles; this gives colloids stable for many months and showing high luminescence quantum yields.[22] Another study reports[23] the preparation of quantum dots with other thiols as stabilizing ligands such as mercaptoethanol [$HS(CH_2)_2OH$], 1-thioglycerol [$HSCH_2CH(OH)CH_2OH$], cysteamine (2-aminoethanethiol, $HSCH_2CH_2NH_2$), 2-dimethylaminoethanethiol ($HSCH_2CH_2NMe_2$), 2-mercaptopropionic acid [$MeCH(SH)CO_2H$], L-cysteine, glutathione, thiopronine, mercaptosuccinic acid, *etc*.

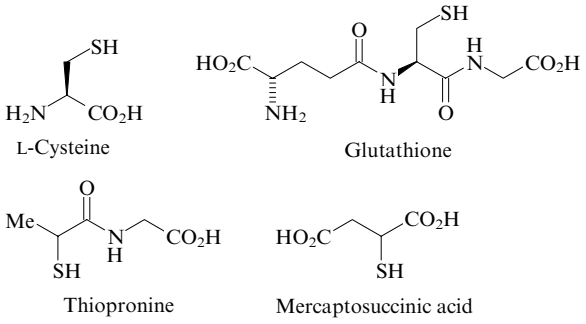


Figure 1 shows a typical example of water-soluble thioglycolic acid-stabilized CdTe QD synthesized under mild conditions. These particles have high degrees of crystallinity and narrow size distribution, which is demonstrated by the transmission electron microscopy (TEM) images.

An advantage of the above-described methods is also rather easy variation of the nanoparticle size. The average particle size and size distribution can be controlled both during the reaction and during the subsequent (post-preparative) treatment.[16] The nucleation time and temperature

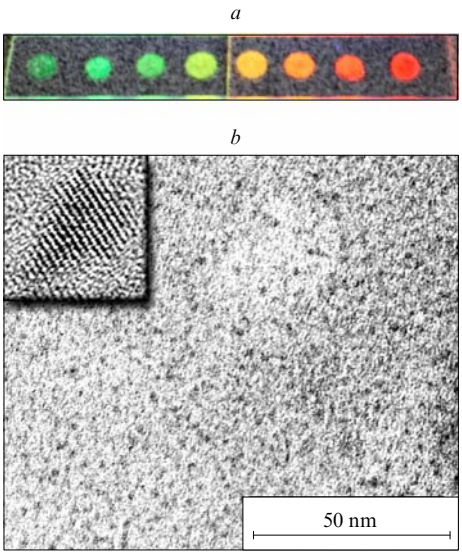


Figure 1. Typical view of chalcogenide quantum dots (our results). (*a*) Optical microscopic image of luminescence of CdTe quantum dots stabilized by thioglycolic acid and synthesized for 5, 10 and 30 min and for 1, 1.5, 3, 10 and 13 h (mercury lamp excitation at 310 nm); (*b*) TEM image of the CdTe quantum dots synthesized for 13 h. Inset: high-resolution TEM image of a single CdTe quantum dot (Titan^{TM} 80–300 transmission electron microscope, National Research Centre 'Kurchatov Institute').

are the key parameters affecting the size of nanocystals.[10] In particular, Figure 2 shows the particle size plotted *vs*. the time of synthesis for the CdTe quantum dots stabilized by thioglycolic acid. The particle size was determined by optical spectroscopy and by statistical processing of the high-resolution TEM images. These curves represent an example of the important calibration dependence that is suitable for determining the time needed for the synthesis of nanocrystals of the desired size and, hence, with the desired optical characteristics.[35]

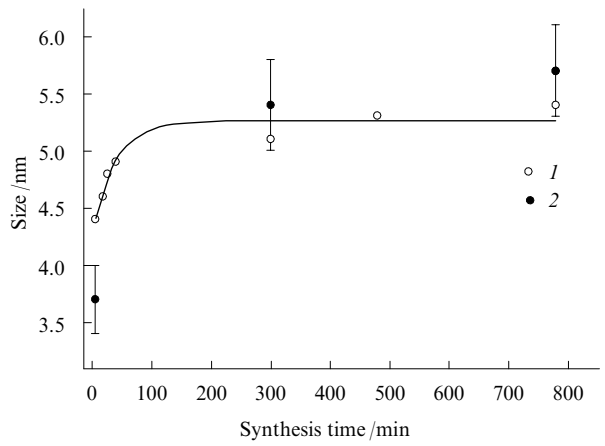


Figure 2. Size of quantum dots *vs*. time of synthesis (our data). (*1*) Optical spectroscopy data, (*2*) results of statistical processing of TEM images.

2. Purification of quantum dots

A major problem of the practical application of QD is the need to develop efficient procedures for QD purification from excess reactants and stabilizers, which can be toxic, decrease the luminescence quantum yield or induce fast chemical or photochemical degradation of the nanoparticles. Currently the size-selective precipitation is the most widely used method for the purification of quantum dots and preparation of sols with a narrow size distribution.[16] This procedure comprises slow portionwise addition of a salting-out solvent with a different polarity to a nanoparticle sol; this results in partial precipitation of QD, most often as flakes composed of aggregates. The precipitated nanoparticles can be again dissolved in a pure solvent to give sols free from low-molecular-mass impurities. By selecting the precipitation conditions in such a way that larger nanoparticles would be the first to precipitate and smaller particles would remain as a stable suspension in the solution, it is possible to separate nanoparticles according to their size to obtain fractions with a very narrow distribution. However, this approach is based on destabilization of QD sols, and this leads to washing-out of the stabilizer away from their surface and, as a consequence, to QD coagulation, oxidation, *etc*. The size-selective precipitation of the semiconductor nanoparticles results most often in a considerable decrease in the luminescence quantum yield and decrease in their colloidal stability in solution.

An alternative method proposed quite recently (in particular, by the authors of this review) for purification of quantum dots is dialysis. During dialysis, the molecules of the low-molecular-mass solute pass through a membrane, while larger colloidal particles remain in the initial sol. The concentrations of the diffusing substance in the dialysis fluid and in the solvent gradually become equal. By repeatedly replacing the solvent, the sol can be purified almost completely from the undesired impurities.

Today dialysis is one of the most mild and efficient methods for purification of quantum dots from low-molecular-mass impurities. A more than fourfold increase in the quantum yield of QD luminescence without a change in the position of the emission maximum was observed [35, 36] after dialysis. The luminescence quantum yield of semiconductor nanocrystals is known to be highly dependent on the pH and the presence of admixed ions in the suspension. An increase in the quantum yield for thioglycolic acid-stabilized CdTe nanoparticles to 18% upon acidification of the solution to pH 4.7 was reported.[36] This effect was attributed to two factors: first, a thin passivating film of a cadmium thiol complex may form on the QD surface and serve similarly to a broadband CdS or ZnS shell; and second, thioglycolic acid adsorbed on the QD surface in an acid medium may favour elimination of surface defects and, hence, increase the lifetime of the excited state.

To confirm this assumption, Kocherginskaya and Itkis [37] studied the luminescence decay kinetics of quantum dots of various diameters before and after the dialysis. For all samples, two-component temporal dependences were obtained with characteristic lifetimes of charge carriers of 70–100 ps and 1.5–9.5 ns. The investigation disclosed the effect of dialysis on the 'slower' component: purification resulted in a twofold increase in the lifetime of nanoparticles and a decrease in their contribution to the total fluorescence of the 'fast' component. The increase in the lifetime of the excited state attests to a decrease in the number of charge

Table 1. Luminescence properties of cadmium telluride quantum dots.

Size /nm	Quantum yield (%)		Lifetime /ns		Fluorescence contribution (%)	
	before dialysis	after dialysis	before dialysis	after dialysis	before dialysis	after dialysis
4.0(5)	7	17	2.78	5.70	94	99
4.6(5)	14	23	4.67	6.95	97	99
5.2(6)	17	35	5.62	9.22	98	99
5.4(7)	15	33	6.78	9.24	98	99

Note. The lifetimes and fluorescence contribution are given for the 'slower' component.

carrier recombination sites and, hence, can serve as evidence for the hypothesis of decrease in the degree of nanocrystal imperfection.

The corresponding luminescence quantum yields and luminescence lifetimes for thioglycolic acid-stabilized quantum dots of various sizes before and after dialysis are presented in Table 1 (authors' unpublished results). Partial removal of the stabilizer from the quantum dot surface followed by spontaneous assembly of ribbon-like one-dimensional structures can be regarded as a side effect of the purification (Fig. 3).

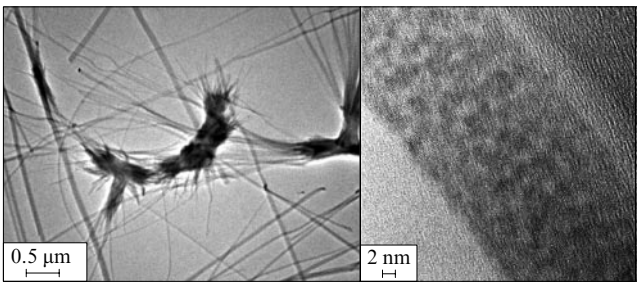


Figure 3. Formation of nanoribbons from CdTe quantum dots stabilized by thioglycolic acid after dialysis (TEM images on different scales).[38]

3. Determination of the concentration of quantum dots

Biomedical applications require certification and standardization of the samples used, which in the case of QD, implies not only the necessity to determine the composition and to study the stability and optical properties but also to determine their concentration. The concentration of QD can be estimated from known nanoparticle size and total concentrations of the elements that compose the nanoparticles. However, it is much more reasonable to determine the QD concentration from the optical characteristics exhibited by the sol.

Peng and co-workers[39] proposed using the calibration plots of the extinction coefficient of the cadmium chalcogenide QD stabilized by phosphine-containing long-chain organic ligands *versus* their size (Fig. 4, curve *1*). In this case, the molar concentration of nanoparticles is determined from the Bouguer – Lambert – Beer law:

$$A = \varepsilon \cdot C \cdot l,$$

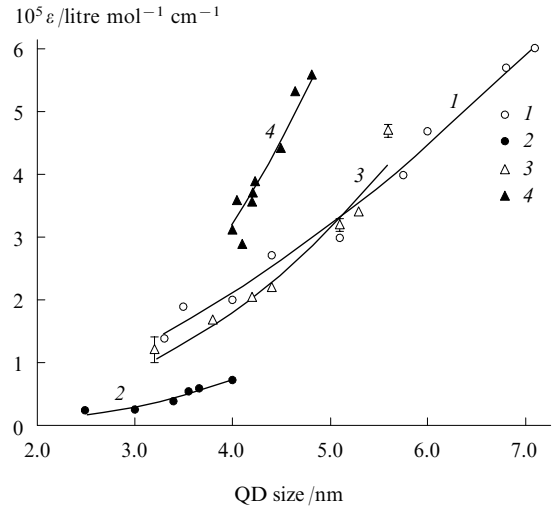


Figure 4. Extinction coefficient *vs*. size (d) for CdTe quantum dots stabilized by tri-n-octylphosphine oxide (*1*, $\varepsilon = 10043\,d^{2.12}$),[39] 2-mercaptopropionic acid (*2*, $\varepsilon = 5982\,d^{2.46}$),[37,40] thioglycolic acid (*3*, $\varepsilon = 5916\,d^{2.89}$) and 2-aminoethanethiol (*4*, $\varepsilon = 1745\,d^{2.66}$) (our data).

where A is the absorbance in the first exciton maximum; ε is the extinction coefficient (litre mol^{-1} cm^{-1}); l is the optical path length (cm); C is the molar concentration of the QD (mol litre^{-1}).

The extinction coefficients of the CdTe quantum dots stabilized by tri-n-octylphosphine oxide (TOPO), thioglycolic acid, 2-aminoethanethiol or mercaptopropionic acid with 2.5 to 7.5 nm size are in the range of $(0.5-8)\times10^5$ litre mol^{-1} cm^{-1}.[37,39,40] Sun and Goldys[41] attributed the increase in the extinction coefficient to an increase in the absorption cross-section, *i.e*., in the probability of absorption of photons by the sample. The tendency of the extinction coefficient to increase with an increase in the QD size is a consequence of decreasing ratio of the particle surface area to volume. The researchers approximated the obtained data by a power law function according to the theoretical views on the change in the absorption cross-section following an increase in the semiconductor nanocrystals.[39] This approximation can be used as the calibration plot suitable for fast and reliable determination of the concentration of aqueous solutions of CdTe with various types of stabilizers (see Fig. 4, curves *1* – *4*).

4. Surface modification of quantum dots

Modification of the surface of quantum dots is needed for their subsequent use as biological objects, for example, to prepare bioconjugates. In the first stage of biofunctionalization of QD by various functional groups or biomolecules, ligand exchange is often used.[25] This method comprises replacement of the initial stabilizer on the quantum dot surface by ligands that ensure a greater degree of surface binding and provide additional options for the introduction of reactive groups.[16] As such ligands, one can use monodentate and bidentate thiols, disulfides and amines and molecules containing carboxy groups, for example, polydentate oligomers and polymers. One more approach to the preparation of nanoparticles with functional groups is

modification of the initial ligands *via* reactions on the nanoparticle surface.[1,3,31,32,42–44]

a. Monodentate ligands

Monodentate thiols contain one thiol group, and *via* this group they can be immobilized on the QD surface. The preparation of water-soluble QD and their conjugates can be performed using various monodentate thiols: thioglycolic acid, ω-mercaptoundecanoic acid [$HS(CH_2)_{10}CO_2H$], cysteine, mercaptoalkyloligo(ethylene glycol)carboxylic acid [$HS(CH_2)_n(CH_2CH_2O)_mCO_2H$] and so on.

The functionalization of QD with thioacids makes them water-soluble and suitable for bioconjugation. For example, quantum dots modified with thioglycolic acid are able to form conjugates with transferrin, which serve for the fluorescent identification of leukemia cells.[45]

Despite the satisfactory biocompatibility and the possibility of performing reactions on the surface, the monothiol-stabilized quantum dots have low stability as a result of the dynamic sorption/desorption of thiol from the nanoparticle surface. For the thiol ligand-coated QD, the stability of sols decreases also due to the photooxidation of thiols to disulfides, which occurs in three stages: photocatalytic oxidation of thiol ligands on the surface, photooxidation of the proper QD and its precipitation. The degradation processes lead to the loss of luminescence properties of the starting QD.[25] For increasing the stability of the optical and electronic properties of quantum dots and their conjugates, polydentate ligands containing several functional groups capable of binding to the nanoparticle surface are used more often.

b. Polydentate ligands

The introduction of polydentate ligands such as dithiols, various oligomers and polymers with one or two amino, carboxy or phosphonic anchoring groups substantially increases the stability of QD in various media and extends the possibilities of their subsequent modification or biofunctionalization.[41]

Quantum dots stabilized by dihydrolipoic acid [$HS(CH_2)_2CH(SH)(CH_2)_4CO_2H$, DHLA] residue that were stable during agarose gel electrophoresis in a Tris-acetate buffer (pH 8.5) were prepared by ligand exchange.[42,46] Quantum dots stabilized by monothiols degrade during electrophoresis, which is attributable to desorption of the stabilizer and agglomeration of nanoparticles. However, the use of a polydentate stabilizer provides semiconductor nanocrystals that migrate in a gel matrix during electrphoresis without loss of stability.[42,46]

We studied the agarose gel electrophoretic mobility of CdTe quantum dots stabilized by thioacids, namely, thioglycolic and dihydrolipoic acids (Fig. 5). TGA-modified QD of 4 nm size (lane *1*) and quantum dots of four different sizes (3.9, 4.5, 5.2 and 5.5 nm) obtained by replacement of TGA ligands by DHLA (lanes *2*, *3*, *4* and *5*, respectively) were applied in the gel. It can be seen that the unique stability of quantum dots is caused by the bidentate coordination of the ligand.

Other dithiols including DHLA derivatives, dithiothreitol [*threo*-$HSCH_2CH(OH)CH(OH)CH_2SH$, DTT], thiol-modified β-cyclodextrin and so on can also be used as bidentate stabilizing ligands.

Gao *et al.*[47] reported the conjugates of the quantum dots stabilized by dihydrolipoic acid with the maltose-binding protein (MBP) and the β-cyclodextrin-QSY9 quencher dye in which the fluorescence resonance energy transfer (FRET) was observed. The possibility of utilization of these conjugates in chemical sensors meant for determining sugar concentration was demonstrated. In these sensors, MBP was bound to the QD surface by terminal oligohistidine segments and the fluorescence of nanoparticles was efficiently quenched in the presence of dye QSY9. Upon the addition of maltose, the luminescence of QD increased due to the replacement of β-cyclodextrin-QSY9.

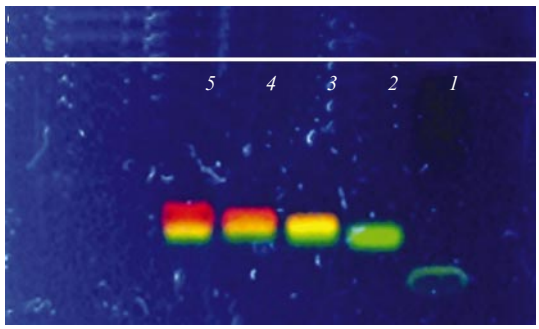
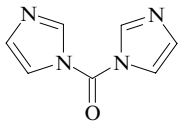


Figure 5. Demonstratione of the agarose gel electrophoretic mobility of quantum dots (see text).
The photograph was obtained by UV irradiation of the gel at 320 nm; the white band shows the start line.

Pathak *et al.*[48] replaced the tri-n-octylphosphine oxide ligand on the CdSe QD surface by the DTT residue (in 1,4-dioxane). The nanoparticles thus formed contained terminal hydroxy groups and were conjugated with oligonucleotides in the presence of 1,1′-carbonyldiimidazole (CDI).

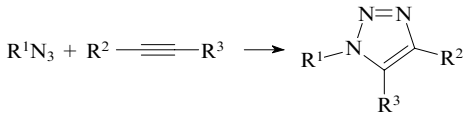


CDI

These bioconjugates were shown to be suitable for detecting abnormal or mutated chromosomes by fluorescent hybridization.

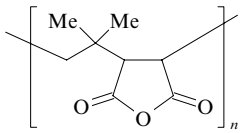
c. Click reactions

A highly promising approach to modification of biological molecules (antibodies, nucleic acids and so on) and to preparation of bioconjugates with them is 1,3-dipolar cycloaddition of azide to alkyne catalyzed by copper(I) compounds. This transformation is called the Huisgen–Meldal–Sharpless or click reaction.



Janczewski *et al.*[43] demonstrated the possibility of using this reaction for the self-assembly of complex structures and superlattices from semiconductor CdSe/ZnS quantum dots and magnetic Fe_3O_4 nanoparticles. Acetylene (or azide) groups were introduced on the nanoparticle surface and the QD were transferred into the aqueous phase using new

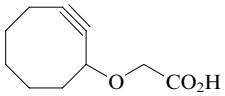
ampliphilic polymers based on poly(isobutylene-*alt*-maleic anhydride).



The process was catalyzed by copper(I) acetate. The QD luminescence was found to be quenched almost completely within several minutes after initiation of the reaction.' However, preliminary addition of sodium ascorbate into the reaction mixture resulted in the retention of high level of luminescence.

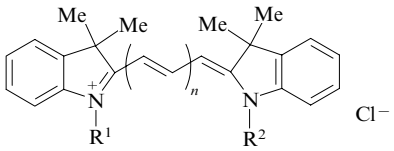
Quenching of luminescence of the CdSe/ZnS quantum dots after the addition of Cu^{I} salt was attributed [43] to partial replacement of Zn^{2+} cations in the shell by Cu^{2+} ions having a very similar ionic radius (0.72 Å).

Bernardin *et al.*[44] used an alternative method for performing click reactions, namely, the Bertozzi modification, known as copper-free click addition. For this purpose, the QD surface was modified with cyclooct-1-yne-3-glycolic acid.



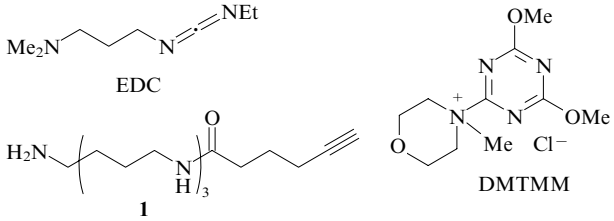
The click addition of the modified dots to the azido mannosamine derivative was carried out with and without copper compounds. The completion of the modification of the nanoparticle surface and the successful proceeding of the reaction were detected from the change in the size and the electrokinetic potential of the nanocrystals and from their electrophoretic mobility measured by agarose gel electrophoresis. It was found that conjugates with the same structure are formed in the copper-initiated and copper-free reactions. However, in the copper-initiated reaction, a more than twofold decrease in the luminescence quantum yield of the conjugates was observed.

For preparing the conjugates with azido derivative of the Cy5 cyanine dye [$R^1 = (CH_2)_5C(O)NH(CH_2)_3N_3$, R^2 = Me]



R^1, R^2 = Alk: n = 1 (Cy3), 2 (Cy5).

the 4.5 nm CdTe quantum dots stabilized by DHLA were modified by treatment with mono- or polyamines with a terminal triple bond.[42] The carboxy groups located on the nanoparticle surface reacted with amino groups in the presence of a condensing reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) or *N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)-*N*-methylmorpholinium chloride (DMTMM).



The reaction pattern involving triamine **1** is shown in Fig. 6. The use of this amine is attractive for the possibility of multicentre functionalization of QD *via* the formation of three amide bonds between the amino groups of the modifying agent and the stabilizer carboxy groups.

Using agarose gel electrophoresis, we demonstrated that in this case, covalent conjugates are formed (Fig. 7). The agarose gel was loaded with the initial azido derivative of dye Cy5 (lane *1*), initial QD (*2*), conjugates of dye Cy5 with quantum dots modified by triamine **1** in the presence of EDC (*3* and *4*) and DMTMM (*5*) and modified by monoamine in the presence of EDC (*6*, *7*). It can be seen from Figure 7 that the highest stability is inherent in the complexes obtained using triamine **1**.

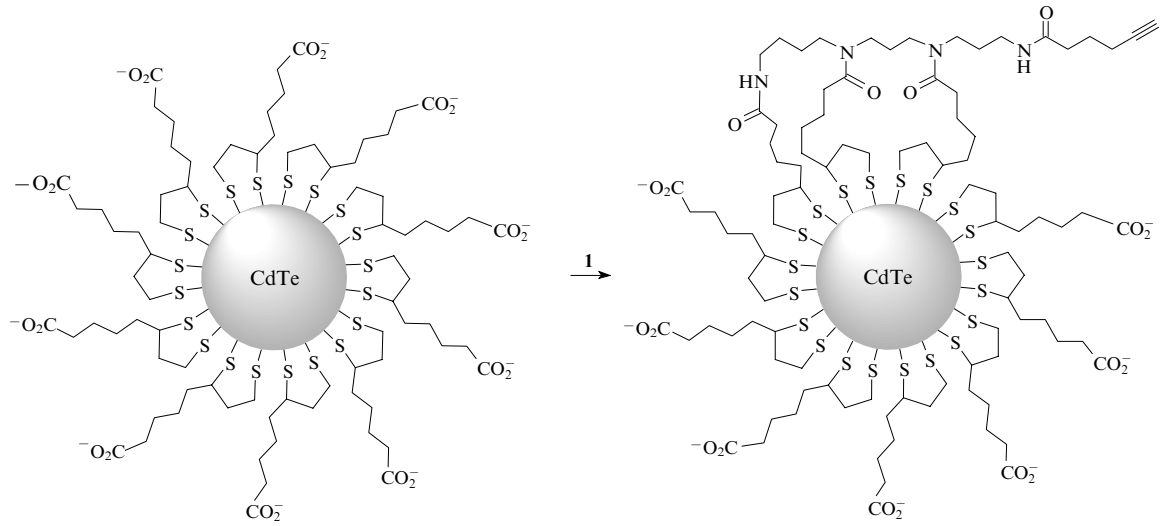


Figure 6. Surface functionalization of CdTe quantum dots stabilized by DHLA.

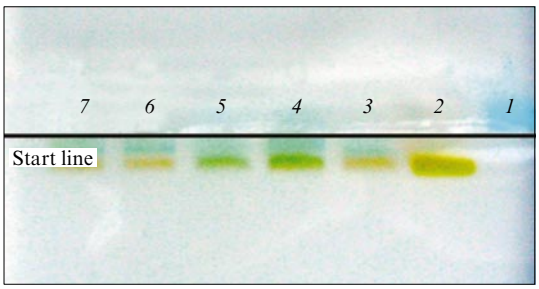


Figure 7. Study of the electrophoretic mobility of the conjugates of fluorescent dye Cy5 and CdTe quantum dots modified by mono- and polyamines (see text).

III. Conjugates of quantum dots and nucleic acids

The key approaches to functionalization of quantum dots by oligonucleotides and double-stranded DNA molecules are shown in Fig. 8. They can be classified according to the type of bond between the nanoparticle and nucleic acid. Two types of complexes are distinguished depending on whether they are formed through covalent or non-covalent addition of the biomolecule to the QD, the stability of the covalent conjugates being much higher.

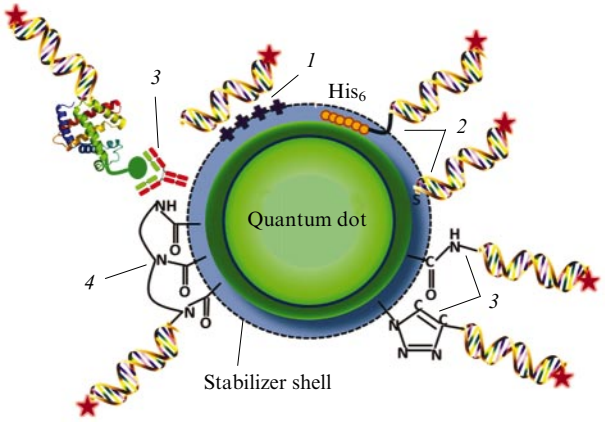


Figure 8. Common methods of functionalization of the semiconductor nanocrystal by nucleic acids: (*1*) formation of a biocompatible or stabilizing shell followed by electrostatic interaction, (*2*) covalent binding of histidine or thiol groups, (*3*) formation of peptide or specific key–lock-type bonds, (*4*) polydentate bonding (for explanations, see text).

Unlike other biological macromolecules (proteins, antibodies, *etc*.), which contain large numbers of functional groups (*e.g*., amino groups) able to form several bonds with one nanoparticle, nucleic acids usually have only one reaction site involved in conjugation. Single-site binding of a DNA molecule does not produce stable conjugates because DNA can be removed from the quantum dot surface upon desorption of the stabilizer. However, there are approaches that allow one to markedly enhance the stability of QD–DNA bioconjugates. Some of them are considered below.

1. Non-covalent addition of nucleic acids to quantum dots

a. The use of nucleic acids as ligands

Procedures for the preparation of conjugates of the CdSe/ZnS quantum dots and oligonucleotides containing a thiol group were developed.[49,50] They are specific because the formation of a zinc sulfide shell on the surface of the CdSe core and stabilization of the particles thus formed by nucleic acids are performed as one stage. The oleylamine-stabilized CdSe quantum dots were used as cores. They were added at room temperature to a DMSO solution containing a thiolating reagent (P_2S_5), 2-mercaptopropionic acid and modified oligonucleotide. Then a zinc chloride solution in DMSO was added to the reaction mixture, and the mixture was kept at 70 °C for 1 h to buildup the shell. Nanoparticles of two sizes fluorescing in green (λ_{max} = 548 nm) and red (λ_{max} = 617 nm) spectral regions were used as the QD cores.[49] According to the transmission electron microscopy, the size of the nucleic acid-stabilized nanoparticles obtained after the treatment was 4.9 ± 0.2 and 6.9 ± 0.3 nm for small and large cores, respectively.

The presence of nucleic acids on the nanoparticle surface was detected based on the change in the optical spectra of the conjugate caused by the resonance energy transfer. The addition of a complementary nucleotide sequence containing an acceptor dye at the 3′-end † to the nanoparticles stabilized by oligonucleotides induced a change in the fluorescence intensity, which decreased for the quantum dots (donors) and increased for the dye (acceptor). It was found that a too long distance between the donor and the acceptor (> 10 nm) decreases the efficiency of the energy transfer, which can be increased *via* simultaneous binding of the donor to several acceptors.

The quantum dots obtained in this way can serve for the self-assembly of nanostructures with the participation of metallic nanoparticles, *i.e*., promising objects for the design of modern optoelectronic devices.[51]

b. Addition of nucleic acids through electrostatic interactions

Quantum dots having a surface charge (positive or negative) can be used in the self-assembly of various complex structures through electrostatic interactions, can be deposited layer-by-layer on silicon, glass and other substrates (for example, by means of polyelectrolytes) and can be manipulated in an electric field. In view of the fact that nucleic acids contain phosphate groups, which make them negatively charged, there is a possibility to attach DNA to positively charged luminescent nanoparticles.

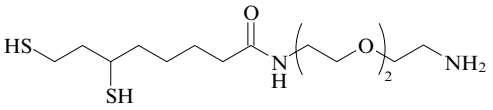
Chen *et al*.[52] obtained conjugates based on water-soluble positively charged CdTe quantum dots stabilized by 2-mercaptoethylamine in which nucleic acids were attached to the nanoparticle surface through electrostatic interactions. The addition of DNA molecules to the surface of nanocrystals induced a red shift of the luminescence maximum of the nanoparticles and a considerable increase in the luminescence intensity as a result of passivation of the surface defects.

Thioglycolic acid-stabilized CdSe/ZnS quantum dots were used [53] for electrostatic deposition of DNA on the surface of semiconductor particles. The carboxy groups on

† Dye Cy3 was used for smaller particles and Cy5 was used for larger QD.

the nanoparticle surface were activated by EDC and *N*-hydroxysuccinimide (NHS) ester in order to attach to them the antibacterial protein lysozyme, which favours the formation of the positive charge on the surface. The quantum dot–nucleic acid conjugates were formed upon the addition of nucleic acids modified by the dye rhodamine X to a solution of positively charged quantum dots. The attachment of nucleic acid was evidenced by a decrease in the luminescence intensity of quantum dots and the growth of the dye fluorescence intensity caused by the resonance energy transfer. These conjugates were used to quantitatively determine the activity of the micrococcal nuclease, the enzyme able to cleave the primary structure of DNA. The detection limit was 1.6×10^3 units ml^{-1}.

The amine-containing dihydrolipoic acid amide (DEDEA)



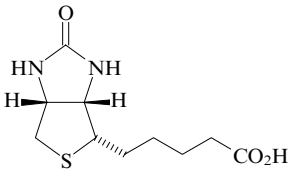
DEDEA

was used[29] as the stabilizer to form positive charge on the surface of CdSe/ZnS core–shell semiconductor particles.

For increasing the solubility of DEDEA-modified quantum dots, the surface was partially coated by polyethylene glycol (PEG). The conjugates were formed by the addition of a 25-mer nucleic acid modified by tetramethylrhodamine to a suspension of nanoparticles in different molar ratios (2 : 1, 5 : 1, 10 : 1). It was found that one QD can contain up to five DNA molecules on the surface. The efficiency of energy transfer in these conjugates reaches 90%, which is due to the small distance between the donor and the acceptor.

c. The use of proteins for the preparation of conjugates

A common approach to the synthesis of QD–nucleic acid conjugates is the use of various protein linkers and also specific protein interactions (antibody–antigen, enzyme–substrate), which allow specific addition of DNA to nanoparticle. In some works,[54–64] the high-affinity binding of biotin to streptavidin (the protein produced by *Streptomyces avidinii*) or avidin (glycoprotein present in the egg white of birds and reptiles) was used to form these conjugates.



Biotin

Huang *et al.*[54] proposed to modify CdSe/ZnS quantum dots stabilized by thioglycolic acid by adding streptavidin in the presence of carbodiimide and succinimide ester. The nucleic acid conjugates were obtained by reactions of streptavidin-coated quantum dots and single-stranded DNA (5′-TAT ATG GAT GAT GTG GTA TT-3′) modified by biotin at the 3′-end and by the acceptor dye rhodamine X at the 5′-end.

The formation of the conjugate was indicated by a change in the luminescence colour of the system from green to orange-red due to fast resonance energy transfer. The possibility of using a conjugate of this type as a biological probe for determination of the micrococcal nuclease was demonstrated. The introduction of micrococcal nuclease into the system resulted in destruction of the conjugate and a shift of the luminescence maximum back to the green region. The detection limit was 0.06 units ml^{-1}.

Maye *et al.*[57] employed QD–nucleic acid conjugates obtained by biotin–streptavidin interaction to determine DNA by time-resolved fluorescence spectroscopy with estimation of the excited state relaxation time.

The advantages of using conjugates based on specific biotin–streptavidin interactions in the multiplex analysis were demonstrated in relation to detection of the anthrax pathogen.[55] This was done using CdSe quantum dots of three different sizes with luminescence maxima at 525, 605 and 705 nm, respectively. The quantum dots were modified with oligonucleotides in such a way that the addition of the nucleotide sequence to be analyzed induced their pairwise nanoassembly (localization), resulting in a change in the luminescence wavelength. The nanoparticles were detected by UV laser excitation fluorescence microscopy. Due to the pairwise assembly, quantum dots of three sizes can encode three different nucleotide sequences. An advantage of QD in this case is the possibility of using one wavelength for the excitation of nanoparticles of different size. The authors assumed that the use of 10 quantum dots of different size and the combinatorial approach would allow for the detection of more than 45 components.

The use of optical-fibre-immobilized quantum dots as biological probes for detecting the DNA hybridization was reported.[56] The surface of semiconductor nanocrystals was coated by avidin through physical sorption. The conjugates were formed upon the subsequent treatment of fibres with a solution containing oligonucleotide probes with biotin at the 5′-end.

Wang *et al.*[27] directly attached the protein UvrB (isolated from *Bacillus caldotenax*), which is able to recognize damage in the DNA strand in the presence of the protein UvrA, to the quantum dots containing activated carboxy groups on the surface. However, the conjugates obtained in this way were almost unable to bind to DNA, which was caused by either steric hindrance or the loss of activity of the protein UvrB upon the addition of thiol. Therefore, the authors proposed a sandwich pattern of quantum dot coating by UvrB by means of murine antibodies. The formation of the conjugate with the DNA molecule was confirmed by changes in the electrophoretic mobility of quantum dots as a result of addition of nucleic acid molecules and by atomic force microscopy data.

d. Conjugation with histidine residues

An important approach to the formation of QD conjugates with nucleic acids is self-assembly of DNA molecules containing a polyhistidine moiety on the nanoparticle surface. It was shown[26, 28, 65, 66] that nucleic acids modified by terminal polyhistidine (His_n) can add to semiconductor nanocrystals *via* the coordination of histidine to metal atoms on the particle surface.

Boeneman *et al.*[26] functionalized QD by a DNA molecule using a protein linker containing six histidine moieties. The conjugates thus obtained were studied for the efficiency of resonance energy transfer depending on the distance

(Förster radius ‡) between the donor (quantum dot) and the acceptor (the dye attached to different regions of the DNA sugar phosphate backbone) and depending on the ratio of the acceptor to donor numbers. The efficiency of energy transfer in the conjugate formed with a histidine linker was found to be determined by the Förster radius, unlike that in the conjugate formed by biotin – streptavidin interaction. In the latter case, the efficiency of the energy transfer is determined only by the molar ratio between the acceptors and the donors. The authors studied the quantum dots – DNA conjugates that were modified by polyhistidine and dye Cy3 attached to different regions of the DNA sugar phosphate backbone. Finally, it was found that the efficiency of energy transfer between the QD and the nearest acceptor reaches 80% if eight acceptors are attached to one donor, whereas the efficiency of energy transfer between the nanoparticle and the dye that is most remote from the QD does not exceed 10%.

Modification of QD was performed also using a bifunctional protein linker containing six histidine residues.[28] This linker was pre-attached to a thiol-functionalized DNA molecule through formation of a disulfide bond.

Yeh *et al.*[65] used a protein linker containing six histidine moieties to obtain conjugates of quantum dots, gold nanoparticles and DNA (molecular flashlight) modified by a thiol group and an amino group. The protein linker was functionalized by maleimide for the subsequent addition of its residue to the thiol group attached to the 5′-end of the nucleic acid to give thioester. The conjugates with a gold nanoparticle were formed by reaction of the amino group of 'molecular beacon' with the activated ester on the particle surface. The subsequent self-assembly of the gold nanoparticle – DNA complex on the surface of the CdSe/ZnS quantum dot stabilized by dihydrolipoic acid was due to the affinity of zinc atoms (the shell of the semiconductor nanoparticle) for the histidine moieties. The formation of the conjugate was accompanied by substantial quenching of the QD luminescence, which reached 40% in the case of equivalent amounts of gold and semiconductor nanoparticles and 91% for the interaction of six gold nanoparticles with one quantum dot. It was shown that the conjugate formation of the semiconductor nanoparticle with 'molecular beacon' does not affect the hybridization kinetics of the latter.

e. Adsorption of oligonucleotides on quantum dots

The attachment of nucleic acids to the QD surface by nonspecific adsorption was described.[42, 67] This effect may considerably complicate the conjectural procedures of DNA diagnostics by fluorescent nanoparticles. However, the adsorption of oligonucleotides on quantum dots can be advantageously used to prepare conjugates. It was found [67] that pH of the buffer solution in which conjugation takes place affects the efficiency of adsorption of single- and double-stranded nucleic acids on the surface of thioglycolic acid-stabilized CdSe/ZnS quantum dots. The strongest adsorption was found in the acetate buffer at pH 4.8. At this pH, the surface carboxy groups are fully protonated, whereas in the borate buffer (pH 9.5), adsorption was more that 10 times weaker. At neutral pH values, the adsorption efficiency was 3 – 4 times higher than in the borate buffer due to partial deprotonation of the carboxy groups. In the authors' opinion, the adsorption of nucleic acids is due to hydrogen bonding between the carboxy groups of the stabilizer and DNA. This is also confirmed by the pronounced decrease in the adsorption efficiency down to zero after the addition of formamide during conjugation. Note that formamide is often used as a denaturing agent inducing cleavage of hydrogen bonds.

‡ The distance at which the efficiency of energy transfer is 50%.

2. Covalent attachment of nucleic acids to quantum dots

A common method for carboxy group activation toward the addition of amine to give amide bond is conducting the reaction in the presence of coupling reagents, most often, carbodiimides and *N*-hydroxysuccinimide esters. This approach ensures covalent attachment of nucleic acids and other biomolecules to different nanoparticles (metallic, semiconductor, magnetic) and is advantageously used for functionalization of quantum dots with single- or double-stranded DNA containing an amine group at one end.[32, 68 – 75]

This process is performed in two stages. In the first stage, carbodiimide reacts with the carboxy group on the pre-modified QD surface to give highly reactive intermediate that can react with various nucleophiles to give off the corresponding urea derivative. The carboxy group is activated most often in a weakly acidic medium (pH $\sim$5 – 6). The resulting activated carboxy intermediate easily reacts with amines to give amide bond (second stage). The addition reaction is usually carried out in a weakly alkaline solution (pH $\sim$8). Due to the high reactivity, the activated carboxylic intermediate can undergo side reactions, which decrease the yield of the target product. For increasing the yield of the conjugate, *N*-hydroxysuccinimide,[32, 71] which increases the lifetime of the activated intermediate, is usually added to the reaction mixture. By varying the molar ratio of the components (quantum dots, nucleic acids, carbodiimide), it is possible to obtain conjugates with different stoichiometry (the ratio of the numbers of nucleic acid and QD molecules).The reaction time varies from several to 24 hours. After the process has been completed, the unreacted excess of carbodiimide is removed by re-precipitation and subsequent dissolution of the conjugates.

Jiang *et al.*[68] described the addition of the nucleic acid obtained by reverse transcription with the ribosomal rRNA modified by the amino group at the 5′-end to the carboxy groups on the surface of CdSe/ZnS quantum dots stabilized by mercaptopropionic acid. The reaction was carried out in the presence of a 1.5-fold excess (relative to the QD) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in a neutral medium (pH 7.4). The complexes with different stoichiometry were formed using six different DNA : QD molar ratios (1 : 1, 1 : 20, 1 : 40, 1 : 60, 1 : 80, 1 : 100). The conjugates were studied during hybridization with complementary DNA containing the dye ROX (QD fluorescence acceptor) at the 3′-end.

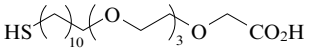
The efficiency of energy transfer between the quantum dot and the dye was studied as a function of DNA : QD ratio. The fluorescence intensity ratio of the acceptor and the donor was found to increase with increase in the amount of amino group-containing nucleic acid used in the addition reaction. An increase in the energy transfer efficiency indicates an increase in the degree of functionalization of the QD. However, for conjugates with DNA : QD ratios of

1 : 60, 1 : 80 and 1 : 100, the fluorescence intensity ratio of the acceptor and the donor (Fa/Fd) remains invariable (~30%), *i.e.*, the efficiency of the FRET process reaches a maximum. This was attributed to the effect of steric hindrance or to relatively low yield of the carbodiimide-catalyzed addition product.

Algar and Krull[69] controlled the degree of functionalization of the CdSe/ZnS quantum dots stabilized by thioglycolic acid by varying the amount of carbodiimide introduced into conjugation. It was found that the activation of the carboxy group is accompanied by partial agglomeration of quantum dots, which can be diminished by introducing less carbodiimide (molecular excess of 10^4-10^5 times relative to QD). As the conjugate formation stoichiometry increased, the luminescence quantum yield of quantum dots somewhat increased due to passivation of the nanoparticle surface by the addition of the oligonucleotide. The authors were able to obtain conjugates using quantum dots of two sizes with fluorescence maxima at 515 and 600 nm. The stoichiometry of the oligonucleotide addition amounted to 2 : 1 for smaller nanoparticles and 6 : 1 for greater quantum dots.

A covalent conjugate was also prepared using CdSe/ZnS quantum dots encapsulated in a polymeric shell.[75] The addition of DNA modified by an amino group at the 5′-end was performed in a borate buffer at pH 8.0. The conjugate thus formed was separated from the unreacted DNA by ultrafiltration. The amount of DNA added to the quantum dots was determined from the absorption spectroscopy data. The reaction gave the conjugates of quantum dots containing from 7 to 15 or from 2 to 5 nucleic acid molecules per nanoparticle for the QD with the luminescence maximum at 512 or 605 nm, respectively. During the addition of DNA, some luminescence quenching of the quantum dots was observed (by ~25% – 35%). The electrophoretic mobility of the conjugates of QD having different sizes with nucleic acids relative to the mobilities of the initial DNA and the nanoparticles was measured. It was found that the effect of DNA addition on the nanoparticle mobility is caused only by the increase in the particle size upon conjugation.

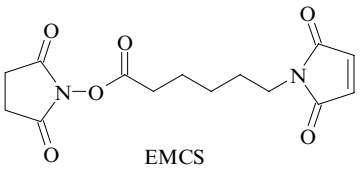
The conjugates of CdSe/ZnS quantum dots stabilized by 11-mercaptoundecyltri(ethylene glycol) acetic acid (EG_3CO_2H) with a 30-mer oligonucleotide amino-group modified at the 5′-end were reported.[32]



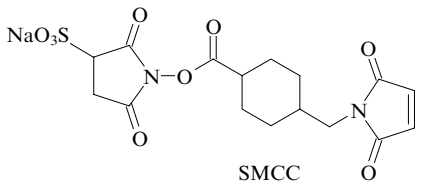
EG_3CO_2H

The carboxy groups on the QD surface were activated at pH 6 in a phosphate buffer containing a mixture of EDC and NHS in 2 : 1 mass ratio. Then an 11-fold excess of DNA (with respect to QD) was added at pH 9. The calculated degree of functionalization of QD was 2.2, and the yield of the reaction product was 20%.

It was demonstrated[76,77] that thiol-modified nucleic acids can be attached to the quantum dots coated by amino groups in the presence of cross-linking reagents such as succinimido(6-maleimidohexanoate) (EMCS) and 3-sulfosuccinimido-[4-(maleimidomethyl)cyclohexanecarboxylate] as the sodium salt (SMCC). The obtained conjugates were stable for 4 weeks without deterioration of luminescent properties.



EMCS



SMCC

It was proposed[48] to use dithiothreitol to introduce hydroxy groups on the surface of CdSe/ZnS quantum dots. The OH groups are convenient functional groups for the addition of oligonucleotides modified by amines to give an amide bond. The hydroxy groups were activated by treatment with 1,1′-carbonyldiimidazole to give imidazolecarbamate moieties on the QD surface. The subsequent addition of oligonucleotide having amino group at the 5′-end to the activated nanoparticles afforded the conjugate. The authors demonstrated that these conjugates can be used for multiplex determination of chromosome mutations.

3. The use of quantum dot – nucleic acid conjugates for diagnostics

A promising trend of the modern nanotechnologies is the use of quantum dot – nucleic acid conjugates in biology and medicine (Table 2). Semiconductor nanoparticles serve as fluorescent labels in widely used DNA diagnostic procedures such as fluorescence *in situ* hybridization (FISH) and biological microchip technology (Fig. 9 *a*,*c*). The QD – nucleic acid conjugates help to increase the sensitivity and specificity of the analysis, the sample volume being decreased to almost single molecules.[2,3,5,7,8,30]

The presence of a broad band in the absorption spectra and a narrow symmetrical luminescence peak makes QD convenient objects for the multiplex analysis.[4] In addition, the excitation wavelength can be varied over a broad spectral range and, hence, it can be chosen far from the emission wavelength, which reduces the background scattering and increases the signal-to-noise ratio. The key feature of all submicron-size particles is a large surface area to volume ratio. For example, the surface area of a 6 nm nanoparticle is 113 nm^2 (small organic luminophores are ~1 nm in size), and this allows for up to 30 oligonucleotide probes to be accommodated on the QD surface, and the use of nanoparticle surface modification techniques allows them to be complexed simultaneously with several different biomolecules. The fluorescence quantum yield of the quantum dots is comparable to that of organic dyes; however, the extinction coefficient is 10 – 50 times higher for QD. Therefore, QD are characterized by high luminescence brightness (10 – 20 times higher) and photostability (100 – 200 times more stable to light).[8]

The use of quantum dots in the FISH diagnostics demonstrated that as regards the colouring efficiency, the semiconductor nanocrystals are not merely not inferior to organic dyes such as FITC, Texas Red or Cy3 but are even superior in the brightness and are much more stable.[2]

Methods for the detection of oligonucleotide hybridization based on the resonance energy transfer in which QD are used as donors have been developed (see Fig. 9 *b*). In this case, DNA hybridization is indicated by the energy

Table 2. Biofunctionalization of quantum dots.

Quantum dot, stabilizer	Surface modification type	Mode of addition	Application	Ref.
CdSe, TOPO	ligand exchange for dithiothreitol	covalent addition of the amino derivative of nucleic acid in the presence of CDI	fluorescence *in situ* hybridization	48
CdSe/ZnS, TOPO	amphiphilic polymers based on poly(isobutylene-*alt*-maleic anhydride)	covalent self-assembly by 1,3-dipolar cycloaddition	–	43
CdSe/ZnS, PEG-NH_2	modification with cyclooct-1-yne-3-glycolic acid in the presence of EDC	copper(I)-free 1,3-dipolar cycloaddition of mannosamine	cell visualization	44
CdTe, TGA	ligand exchange for DHLA, modification with mono- or polyaminoalkynes in the presence of condensing reagents	copper(I)-initiated 1,3-dipolar cycloaddition of fluorescent dyes	FRET-based analyses	42
CdSe/ZnS, oleylamine	mercaptopropionic acid and oligonucleotide containing a thiol group	oligonucleotides serve as ligands	FRET-based DNA probes	49
CdTe, 2-amino-ethanethiol	–	attachment of oligonucleotides through electrostatic forces	DNA probes	52
CdSe/ZnS, TGA	surface modification by the anti-bacterial protein lysozyme giving rise to a positive charge	the same	FRET-based quantitative determination of micrococcal nuclease	53
CdSe/ZnS, TOPO	ligand exchange for ethylamino derivative of dihydrolipoic acid with addition of PEG	"	DNA probes	29
CdSe/ZnS, TGA	surface modification by strepta-vidin in the presence of EDC	reaction with biotin-modified single-stranded DNA	DNA probes, FRET-based quantitative determination of micrococcal nuclease	54
CdSe	–	use of biotin – streptavidin interaction	multiplex analysis by fluorescence microscopy	55
	surface modification by the murine antibody	use of antibody – antigen interaction	DNA probes	27
CdSe/ZnS	–	nucleic acid addition through a poly-histidine linker	DNA probes, FRET-based DNA detection	8, 26, 28, 65, 66
CdSe/ZnS, 2-mercapto-propionic acid	–	oligonucleotide addition in the presence of EDC	DNA probes	68
CdSe/ZnS, thioglycolic acid	–	the same	the same	69
CdSe/ZnS, EG_3CO_2H	–	"	"	32
CdSe/ZnS, TOPO	methacrylic acid copolymer modified by hexahistidine	addition of oligonucleotide containing chelated Ni^{2+}	"	66
CdTe, DHLA	surface modification by tria-minoazide	1,3-dipolar cycloaddition of DNA	"	42

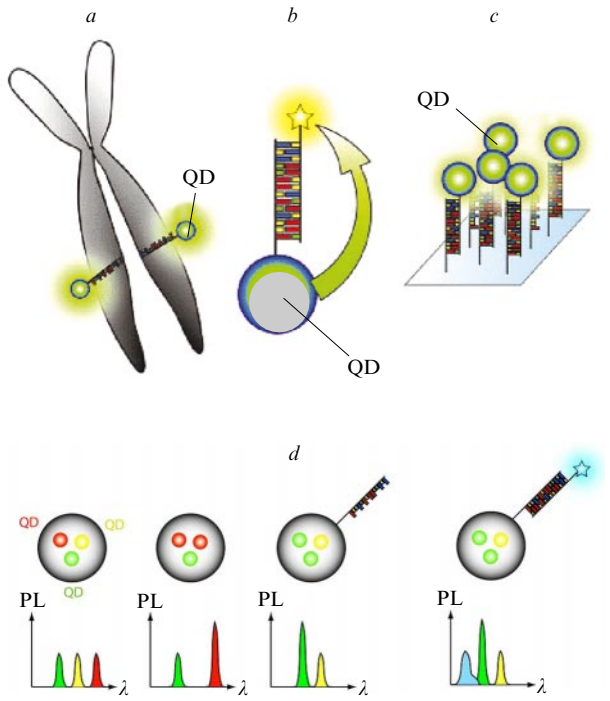


Figure 9. Application of quantum dots in the diagnostics of nucleic acids.[2]
(*a*) Quantum dots as fluorescent labels for FISH diagnostics; (*b*) quantum dots as donors for FRET detection of hybridization; (*c*) quantum dots as labels for hybridization on microchips; (*d*) encapsulated quantum dot assemblies for spectral bar coding (λ is the wavelength, PL is the photoluminescence intensity).

transfer between the nanocrystal and the corresponding acceptor fluorophore (organic dye). Due to the large surface area, one quantum dot can interact simultaneously with several acceptor molecules. As the number of acceptors increases, the cross-sectional area of the FRET (overlap integral) increases and, hence, the overall efficiency of energy transfer increases. The dependence of the energy transfer efficiency on the number of acceptors is expressed as follows:

$$E = \frac{nR_0^6}{nR_0^6 + r^6},$$

where n is the total number of acceptors that interact with one donor, R_0 is the Förster radius, r is the fixed distance between the donor and the acceptor.[3]

The key factors that affect the efficiency of FRET in the quantum dot-based structures include the donor to acceptor molar ratio, the donor to acceptor distance and the denticity of oligonucleotide addition. Thus it was shown [3] that in the case of non-specific electrostatic addition of DNA to the QD surface, the use of this conjugate as a probe becomes difficult.

Giraud *et al.*[30] demonstrated the good prospects of polymer-coated CdSe/ZnS quantum dots for the quantitative determination of biomolecules through the multiplex analysis. The use of 10 different intensity levels and 6 colours is theoretically suitable for encoding about 1 million nucleic acid or protein sequences. These polymeric species are identified by fluorescence spectroscopy and microscopy to an accuracy of $>99.99\%$.

Figure 9 *d* shows the preparation of quantum dot aggregates with a set of narrow luminescence spectra that form a colour blend for an external observer (one colour). This colour code is unique (the same principle but at a much coarser level is used to encode 'millions of colours' in data display devices).

It is noteworthy that excitation of quantum dots of different size (with different fluorescence maxima) may be performed by a light source at one wavelength, which is impossible when organic dyes are studied by fluorescence microscopy.[30]

IV. Conclusion

The nanoparticle size and the resonance energy transfer processes are the key factors that influence the optical characteristics of QD conjugates. The known problem of toxicity of heavy metal-containing quantum dots is being currently solved fairly successfully by the integrated approach based on biofunctionalization of semiconductor nanocrystals. Analysis of the publications devoted to the preparation and applications of quantum dots indicates that the biofunctionalized quantum dots offer broad opportunities for varying their physical characteristics and increasing the biocompatibility. This allows one to hope that they would serve for the design of fundamentally new biomedical diagnostic devices.[78–80] Special mention should be made of the regular appearance of new ideas concerning stabilization and conjugation of quantum dots that would provide the formation of stable bioconjugates and considerably increase the chance for their practical use as biomarkers and as parts of highly sensitive biosensors.

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References

1. P Zrazhevskiy, M Sena, X Gao *Chem. Soc. Rev.* **39** 4326 (2010)
2. W R Algar, M Massey, U J Krull *Trends Anal. Chem.* **28** 292 (2009)
3. I L Medintz, H Mattoussi *Phys. Chem. Chem. Phys.* **11** 17 (2009)
4. M Han, X Gao, J Z Su, S Nie *Nature Biotechnol.* **19** 631 (2001)
5. C P Soman, T D Giorgio *Langmuir* **24** 4399 (2008)
6. A Merkoci *Biosens. Bioelectron.* **26** 1164 (2010)
7. W Zhong *Anal. Bioanal. Chem.* **394** 47 (2009)
8. G Rousserie, A Sukhanova, K Even-Desrumeaux, F Fleury, P Chames, D Baty, V Oleinikov, M Pluot, J H M Cohena, I Nabiev *Crit. Rev. Oncol. Hematol.* **74** 1 (2010)
9. V I Klimov *Los Alamos Sci.* **28** 214 (2003)
10. *Semiconductor and Metal Nanocrystals. Synthesis and Electronic, and Optical Properties* (Ed. V I Klimov) (New York: Marcel Dekker, 2004)
11. A D Yoffe *Adv. Phys.* **50** 1 (2001)
12. L J Lauhon, M S Gudiksen, D Wang, C M Lieber *Nature (London)* **420** 57 (2002)
13. Y Yin, P Alivisatos *Nature (London)* **437** 664 (2005)

14. C de Mello Donega, S G Hickey, S F Wuister, D Vanmaekelbergh, A Meijerink *J. Phys. Chem. B* **107** 489 (2003)
15. P N Tananaev, S G Dorofeev, R B Vasil'ev, T A Kuznetsova *Inorg. Mater.* **45** 347 (2009)
16. C B Murray, S Sun, W Gaschler, H Doyle, T A Betley, C R Kagan *IBM J. Res. Devel.* **45** 47 (2001)
17. P Reiss, M Ptoriere, L Li *Small* **5** 154 (2009)
18. C B Murray, D J Norris, M G Bawendi *J. Am. Chem. Soc.* **115** 8706 (1993)
19. X Peng, J Wickham, A P Alivisatos *J. Am. Chem. Soc.* **120** 5343 (1998)
20. D Pan, Q Wang, S Jiang, X Ji, L An *J. Phys. Chem. C* **111** 5661 (2007)
21. Y Wei, J Yang, A W H Lin, J Y Ying *Chem. Mater.* **22** 5672 (2010)
22. N Gaponik, D V Talapin, A L Rogach, K Hoppe, E V Shevchenko, A Kornowski, A Eychmüller, H Weller *J. Phys. Chem. B* **106** 7177 (2002)
23. N Gaponik, A L Rogach *Phys. Chem. Chem. Phys.* **12** 8685 (2010)
24. N Gaponik, S G Hickey, D Dorfs, A L Rogach, A Eychmüller *Small* **6** 1364 (2010)
25. J K Oh *J. Mater. Chem.* **20** 8433 (2010)
26. K Boeneman, J R Deschamps, S Buckhout-White, D E Prasuhn, J B Blanco-Canosa, P E Dawson, M H Stewart, K Susumu, E R Goldman, M Ancona, I L Medintz *ACS Nano* **4** 7253 (2010)
27. H Wang, I Tessmer, D L Croteau, D A Erie, B Van Houten *Nano Lett.* **8** 1631 (2008)
28. I L Medintz, L Berti, T Pons, A F Grimes, D S English, A Alessandrini, P Facci, H Mattoussi *Nano Lett.* **7** 1741 (2007)
29. J Lee, Y Choi, J Kim, E Park, R Song *ChemPhysChem* **10** 806 (2009)
30. G Giraud, H Schulze, T T Bachmann, C J Campbell, A R Mount, P Ghazal, M R Khondoker, S W J Ember, I Ciani, C Tlili, A J Walton, J G Terry, J Crain *Chem. Phys. Lett.* **484** 309 (2010)
31. R Shen, X Shen, Z Zhang, Y Li, S Liu, H Liu *J. Am. Chem. Soc.* **132** 8627 (2010)
32. D Zhou, L Ying, X Hong, E A Hall, C Abell, D Klenerman *Langmuir* **24** 1659 (2008)
33. V K La Mer, R H Dinegar *J. Am. Chem. Soc.* **72** 4847 (1950)
34. Z Fang, L Liu, L Xu, X Yin, X Zhong *Nanotechnology* **19** 235603 (2008)
35. A V Romanova, P B Kocherginskaya, in *XXI Mendeleevskii Konkurs Studentov-Khimikov (Tezisy Dokladov)* [The XXIst Mendeleev Competition of Chemist-Students (Abstracts of Reports), Dubna, 2011] p. 17
36. A L Rogach, A Kornowski, M Gao, A Eychmüller, H Weller *J. Phys. Chem. B* **102** 8360 (1998)
37. P B Kocherginskaya, D M Itkis, in *Nanotechnology International Forum (Book of Abstracts), Moscow, 2009* p. 698
38. S Srivastava, A Santos, K Critchley, K-S Kim, P Podsiadlo, K Sun, J Lee, C Xu, G D Lilly, S C Glotzer, N A Kotov *Science* **327** 1355 (2010)
39. W W Yu, L Qu, W Guo, X Peng *Chem. Mater.* **15** 2854 (2003)
40. S Dong, J Ren *Analyst* **135** 1395 (2010)
41. J Sun, E M Goldys *J. Phys. Chem. C* **112** 9261 (2008)
42. P B Kocherginskaya, I A Prokhorenko, A V Romanova, E A Obraztsova, D V Klinov, A V Ustinov, T S Zatsepin, D Yu Ryazantsev, A A Formanovskii, I A Stepanova, N B Pestov, D M Itkis, E A Goodilin, V A Korshun, in *Tezisy 2-oi Mezhdunarodnoi Konferentsii 'Fiziko-khimicheskaya Biologiya'* (Abstracts of the 2nd International Conference 'Physicochemical Biology', Novosibirsk, 2011) p. 91
43. D Janczewski, N Tomczak, S Liu, M-Y Hanab, G J Vancso *Chem. Commun.* 3253 (2010)
44. A Bernardin, A Cazet, L Guyon, P Delannoy, F Vinet, D Bonnaffe, I Texier *Bioconjugate Chem.* **21** 583 (2010)
45. Z Zhelev, H Ohba, R Bakalova, R Jose, S Fukuoka, T Nagase, M Ishikawa, Y Baba *Chem. Commun.* 1980 (2005)
46. W Liu, M Howarth, A B Greytak, Y Zheng, D G Nocera, A Y Ting, M G Bawendi *J. Am. Chem. Soc.* **130** 1274 (2008)
47. X Gao, Y Cui, R M Levenson, L W K Chung, S Nie *Nature Biotechnol.* **22** 969 (2004)
48. S Pathak, S-K Choi, N Arnheim, M E Thompson *J. Am. Chem. Soc.* **123** 4103 (2001)
49. Q Wang, Y Liu, Y Ke, H Yan *Angew. Chem., Int. Ed.* **47** 316 (2008)
50. M Xing, H Shen, W Zhao, Y Liu, Y Du, Z Yu, X Chen *BioTechniques* **50** 259 (2011)
51. Q Wang, H Wang, C Lin, J Sharma, S Zou, Y Liu *Chem. Commun.* 240 (2010)
52. Q Chen, D Yang, S Xu *Anal. Lett.* **41** 1964 (2008)
53. T Qiu, D Zhao, G Zhou, Y Liang, Z He, Z Liu, X Peng, L Zhou *Analyst* **135** 2394 (2010)
54. S Huang, Q Xiao, Z K He, Y Liu, P Tinnefeld, X R Su, X N Peng *Chem. Commun.* 5990 (2008)
55. Y-P Ho, M C Kung, S Yang, T-H Wang *Nano Lett.* **5** 1693 (2005)
56. W R Algar, U J Krull *Anal. Chem.* **82** 400 (2010)
57. M M Maye, O Gang, M Cotlet *Chem. Commun.* 6111 (2010)
58. R-Q Liang, W Li, Y Li, C-y Tan, J-X Li, Y-X Jin, K-C Ruan *Nucleic Acids Res.* **33** e17 (2005)
59. A Crut, B Geron-Landre, I Bonnet, S Bonneau, P Desbiolles, C Escude *Nucleic Acids Res.* **33** e98 (2005)
60. P Chan, T Yuen, F Ruf, J Gonzalez-Maeso, S C Sealfon *Nucleic Acids Res.* **33** e161 (2005)
61. A A Chen, A M Derfus, S R Khentani, S N Bhatia *Nucleic Acids Res.* **33** e190 (2005)
62. A K Chen, M A Behlke, A Tsourkas *Nucleic Acids Res.* **35** e105 (2007)
63. N Luzzietti, H Brutzer, D Klaue, F W Schwartz, W Staroske, S Clausing, R Seidel *Nucleic Acid Res.* **39** e15 (2011)
64. C-Y Zhang, H-C Yeh, M T Kuroki, T-H Wang *Nature Mater.* **4** 826 (2005)
65. H-Y Yeh, M V Yates, A Mulchandani, W Chen *Chem. Commun.* 3914 (2010)
66. H Kwon, S Hong, H Kim, Y Choi, J Kim, R Song *Chem. Commun.* 8959 (2010)
67. W R Algar, U J Krull *Langmuir* **22** 11346 (2006)
68. J F Jiang, Z H Peng, L Deng, G Li, L Chen *J. Fluoresc.* **20** 365 (2010)
69. W R Algar, U J Krull *Anal. Chim. Acta* **581** 193 (2007)
70. Z Dai, J Zhang, Q Dong, N Guo, S Xu, B Sun, Y Bu *Chin. J. Chem. Eng.* **15** 791 (2007)
71. X Wang, X Lou, Y Wang, Q Guo, Z Fang, X Zhong, H Mao, Q Jin, L Wu, H Zhao, J Zhao *Biosens. Bioelectron.* **25** 1934 (2010)
72. Y Li, X Duan, L Jing, C Yang, R Qiao, M Gao *Biomaterials* **32** 1923 (2011)
73. B C Satterfield, M R Caplan, J A A West *Nucleic Acids Res.* **36** e129 (2008)
74. C-S Wu, M K K Oo, J M Cupps, X Fan *Biosens. Bioelectron.* **26** 3870 (2011)
75. Y Choi, H P Kim, S M Hong, J Y Ryu, S J Han, R Song *Small* **5** 2085 (2009)
76. D Bas, I H Boyaci *Anal. Bioanal. Chem.* **400** 703 (2011)
77. E Sharon, R Freeman, I Wilner *Anal. Chem.* **82** 7073 (2010)
78. Yu D Tretyakov, E A Goodilin *Russ. Chem. Rev.* **78** 801 (2009)
79. A A Rempel *Russ. Chem. Rev.* **76** 435 (2007)
80. V A Oleinikov, A V Sukhanova, I R Nabiev *Ros. Nanotekhnol.* **2** 160 (2007) [a]

[a] — *Nanotechnol. Russ. (Engl. Transl.)*