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REVIEW

Quantum dots on electrodes—new tools for bioelectroanalysis

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Abstract The review covers recent developments in which quantum dots (QDs) are combined with electrodes for detection of analytes. Special focus will be on the generation of photocurrents and the possibility of spatially resolved, light-directed analysis. Different modes for combining biochemical reactions with QDs will be discussed. Other applications involve the use of QDs as labels in binding analysis. Different methods have been developed for read-out. In addition to photocurrent analysis, voltammetric detection of metals and electrochemiluminescence (ECL) can be used. In the latter, light is the sensor signal. ECL-based systems combine the advantage of very sensitive analytical detection with rather simple instrumentation.

Keywords Quantum dot · Photocurrent · Electrochemiluminescence · Catalysis

Abbreviations

AChE	Acetycholine esterase
ALP	Alkaline phosphatase
ATP	Adenosine triphosphate
BDT	1,4-Dithiobenzene
CCD	Charge-coupled device
CNTs	Carbon nanotubes
$[Co(Phen)_3Cl_2]$	Cobalt phenanthroline chloride
CRET	Chemical resonance energy transfer
Cy5	Fluorescence dye (for Cy5 Ex=650 nm; Em=670 nm)
cyt c	Cytochrome c
DET	Direct (heterogeneous) electron transfer
ECL	Electrochemiluminescence
ERET	Electrochemical resonance energy transfer
FRET	Förster resonance energy transfer
GDH	Glucose dehydrogenase
GOD	Glucose oxidase
HEPES	4-(2-Hydroxyethyl)-1-piperazineethane sulfonic acid
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
ITO	Indium tin oxide
LAPS	Light-addressable potentiometric sensors
LDH	Lactate dehydrogenase
NAD^+/NADH	Nicotinamide adenine dinucleotide
NPs	Nanoparticles
NR	Nitrate reductase
PAH	Polyallylamine hydrochloride
pAP	*p*-Aminophenol
pAPP	*p*-Aminophenylphosphate
PSA	Prostate-specific antigen
QDs	Quantum dots
$[Ru(bpy)_3]^{2+}$	Ruthenium bipyridine complex
TOPO	Trioctylphosphine oxide
TPrA	Tri-*n*-propylamine

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Introduction

Quantum dots, which are semiconducting nanoparticles, not only have interesting optical properties but also electrical features modulated by light. Because these particles are semiconductors there is a gap between the energy states of electrons in the valence and conduction bands. By interaction with light or by (electro)chemical reactions electrons

can acquire a higher energetic state. Subsequently a free electron and a hole (or defect electron) are created. This gives rise to different properties, with luminescence as the most prominent on. After photoexcitation the nanoparticles return to the original state by light emission. Because the electronic situation of QDs is size-dependent, the wavelength of emitted photons is also shifted to higher values with increasing size. In contrast to absorption, the emission spectra of quantum dots have a rather sharp peak if the size distribution of the prepared nanoparticles is not very broad. Thus, quantum dots have become very popular fluorescence labels in biology and bioanalysis; molecules coupled to quantum dots of different size can be easily distinguished [1]. Multiplexed analysis is therefore feasible.

The focus of this review is, however, the coupling of QDs with electrodes. This is based on the use of light-generated charge carriers for electron transfer reactions or on the reverse process in which electrochemical reactions are used to excite QDs which subsequently emit light. Directions of research are different, depending on the excitation of charge carriers in quantum dots on electrodes:

- investigation of charge transport through molecules which are used to fix the quantum dots to the electrode [2–4];
- conversion of light energy into electrical energy (solar cell) [5–10];
- photocatalysis (induction of defined reactions on the excited quantum dots with substances in solution) [11–13];
- electrochemiluminescence (stimulation of light emission by electrochemical reactions); and
- light-initiated (bio)analysis (photocurrent measurements).

The last two areas are devoted to analytical applications and, thus, will be considered here.

By analogy with fluorescence, emission of light can be used for analytical applications when the excitation is induced not by illumination but by electrochemical and chemical reactions near the electrode surface [14]. Electrochemiluminescence (ECL) is thus on the borderline between spectroscopy and electrochemistry. Because the intensity of the emitted light depends, among other factors, on the number of QDs, the effect is often used for label detection.

When the excitation is performed by light, however, another coupling with electrochemistry becomes possible. Electron-transfer reactions of the excited QDs with electrodes result in generation of a photocurrent which can be used as analytical tool. Here the QDs act as a light-switchable layer on the electrode surface or as a label which can be detected electrochemically.

Compound semiconductors from the II/VI family are the main materials used for QD electrode construction, for example CdS [3, 15–24], CdTe [25–31], and such other materials as ZnO [32] and TiO_2 [33, 34]. Core–shell nanoparticles can also be used, for example CdSe–ZnS [35–38]. The advantage of the latter nanoparticles is the reduced amount of surface states as a result of the thin shell structure. This leads to an improved quantum yield and reduces charge carrier trapping.

Quantum dots and photocurrent measurements

When quantum dots are immobilized on an electrode surface, a rather insulating layer is prepared, hindering electrochemical conversions. On illumination of such a quantum dot electrode charge carriers are generated and current flow is possible; this current is normally termed “photocurrent”. The charge carriers are electrons in the conduction band and defect electrons (holes) in the valence band. Background for photocurrent generation is electron transfer reactions from and to the illuminated QDs. Thus, this current is affected by the sign and the magnitude of the potential applied to the electrode and by the presence of substances which can act as electron donors or acceptors. This means that under fixed potential conditions concentration analysis is feasible by photocurrent measurement. Here electron flow from the QDs toward the electrode (anodic) and from the electrode to the QDs (cathodic) is observed [35, 39–41]. Such a reaction scheme is illustrated in Fig. 1.

For effective photocurrent generation different factors are important:

1. Efficient electron transfer between the excited QDs and the electrode. Besides the potential applied to the electrode, the distance of the QDs from the surface and the nature of the molecules used for fixation of the QD to the electrode determine the rate of electron exchange.
2. Reaction rates for oxidation or reduction of substances at the surface of excited QDs. The redox properties (redox potential) of donor or acceptor compounds in solution and the surface properties of the QDs, including capping agents, will affect the reaction efficiency and thus the overall photocurrent.
3. Charge recombination inside the QDs. The light -induced charge carriers can also recombine in the QDs without participating in external redox reactions.

It must be mentioned here that the quality of quantum dot synthesis is crucial to the magnitude and potential dependence of the photocurrent [42]. Surface states can act as traps for the light-generated charge carriers. This can lead to systems with a limited potential dependence of the photocurrent and, thus, limited applicability to the detection of donor and acceptor compounds. Such effects were mainly observed in the early years of quantum dot combination

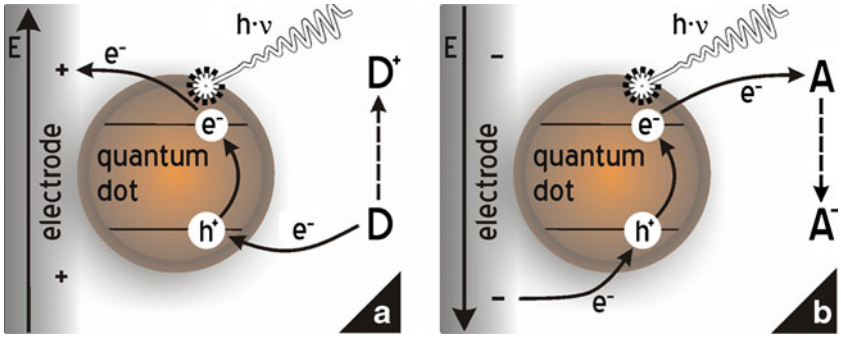

Fig. 1 Electron transfer steps at a quantum dot-modified electrode after illumination: (**a**) oxidation cascade and (**b**) reduction cascade. "*D*" represents a donor molecule which will be oxidized and "*A*" represents an acceptor which is reduced at the QD surface

with electrodes [2, 4, 15, 43]. However, when the light-induced charge carrier generation in the valence and conduction bands is followed by rapid reaction with the electrode on the one hand and with species in solution on the other hand then charge carrier recombination can be limited and well defined photocurrents, which follow the applied potential, can be obtained.

Different procedures have been developed for QD-immobilization. Dithiol or disulfide compounds (e.g. 1,4-dithiane, 1,6-hexanedithiol, 1,4-dithiobenzene, *trans*-stilbenedithiol) are most often used for fixation on gold, via chemisorption. The quality of such a thiol layer and the properties of the compound used strongly affect the photocurrent output. Compounds which can form well ordered and rigid monolayers, for example tetrahydro-4*H*-thiopyranylidene [2] or stilbenedithiol [44], will result in a well defined photocurrent response. For such systems, clear dependence of the current on the tunneling distance can also be shown. In addition, the conducting properties are important; conjugated π systems are advantageous for effective electronic coupling compared with aliphatic chains [2]. As an alternative to electrode modification and subsequent QD immobilization, the quantum dots can be coated with different capping agents and then fixed to the electrode. The capping agents are also important for adjustment of the surface properties for different sensing applications. For example, this might be the direct coupling of biomolecules. These agents can be used as a starting point for covalent coupling strategies [45], for the application of bioaffinity binding (for example biotin–avidin reaction [46] or DNA hybridization [16, 18, 47, 48]) or for use of polyelectrolytes for layer-by-layer build up. The last technique can also be used to fix quantum dots to electrodes. The advantage here is the tuning of the number of QDs on the surface and, subsequently, the magnitude of the photocurrent [49–52]. Multilayer systems can alternatively be obtained by alternating incubation steps of the electrode in dithiol and QD-containing solutions, respectively [15]. A rather unique system has been reported in which covalent bonds were established by electropolymerization of *p*-aminophenol-capped QDs to an aminophenol-modified Au electrode [53]. In addition to gold, QDs can also be immobilized on ITO electrodes by means of mercaptosilane compounds [41]. A different concept can be seen in the use of polymers, for example Nafion, for QD fixation [54].

Nanoparticles can be efficiently used to achieve rapid electron transfer from the excited quantum dots to the electrode. Examples include Au nanoparticles [8, 55], carbon nanotubes [7, 56], TiO_2 nanocrystals [9], graphene [30, 57]. This can help to avoid charge carrier recombination and thus, will increase the sensitivity for the detection of redox species in solution.

Compared with an unmodified electrode the quantum dot electrode provides an additional tool for regulation of a specific kind of reaction—the light. This also means that only the area of the electrode which is illuminated is evaluated. When different biochemical systems are immobilized at different positions on the electrode surface, parallel analysis becomes feasible. An analyte molecule can only be detected when the respective area is photoexcited. The light must then be focused on another area and the next reaction will be analyzed. Figure 2 illustrates the concept of this kind of measurement.

Resolution depends on the immobilization of the biological receptor molecule and the light system used. These measurements reflect the concept of light-addressable sensors which have been developed for chemically sensitive semiconductor devices and which are called LAPS (light addressable potentiometric sensors) [58, 59]. In contrast with LAPS, quantum dot electrodes have only a thin, semiconducting particle layer and enable electron transfer reactions to occur. As for all kinds of amperometric sensor, this provides better resolution for analyte detection and gives access to detection schemes for which no potential forming step is available as required for LAPS. This substantially increases the applicability of such systems.

Last, but not least, it should be mentioned that photocurrent measurements can be performed comparatively easily, because a three or two-electrode arrangement can be operated in an amperometric mode with the QD electrode as the working electrode [35]. However, to eliminate the background current the lock-in amplifier technique is advantageous [60]. To reduce the noise a chopped light source can be used [60]. Because of the rather wide absorption spectra of quantum dots a white light source is often used; only for mechanistic studies

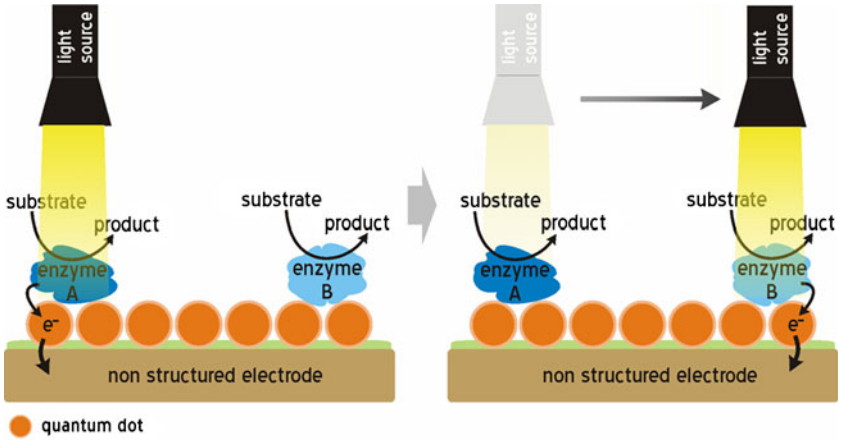

Fig. 2 Spatially resolved readout of a sensor surface with different biochemical systems immobilized on the QD electrode by illumination of specific areas

is variation of the defined wavelengths desirable [61]. Illumination power can vary in the mW range [25].

QD electrodes for detection of redox-active substances

Concentrations of redox-active substances can be detected by photocurrent measurements by choosing an appropriate potential and illumination of the electrode surface. However, the substance must react with the excited QDs. This has been shown, e.g., with the ferri–ferrocyanide system. An enhanced reduction current is observed for a CdSe–ZnS electrode under negative polarization when hexacyanoferrate (III) ions are present in solution. Thus, electron transfer from the electrode via the quantum dots to ions in solution—acting here as an electron acceptor—is achieved. As expected, no change in photocurrent is measurable at +0.25 V vs. Ag/AgCl, because all the ions are already in the oxidized state [35].

Hydrogen peroxide is a biologically active molecule, which is also the byproduct of several enzyme reactions. However, investigations at CdS and CdSe–ZnS electrodes show that detection is not feasible. To add new functionality to the light-switchable QD layer two different approaches have been tested [44]. Here the catalytic activity for hydrogen peroxide conversion of another kind of nanoparticle, FePt, is used:

1. The combination of CdS and FePt NPs by simple co-immobilization on the Au electrode surface leads to a photocurrent, the amplitude of which depends on the concentration of H_2O_2. It is found that reduction is preferred in the acidic pH region (pH5) and can be facilitated by reducing the electrode potential from −0.2 to −0.6 V vs. Ag/AgCl [44].
2. Modern synthetic procedures also enable production of hybrid NPs, for example CdS NPs grown on top of FePt NPs [62, 63]. In this way, a combination of two materials with different functionality in one particle is possible. In comparison with the mixed assembly of CdS and FePt NPs (1, above) a 2–3 times larger response to H_2O_2 has been observed. Electrons are effectively injected from the Au electrode for the reduction of H_2O_2, which results in a photocurrent with a negative sign. For CdS–FePt NPs, electrons can flow directly from the CdS to the FePt domain without crossing an external interface. Sensitivity in the range from 1 to approximately 30 $\mu mol L^{-1}$ can be achieved [44].

This example indicates one is not limited to the chemical and/or electrochemical properties of the quantum dots obtained from synthetic procedures; one can design nanoparticles in such a way that a desired conversion is facilitated.

With regard to small molecules, it has also been demonstrated, that detection of copper and silver ions is feasible at an ITO electrode modified with CdS nanoparticles. Here the photocurrent is found to decrease with increasing metal ion concentration, because of the formation of new compounds on the QD surface (e.g. Cu_xS) with energy states within the bandgap of CdS [32, 64]. Another example is the detection of cysteine with a CdS functionalized ITO electrode. In this case, however, a mediator (methyl viologen) must also be applied, which can cause side reactions [54].

QD electrodes and enzymatic signal chains based on first sensor generation

Classical biosensors of the first generation exploit the specificity of an enzyme reaction and detect conversion by following the formation of an electro-active product or the consumption of a co-substrate [65]. Following on from this idea, several enzyme reactions could be coupled with the QD electrode if a product or co-substrate can be effectively converted at the illuminated nanoparticles.

Substrates of the enzyme acetylcholine esterase can be detected in this way [45]. The enzyme catalyzes the hydrolysis of acetylthiocholine, liberating thiocholine and acetate.

The former can be oxidized at the illuminated CdS quantum dot electrode. The photocurrent reflects the substrate concentration in solution and is modified by the presence of enzyme inhibitors (at constant substrate concentration). Because acetylthiocholine is an artificial substrate, this approach is mainly directed toward analysis of potential inhibitors of acetylcholine esterase. The scheme in Fig. 3a illustrates the reaction cascade.

Another example is the electrocatalytic oxidation of NADH at CdSe–ZnS QDs [66]. NADH detection is possible in a rather wide potential range around 0 V vs. Ag/AgCl. At positive electrode potentials the anodic photocurrent increases substantially in the presence of NADH. At potentials at which a cathodic photocurrent is generated, this current decreases in the presence of NADH, indicating that electrons transferred from the electrode to the nanocrystals seem to compete with electrons from NADH. This also means that the direction of the photocurrent generated can be reversed by chemical reaction. For NADH, a cathodic photocurrent can eventually be transformed into an anodic current at an appropriate potential and high enough NADH concentration.

Because NADH is involved in many dehydrogenase reactions the quantum dot electrode system can be used to follow such catalytic reactions. The feasibility of the concept has been shown with glucose dehydrogenase (*Pseudomonas* sp.) as biocatalyst. This dehydrogenase catalyzes the reaction of β-D-glucose to D-glucono-1,5-lactone while reducing its cofactor NAD^+ to NADH. It must be mentioned here that glucose cannot be oxidized directly on the CdSe–ZnS gold electrode at low potential. Only in the presence of the enzyme a concentration-dependent photocurrent can be detected. The principle is illustrated in Fig. 3b.

Because the photoluminescence of QDs is affected by the concentration of oxygen in solution [67–70], studies have been conducted to determine whether oxygen can act as electron acceptor for excited quantum dots. At CdSe–ZnS quantum dot electrodes the magnitude of the photocurrent is clearly larger in oxygen-containing solution. The cathodic photocurrent is obviously a result of electron flow from the electrode to holes in the nanocrystals and electron transfer from the conduction band to oxygen, which increases with decreasing potential [38]. The highest currents have been observed in solutions at basic and neutral pH.

The observed oxygen sensitivity can be coupled with an oxidase to analyze the substrate of the respective enzyme reaction (Fig. 3c). This was first exemplified with glucose oxidase [38]. Different approaches can be used to achieve coupling, e.g. covalent crosslinking of GOD or layer-by-layer deposition of the enzyme by means of an oppositely charged polyelectrolyte. In both cases a rather high enzyme density must be provided to achieve oxygen depletion in front of the QD surface. Layer-by-layer deposition has the additional advantage that the surface concentration can be tuned by the number of deposition steps. With a four-layer system, sensitivity in the range 100 $\mu mol L^{-1}$ to 5 $mmol L^{-1}$ glucose can be achieved. Figure 4 shows a sensitivity graph and the photocurrent behavior for different substrate

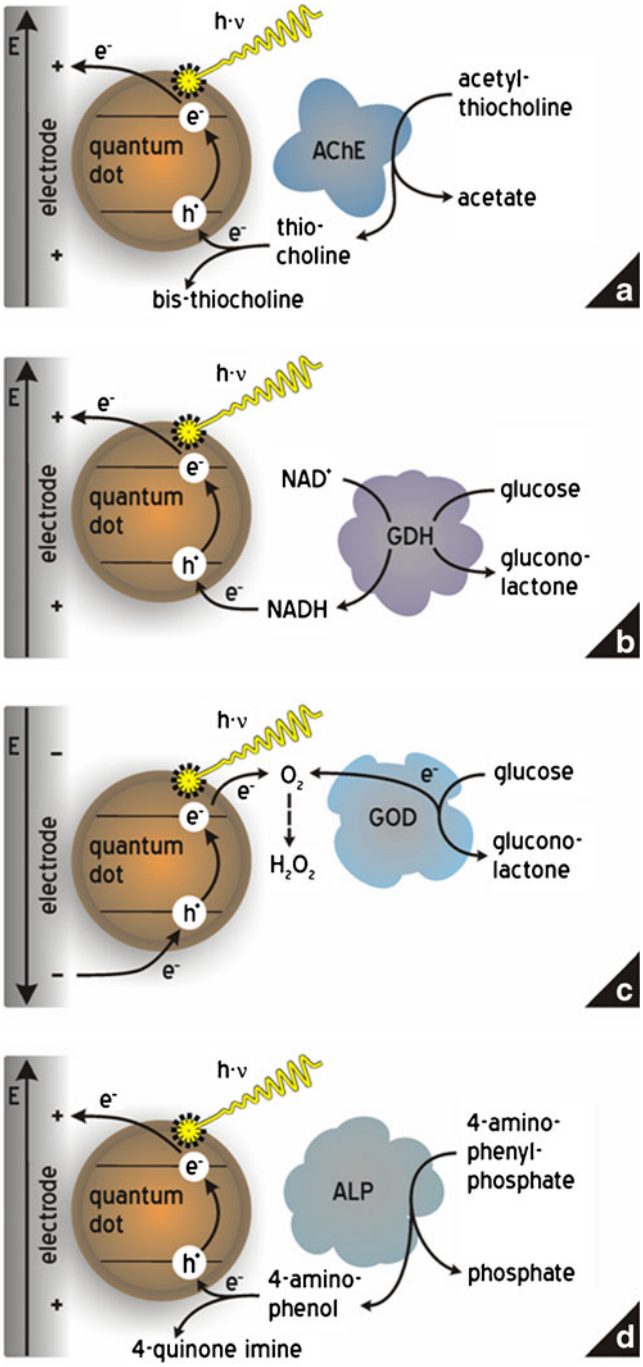

Fig. 3 Different enzymatic signal chains on quantum dot electrodes. These systems rely on enzymatic generation of a product which can be converted at the illuminated QDs (**a**, **b**, **d**) or on competing reactions at the QDs and with the enzyme (**c**). *GOD*, glucose oxidase; *AChE*, acetylcholine esterase; *GDH*, glucose dehydrogenase; *ALP*, alkaline phosphatase

concentrations. Because no substances have to be added during the analysis this is a real reagent-less sensor device. An analogous system can be demonstrated with sarcosine oxidase and sarcosine detection [71].

Another electrode structure has been developed on the basis of the oxidation of phenolic compounds on the surface of illuminated CdS nanoparticles (Fig. 3d). Here *p*-aminophenol (pAP) has been selected because it is the reaction product of the enzymatic action of alkaline phosphatase (ALP) on its substrate *p*-aminophenylphosphate (pAPP). A clear response of the photocurrent to the presence of pAP is observed, indicating that the QD electrode provides a suitable surface for oxidation of the phenolic enzyme product under positive polarization. To create a photobioelectrochemical sensor for pAPP the enzyme has been immobilized by the layer-by-layer approach. Sensitivity for pAPP detection is in the range 25 $\mu mol L^{-1}$ to 1 $mmol L^{-1}$ [60].

QD electrodes and enzymatic signal generation based on mediators

Enzyme electrodes of the so-called "second generation" apply a mediator between the biocatalyst and the electrode and thus enable new sensing schemes with rather low potential. With QD electrodes little research has been devoted to this topic. One example uses glucose oxidase and the tris-1,10-phenanthroline complex of cobalt $[Co(phen)_3]Cl_2$ [72]. A layered assembly on CdSe–CdS core-shell nanoparticles on a TiO_2 modified electrode has been created. A rather high potential was used for the analysis (+0.4 V vs. Ag/AgCl). Thus, the photocurrent generated is partly attributed to the mediated electron transfer and is affected by direct sugar oxidation.

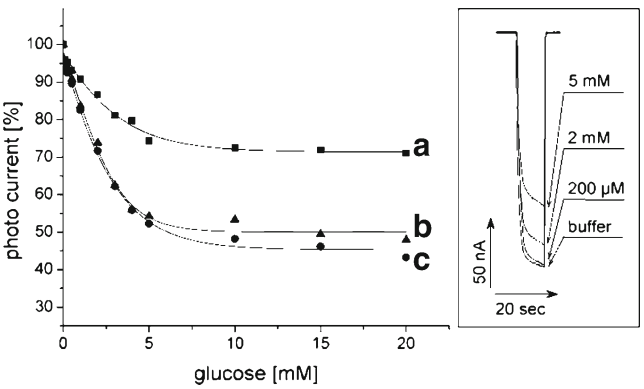

Fig. 4 *Left*: relative change of the photocurrent of Au-[QD-BDT] electrodes with immobilized $[GOD/PAH]_{n\ layers}$ with increasing glucose concentration: (*a*) $[GOD/PAH]_2$, (*b*) $[GOD/PAH]_4$, (*c*) $[GOD/PAH]_6$ (100 $mmol L^{-1}$ HEPES pH6.8; illumination time t_L=10s; E=−350 mV vs. Ag/AgCl, 1 $mol L^{-1}$ KCl). *Right*: Photocurrent behavior of an electrode with two bilayers, $[GOD/PAH]_2$, in air-saturated buffer with 200 $\mu mol L^{-1}$, 2 $mmol L^{-1}$, and 5 $mmol L^{-1}$ glucose (corresponding to curve (*a*) on the *left*)

QD electrodes and direct protein electrochemistry

Progress in direct protein electrochemistry, particularly in the last decade, has shown the benefits of direct signal transfer from the converting biomolecule to the electrode by establishing direct heterogeneous electron transfer (DET) between the electrode and the redox centre of the protein [73–75]. Nanostructures, in particular, have been found to be beneficial as modifier of electrode surfaces, because their size and properties are often suited to oriented interaction with the biomolecule and, thus, may facilitate DET reactions. Although gold nanoparticles, carbon nanotubes, and graphene have mainly been used [76–79], quantum dots as semiconducting nanoparticles have also been studied. It has been found that appropriate surface properties are essential for observation of DET with a protein. With an organic capping agent (e.g. trioctylphosphine oxide, TOPO) on the QD surface no electron transfer with the redox protein cytochrome c occurs. However, when the layer of hydrophobic surfactant is converted to a hydrophilic layer by ligand exchange using, e.g., mercaptopropionic acid or mercaptosuccinic acid, electron-transfer reactions become feasible [35]. Cytochrome c is the first redox protein for which a DET to an activated semiconducting nanoparticle has been observed. It provides the basis for construction of analytical signal chains which enable quantification of analyte molecules in solution.

As a first example the well-defined reaction of superoxide radicals with cyt c has been used [61]. This reactive species is produced under several physiological but also pathophysiological conditions; its detection is, consequently, intensively studied [80, 81]. When the superoxide radical is produced in solution, it can rapidly interact with the redox protein [82]. The reduced protein can be re-oxidized at the quantum dot electrode if the QDs are photoexcited and the electrode potential is high enough to extract electrons from the conduction band (Fig. 5a). This results in an enhanced photocurrent which can follow different radical concentrations in the nanomolar range [61].

Another means of construction of signal chains is based on the reaction of cyt c with enzyme molecules. Such biocatalytic cascades can amplify the photoelectrochemical process. One example is the coupling of a QD electrode with cyt c and the enzymatic system of lactate dehydrogenase (LDH) and lactate [17]. The anodic photocurrent is enhanced as the lactate concentration increases, and it levels off to saturation at a lactate concentration of approximately 70 $mmol L^{-1}$. The saturated photocurrent value in the system is approximately ninefold higher than the value observed with reduced cyt c only.

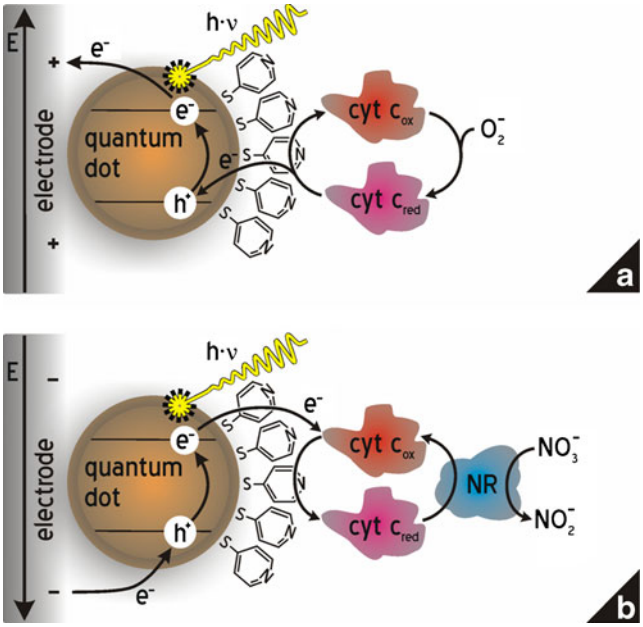

Fig. 5 Light-initiated signal chains on quantum dot electrodes which are based on direct protein electrochemistry. (**a**) Superoxide can be detected as an anodic photocurrent via cytochrome c according to Stoll et al. [61]. (**b**) Conversion of cytochrome c can be amplified by the enzyme nitrate reductase (*NR*) which can oxidize cytochrome c in the presence of nitrate. This results in a nitrate-dependent cathodic photocurrent according to Katz et al. [17]

A photo-switchable bioelectrocatalytic system with current flow in the opposite direction can be created by combining a QD electrode with cyt c and nitrate reductase (NR) [17], as illustrated in Fig. 5b. Here, also, an increased photocurrent is observed with increasing substrate (NO_3^-) concentration. The saturated cathodic photocurrent is approximately eightfold greater than in the presence of oxidized cyt c only. The amplification of the cathodic photocurrent in the presence of NR and NO_3^- is attributed to biocatalytic regeneration of oxidized cyt c at the CdS NPs interface.

It has been also suggested that embedding glutamate dehydrogenase in a composite of multiwalled carbon nanotubes, poly-diallyldimethylammonium chloride, and CdS nanoparticles can result in a photoelectrochemical sensor for glutamate for which addition of the enzyme cofactor NAD^+ is not necessary [83]. This is based on earlier work using formaldehyde dehydrogenase as biocatalyst [40]. However, in such a complex system clarification of the electron pathway needs further investigation because substances can also interact directly with the excited quantum dots as has been shown, for example, with glucose [72].

Another reported approach is coupling of horseradish peroxidase (HRP) to CdSe nanoparticles embedded in mesoporous silica spheres [84]. Here hydrogen peroxide detection under illumination is possible without addition of a mediator. An alternative system based on titania nanotubes with adsorbed enzyme HRP has also been reported [85]. Sensitivity is in the concentration range 0.5–35 $\mu mol L^{-1}$.

QDs as transduction layers for analysis of biospecific binding

For detection of binding another concept has been developed. The purpose is label-free analysis of biospecific recognition. The quantum dot electrode is modified by a specific recognition element (DNA, aptamer, or antibody); after capture of the respective reaction partner the change in photocurrent upon binding is evaluated [86]. The principle is illustrated in Fig. 6. With a hybrid system of TiO_2 and CdS, this has been used, e.g., for sensitive analysis of α-fetoprotein [87]. Alternatively QDs multilayers can be prepared by means of a polyelectrolyte, enhancing the generated photocurrent. Thus, for example, recognition of mouse IgG can be detected, without a label, as a reduced photocurrent [51]. The response can also be enhanced by use of a second binding reaction bringing Au-nanoparticles close to the QD electrode and, thus, enabling energy transfer to occur [88]. It has, furthermore, been demonstrated that capture of cells to surfaces can be detected by such an approach [52, 89]. This can be exemplified for human hepatoma carcinoma cells by use of concanavalin A and Ramos cells and a DNA aptamer.

Quantum dots as labels

Because the surface of QDs can be easily modified, immobilization of binding molecules, for example DNA, oligonucleotides, aptamers, or antibodies, is feasible with standard methods. This enables application of QDs as labels which can sense the presence of a specific molecule on the sensor electrode. However, very different modes have been used for the detection of the presence of the QD:

1. If binding brings the quantum dots into close contact with the electrode, a photocurrent can be generated [90]. Further amplification by addition of a donor or acceptor compound to the solution is feasible. If several binding molecules are fixed on the quantum dot surface, it is also possible to create a multilayered network on the electrode. When the distance between the quantum dots is small enough, interparticle electron transfer seems possible, greatly enhancing the photocurrent detected. For rather small binding molecules, for example DNA this system works well [16, 18, 47]. It has been shown that a simple method of photocurrent amplification is possible for detection of DNA. Intercalation of redox-active substances into the DNA duplex can substantially

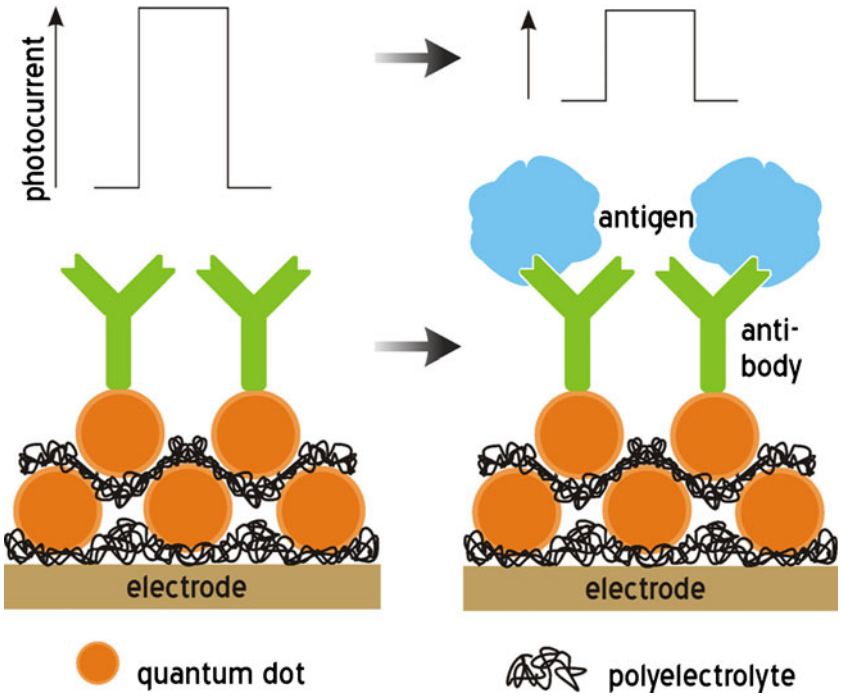

Fig. 6 Photocurrent change of a QD electrode with immobilized capture molecules on analyte (antigen) binding (modified from Refs. [52, 86])

enhance the current output, as a result of improved electronic coupling between the quantum dots and the electrode [91]. This type of approach is limited, however, when multiple protein–quantum dot layers are formed on an electrode. The proteins can hinder interparticle electron transfer, thus resulting in rather small photocurrents [49].

2. An alternative approach has been intensively exploited for detection of different biospecific binding partners. It is based on very sensitive electrochemical detection of the metal ions from which the QD were formed. This is achieved by dissolution of the surface-attached quantum dots and a subsequent voltammetric stripping analysis [92, 93]. Ultrasensitive detection schemes have been reported.
3. Another means of label detection relies on the possibility of electrochemiluminescence (ECL) generation by electrode-fixed QDs. Electrochemical reactions are used to excite the quantum dots, with the emitted light being an analytical signal corresponding to the presence or absence of a specific analyte molecule. This will be treated in more detail in the next section.

Electrochemiluminescence label

As semiconductors, QDs can be excited not only by light but also by chemical and electrochemical reactions. Emission of light is controlled by the applied electrode potential, resulting in subsequent reaction cascades [1, 94]. The advantage of this type of read-out is spatial control of the reaction by the electrode area used. In contrast with photoluminescence, no light source is needed and problems with light scattering and sample absorption can often be avoided. Thus, lower background signals can be obtained. By analogy with photoluminescence, here, the size-tunable properties of QDs can be exploited (positive shift of potential and increase in intensity with increasing size [29]). In principle multiplexing is possible although not yet really exploited. Compared with photocurrent measurements, much higher potentials are applied in ECL systems.

Another advantage of ECL is the rather simple equipment needed for analysis. Usually a three electrode arrangement is used, with Au, ITO, paraffin-impregnated graphite, or glassy carbon electrodes as working electrode (with no clear preference for one material) and an optical window in the electrochemical measurement cell. The light generated can be detected by use of a conventional fluorescence spectrophotometer, but also by a photomultiplier unit or a charge-coupled device (CCD).

Several inherent problems of QD-based ECL have been addressed in recent years. Thus, limitations in the range of solvents which can be used and the applicable pH range have been overcome, and sensitivity has been greatly improved. This seems to be mainly attributed to improved procedures for synthesis, modification, and assembly on electrodes. It provides the basis for much researches to look for analytical applications. Although the mechanisms have

also been investigated, comprehensive understanding has not yet been achieved.

In ECL-based systems, CdS, CdSe–ZnS, and CdTe are usually used as semiconductor material. Mercapto-acids, for example mercaptopropionic acid [22, 26, 95–97] or thioglycolic acid [98, 99], are often applied as capping agents, with PEG [37] or biomolecules, for example DNA or antibodies, immobilized on top. Adsorption [22, 84, 98, 100], polymers [69, 101], and covalent coupling [96, 102] are commonly used for attachment to the electrodes. Additionally QDs have been immobilized via bioaffinity reactions [103, 104]. Preparation of more complex structures including micro and nanoparticles [37, 100, 105], dendrimers [106, 107], and use of the layer-by-layer approach [105, 108] have also been reported.

Considering the mechanisms used for QD electrodes, annihilation ECL and co-reactant ECL are mainly used, with the latter being favored. Annihilation occurs when oxidized and reduced QDs are produced as a result of a change in electrode potential (e.g. by potential pulse or sweep). When electron transfer occurs between these particles, excited QDs are produced. A precondition for high efficiency is sufficient stability of the nanoparticles in the charged states. Co-reactant ECL needs, in addition to the QDs on the electrode, substances which can react with the electrode and the QDs to generate an excited state [1, 109]. Several molecules have been used for this purpose, for example tri-*n*-propylamine (TPrA), 2-(dibutylamino)ethanol, peroxodisulfate, oxygen, hydrogen peroxide, sulfite, and others.

As an example, Fig. 7 illustrates the reaction pathway elucidated for a QD electrode reacting with peroxodisulfate. The reaction is often used in cathodic ECL and normally started by a one-directional potential sweep of the electrode. The reaction cascade shows that there are several reactions occurring and reactive intermediates are formed. This can significantly complicate the mechanism, particularly when more substances are present in solution. One example is the reduction of oxygen, which can be a co-reactant for the ECL. Hydrogen peroxide, however, can also be an appropriate co-reactant, but is, at the same time, an intermediate in oxygen reduction. Thus, it is clear that the electrode material, its modification, and the potential used will affect the dominating reaction pathway [26, 27, 33, 37]. The situation can become even more complicated when the QD electrode is combined with an enzyme reaction and oxygen is catalytically converted to hydrogen peroxide [99, 110]. Very often radicals can be produced as intermediates. Superoxide seems, occasionally, to be involved in generation of the ECL [26, 30], and the hydroxyl radical has also been discussed [98].

Compared with several chemical substances, for example luminol, the ECL efficiency of QDs is rather low.

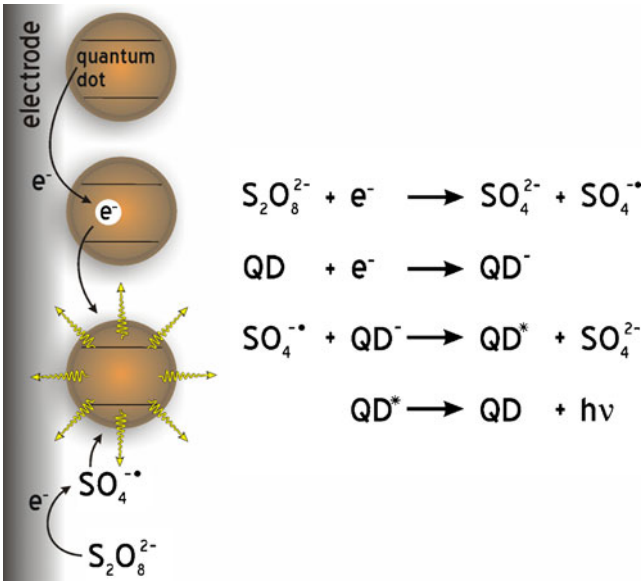

Fig. 7 Schematic illustration of an example of ECL generation by use of a co-reactant. By a cathodic sweep peroxodisulfate is reduced, liberating a radical, which reacts with a reduced QD forming the excited state (modified from Ref. [134])

Consequently, several approaches have been used to enhance the efficiency of light emission.

1. Hierarchical assembly of QDs has been shown to be beneficial compared with disordered immobilization [23, 111]. Ordered TiO_2 nanotubes have been prepared for the same reason [34].
2. Often, the surface of the QDs has been modified, e.g. by preparation of a ZnS shell (core shell particles) [112], by ammonia treatment of the QDs [113], or by means of a polyelectrolyte shell [114].
3. As a result of progress in graphene research, electrode modification with graphene or graphene oxide has also been used, because this will affect electrochemical behavior and can also reduce the magnitude of the potential which must be applied [30, 110, 115, 116].
4. QDs can be combined with other nanoparticles [97]. Carbon nanotubes, in particular, have often been used [19, 24, 28, 101, 117]. CNTs do not only affect electrochemical activity but also the active surface area and the possibility for electron transfer from/to the QDs. Use of Au and Ag nanoparticles for ECL enhancement has also been reported [100, 118, 119], although other studies have demonstrated quenching of luminescence by AuNPs [106, 120]. Thus, more research on mechanisms seems necessary. Some hints can be taken from the study of Shan et al. [22], who report a distance-dependent change from quenching to ECL enhancement.
5. The ECL signal can be enhanced by construction of supramolecular nanoclusters with a large number of QDs on the electrode surface. This has been

demonstrated, for example, with dendrimers [107] and with QD multilayers [105, 108].

Most ECL systems with QDs use cathodic electrode polarization and co-reactants to excite the QDs. However, anodic ECL has also been reported [108, 121, 122]. With both techniques the objective is reduction of the electrode potential necessary for QD excitement [34, 95, 122]. This can help to prevent undesired side reactions and thus reduce the background signal.

Many ECL systems emit light with wavelengths in the visible range. Careful shielding is therefore necessary if problems with external light sources have to be avoided. Thus, on the basis of progress in nanoparticle synthesis, systems have been developed with the objective of producing emission with near-infrared wavelengths [96, 112, 123].

By analogy with light-induced FRET (Förster resonance energy transfer), which can be beneficially used in bioanalysis, CRET can be used with QD electrodes. (E)CRET (or ERET) stands for (electro)chemical resonance energy transfer between an (electro)chemiluminescent donor and a fluorescent acceptor. The same conditions as for FRET are important: overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor and close contact of both partners. In this arrangement the QD can be the electrochemiluminescent donor and the fluorescent acceptor. Corresponding QD partners can be, e.g., Cy5 [124], $Ru(bpy)_3^{2+}$ [125], and luminol [126], but also quenchers, for example AuNP [120]. Naturally this technique is valuable for analysis of protein interactions and binding events, e.g. in immune reactions.

With regard to analytical application, research has been conducted in different directions. First the ECL of QDs has been used for analysis of substances involved as co-reactants in the light-generating process. Examples are hydrogen peroxide [24, 27, 33, 111] and oxygen [33, 110, 127]. On this basis, first-generation biosensors can be constructed. For example, for immobilized glucose oxidase the ECL signal of a QD electrode has been found to decrease with increasing glucose concentration [99]. Figure 8a illustrates the reaction principle schematically. The investigation also demonstrated that oxygen is more efficient than hydrogen peroxide as co-reactant.

In addition to this, molecules which quench or inhibit the ECL signal can be detected. Examples are catechol derivatives (for example dopamine) [26, 33, 128], methimazole [28], glutathione [36], cysteine [29], copper ions [31, 95], or lead ions [129]. However, this approach seems to be of limited practical importance, because the solution composition must be carefully controlled in order to get defined ECL signals and many redox-active substances and scavengers can enhance or inhibit light emission.

Much more attention has been devoted to the use of ECL for detection of biochemical binding events. Here the real sample can be easily separated from the process of detection by the sensor and defined solution conditions can be used. The application of QDs as labels provides access to "signal on" sensors, the signal from which is enhanced in the presence of the analyte only.

Different capture molecules have been used, for example aptamers [103, 105, 130], antibodies [21, 37, 117, 131, 132], carbohydrates and/or lectins [133], or ss-DNA [18, 22, 104]. Numerous detection schemes have been developed, based mainly on a competitive or sandwich type of assay (Fig. 8b). Very sensitive sensor systems have been reported for detection of, e.g., DNA [18, 22, 104, 120], but also metabolites and proteins. For example, ATP can be detected by use of a DNA aptamer which specifically binds to ATP and, thus, is not able to hybridize with a second, QD-coupled, DNA-strand. This signaling strand can only bind to the unbound aptamer on the electrode. The resulting ECL signal is subsequently inversely proportional to the ATP concentration [130]. An aptamer is also used for thrombin detection, on the basis of a sandwich on the electrode surface. The second aptamer, however, is coupled to a polystyrene microbead coated with several layers of CdTe QDs fixed by biotin–streptavidin binding, thus amplifying the ECL signal after recognition [105]. A sensitive immunosensor has been developed on the basis of CdTe–CdS QDs-tagged silica nanospheres as label. The sandwich type analysis is performed on an electrode modified with AuNP and graphene nanosheets [123]. Other examples include sensing schemes for biotin [46], C-reactive protein [37], α fetoprotein [115, 125], and carcinoembryonic antigen [100]. With regard to sensitivity, it has been found to be beneficial to use suprastructures with many active semiconductor nanoparticles instead of single QDs, which are attached to the surface during the binding assay. Alternatively, energy transfer systems (ERET) have been developed. These can be based on a quenching effect [22] (Fig. 8c), but also on the luminescence of a coupled acceptor compound [124, 125].

The properties of different kinds of nanoparticle can be combined advantageously for sensitive and rapid analysis. One example is the preparation of magnetic nanostructures containing CdSe-CdS QDs, thus enabling easy separation from the solution, by use of a magnet, and efficient ECL on the electrode surface [37, 114].

Electrochemiluminescence-active layer

QDs can be immobilized in different ways while maintaining the possibility of electrochemical excitation. The electrochemiluminescence signal can be regarded here as a base signal which will be modified by binding reactions on top of the QD-layer. This approach is rather similar to the change of the photocurrent of a QD electrode when specific binding occurs with a capture molecule which is fixed on the QD

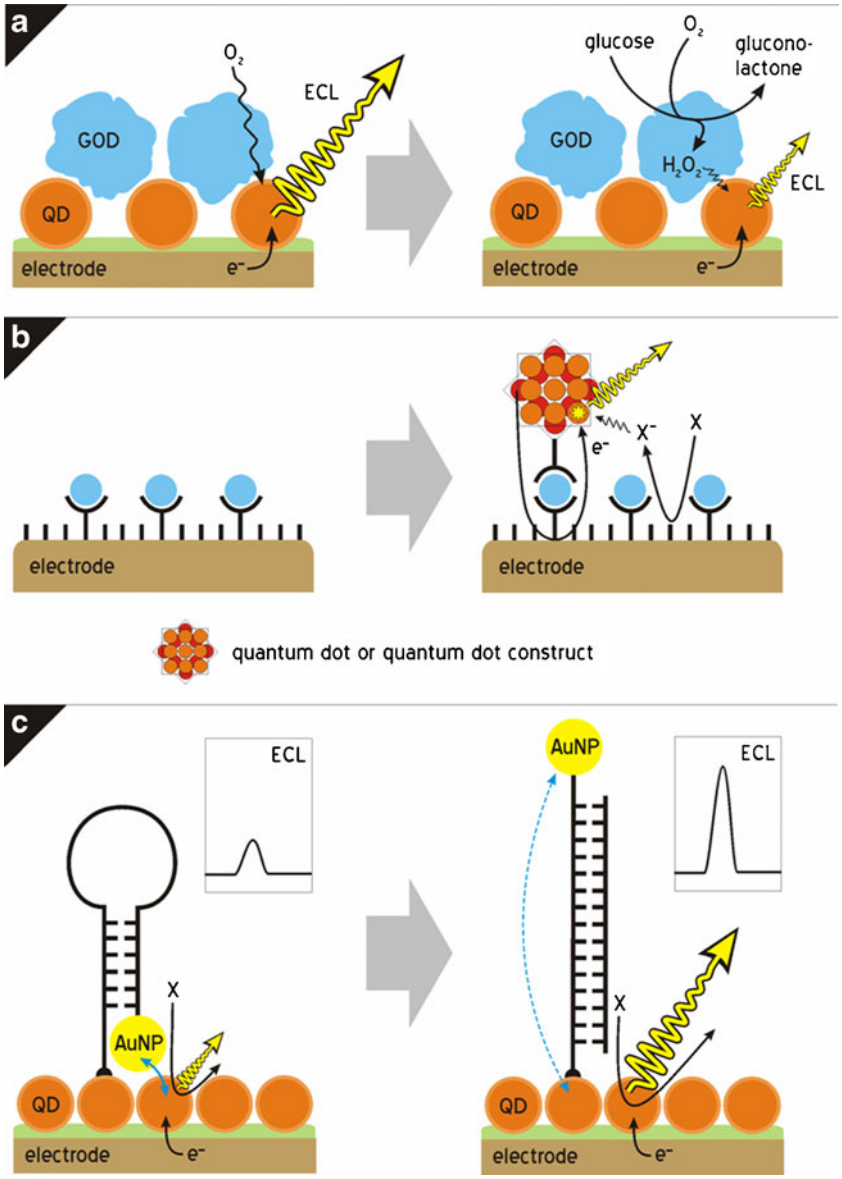

Fig. 8 Examples of the combination of QD electrodes and ECL with biocomponents. (**a**) Schematic diagram of a first-generation biosensor using ECL. Oxygen is used as co-reactant for QD ECL. Consumption of the enzyme substrate enables its detection. A precondition is low-efficiency ECL generation from hydrogen peroxide (modified from Ref. [99]). (**b**) Principle of application of QD as ECL-label in immune detection based on a sandwich-type assay. (**c**) Schematic illustration of binding detection exploiting radiation-less energy transfer. Here Au-NP act as quenchers of the ECL of exited QDs. The binding-induced conformation change results in an increase in distance, reducing the efficiency of energy transfer (modified from Ref. [22])

layer (as discussed in the section "QDs as transduction layers for analysis of biospecific binding"). For ECL systems the binding reaction may hinder the electrochemical reaction of the QD or access of the co-reactants to the electrode surface. As a consequence, the ECL signal is reduced as a result of the specific binding reaction with the immobilized capture molecules [101, 115, 117, 134]. Consequently, these systems can also be regarded as "signal off" sensors. For example, a label-free immunosensor for human IgG has been developed on the basis of a QD layer on the electrode with high ECL intensity exploiting co-immobilization of QDs, CNTs, and chitosan with subsequent coupling of antibodies. A defined decrease in ECL intensity is found after antigen (IgG) binding in the ngmL^{-1} range [101].

In addition to this, the surface-fixed QDs can be involved in energy transfer systems established on the sensing electrode. Thus, upon binding, the position of a quencher can be changed, modifying the efficiency of ECL quenching [22, 102], or resonance energy transfer between a donor and acceptor compound (or particle) is altered. An example, which has already been used in a microfluidic array, is the detection of several antigens, for example α-fetoprotein or prostate specific antigen (PSA), on a CdS nanorod array and $Ru(bpy)_3^{2+}$ as label for the second antibody [125].

Conclusions

Quantum dots are of interest in bioanalysis, not only because of their defined fluorescence properties but also because they can be combined with electrochemical techniques. In the most

simple arrangements, the particles are used solely as a source of metal ions which can be voltammetrically detected with high sensitivity. More advanced schemes exploit the possibility of charge-carrier generation inside the semiconducting nanoparticles. Thus, these systems are at the border between electrochemistry and spectroscopy. Whereas for photocurrent measurement light energy is converted into a current signal, in electrochemiluminescence (ECL) systems the electrical energy is converted into emitted light. Thus, for the former QD electrodes a spatially resolved read-out seems feasible; for ECL systems a sensitive and comparatively simple detection technique can be used for analysis of binding reactions.

The combination of QDs with electrodes is not only attractive in bioanalysis but also for energy-converting systems and catalysis. Thus, further research will focus not only on applications but also on a better understanding of underlying mechanisms. Particularly interesting from current perspectives are combined or hybrid nanoparticles which can integrate different features in one nanostructure.

References

1. Algar WR, Tavares AJ, Krull UJ (2010) Anal Chim Acta 673(1):1–25
2. Bakkers EPAM, Roest AL, Marsman AW, Jenneskens LW, de Jong-van Steensel LI, Kelly JJ, Vanmaekelbergh D (2000) J Phys Chem B 104(31):7266–7272
3. Miyake M, Torimoto T, Nishizawa M, Sakata T, Mori H, Yoneyama H (1999) Mat Sci Eng C 15(8):2714–2718
4. Bakkers E, Kelly J, Vanmaekelbergh D (2000) J Electroanal Chem 482(1):48–55
5. Günes S, Sariciftci NS (2008) Inorg Chim Acta 361(3):581–588
6. Tada H, Fujishima M, Kobayashi H (2011) Chem Soc Rev 40(7):4232–4243
7. Robel I, Bunker BA, Kamat PV (2005) Adv Mater 17(20):2458–2463
8. Kamat PV (2002) J Phys Chem B 106(32):7729–7744
9. Robel I, Subramanian V, Kuno M, Kamat PV (2006) J Am Chem Soc 128(7):2385–2393
10. Kongkanand A, Tvrdy K, Takechi K, Kuno M, Kamat PV (2008) J Am Chem Soc 130(12):4007–4015
11. Rajeshwar K, de Tacconi NR, Chenthamarakshan CR (2001) Chem Mater 13(9):2765–2782
12. Tristão JC, Magalhães F, Corio P, Sansiviero MTC (2006) J Photochem Photobiol A 181(2–3):152–157
13. Rajendran V, Lehnig M, Niemeyer CM (2009) J Mater Chem 19(35):6348–6353
14. Ding Z (2002) Science 296(5571):1293–1297
15. Nakanishi T, Ohtani B, Uosaki K (1998) J Phys Chem B 102(9):1571–1577
16. Willner I, Patolsky F, Wasserman J (2001) Angew Chem Int Ed 40(10):1861–1864
17. Katz E, Zayats M, Willner I, Lisdat F (2006) Chem Commun (13):1395–1397
18. Guo Y, Sun Y, Zhang S (2011) Chem Commun 47(5):1595–1597
19. Ding S, Xu J, Chen H (2006) Chem Commun (34):3631–3633
20. Fang Y, Song J, Zheng R, Zeng Y, Sun J (2011) J Phys Chem C 115(18):9117–9121
21. Jie G, Liu P, Wang L, Zhang S (2010) Electrochem Commun 12(1):22–26
22. Shan Y, Xu J, Chen H (2009) Chem Commun (8):905–907
23. Wang CZ, Yifeng EF, Fan LZ, Wang ZH, Liu HB, Li YL, Yang SH (2007) Adv Mater 19(21):3677–3681
24. Wang X, Zhou Y, Xu J, Chen H (2009) Adv Funct Mater 19(9):1444–1450
25. Yue Z, Khalid W, Zanella M, Abbasi AZ, Pfreundt A, Rivera Gil P, Schubert K, Lisdat F, Parak WJ (2010) Anal Bioanal Chem 396(3):1095–1103
26. Liu X, Jiang H, Lei J, Ju H (2007) Anal Chem 79(21):8055–8060
27. Han H, Sheng Z, Liang J (2007) Anal Chim Acta 596(1):73–78
28. Hua L, Han H, Chen H (2009) Electrochim Acta 54(5):1389–1394
29. Hua L, Han H, Zhang X (2009) Talanta 77(5):1654–1659
30. Wang Y, Lu J, Tang L, Chang H, Li J (2009) Anal Chem 81(23):9710–9715
31. Zhang L, Shang L, Dong S (2008) Electrochem Commun 10(10):1452–1454
32. Shen Q, Zhao X, Zhou S, Hou W, Zhu J (2011) J Phys Chem C 115(36):17958–17964
33. Chen L, Lu L, Mo Y, Xu Z, Xie S, Yuan H, Xiao D, Choi MM (2011) Talanta 85(1):56–62
34. Liu X, Hou Z, Lei J, Ju H, Cai J, Shen J (2011) Electroanal 23(11):2629–2632
35. Stoll C, Kudera S, Parak WJ, Lisdat F (2006) Small 2(6):741–743
36. Dennany L, Gerlach M, O'Carroll S, Keyes TE, Forster RJ, Bertoncello P (2011) J Mater Chem 21(36):13984–13990
37. Wang S, Harris E, Shi J, Chen A, Parajuli S, Jing X, Miao W (2010) Phys Chem Chem Phys 12(34):10073–10080
38. Tanne J, Schäfer D, Khalid W, Parak WJ, Lisdat F (2011) Anal Chem 83(20):7778–7785
39. Willner I, Katz E (2005) In: Katz E, Shipway AN (eds) Bioelectronics. From theory to applications. 11. Molecular optobioelectronics. Wiley–VCH, Weinheim
40. Curri M, Agostiano A, Leo G, Mallardi A, Cosma P, Della Monica M (2002) Mat Sci Eng C 22(2):449–452
41. Hickey SG, Riley D (2000) Electrochim Acta 45(20):3277–3282
42. Amelia M, Impellizzeri S, Monaco S, Yildiz I, Silvi S, Raymo FM, Credi A (2011) Chem Phys Chem 12(12):2280–2288
43. Hickey SG, Riley DJ, Tull EJ (2000) J Phys Chem B 104(32):7623–7626
44. Khalid W, El Helou M, Murböck T, Yue Z, Montenegro J, Schubert K, Göbel G, Lisdat F, Witte G, Parak WJ (2011) ACS Nano 5(12):9870–9876
45. Pardo-Yissar V, Katz E, Wasserman J, Willner I (2003) J Am Chem Soc 125(3):622–623
46. Baş D, Boyacı IH (2009) Electroanal 21(16):1829–1834
47. Baş D, Boyacı IH (2011) Anal Bioanal Chem 400(3):703–707
48. Xu J, Weizmann Y, Krikhely N, Baron R, Willner I (2006) Small 2(10):1178–1182
49. Göbel G, Schubert K, Schubart IW, Khalid W, Parak WJ, Lisdat F (2011) Electrochim Acta 56(18):6397–6400
50. Hojeij M, Su B, Tan S, Mériguet G, Girault HH (2008) ACS Nano 2(5):984–992
51. Wang G, Yu P, Xu J, Chen H (2009) J Phys Chem C 113(25):11142–11148
52. Zhang X, Li S, Jin X, Li X (2011) Biosens Bioelectron 26(8):3674–3678
53. Granot E, Patolsky F, Willner I (2004) J Phys Chem B 108(19):5875–5881
54. Long Y, Kong C, Li D, Li Y, Chowdhury S, Tian H (2011) Small 7(12):1624–1628
55. Sheeney-Haj-Ichia L, Pogorelova S, Gofer Y, Willner I (2004) Adv Funct Mater 14(5):416–424
56. Sheeney-Haj-Ichia L, Basnar B, Willner I (2005) Angew Chem Int Ed 44(1):78–83

57. Zhang X, Li S, Jin X, Zhang S (2011) Chem Commun 47(17):4929–4931
58. Hafeman D, Parce J, McConnell H (1988) Science 240(4856):1182–1185
59. Bratov A, Abramova N, Ipatov A (2010) Anal Chim Acta 678(2):149–159
60. Khalid W, Göbel G, Hühn D, Montenegro J, Rivera-Gil P, Lisdat F, Parak WJ (2011) J Nanobiotechnol 9(1):46
61. Stoll C, Gehring C, Schubert K, Zanella M, Parak WJ, Lisdat F (2008) Biosens Bioelectron 24(2):260–265
62. Gu H, Zheng R, Zhang X, Xu B (2004) J Am Chem Soc 126(18):5664–5665
63. Zanella M, Falqui A, Kudera S, Manna L, Casula MF, Parak WJ (2008) J Mater Chem 18(36):4311–4317
64. Wang G, Xu J, Chen H (2010) Nanoscale 2(7):1112–1114
65. Scheller F, Schubert F, Wollenberger U (1989) Biosensoren. Birkhäuser, Basel
66. Schubert K, Khalid W, Yue Z, Parak WJ, Lisdat F (2010) Langmuir 26(2):1395–1400
67. van Sark WGJHM, Frederix PLTM, van den Heuvel DJ, Gerritsen HC, Bol AA, van Lingen JNJ, de Mello Donegá C, Meijerink A (2001) J Phys Chem B 105(35):8281–8284
68. Frederix PLTM, Bol AA, Gerritsen HC, Meijerink A (2002) Chem Phys Chem 3(10):871–879
69. Koberling FF, Mews AA, Basche TT (2001) Adv Mater 13(9):672–676
70. Müller J, Lupton JM, Rogach AL, Feldmann J, Talapin DV, Weller H (2004) Appl Phys Lett 85(3):381
71. Riedel M, Göbel G, Abdelmonem AM, Parak WJ, Lisdat F (2013) Chem Phys Chem (in press)
72. Zheng M, Cui Y, Li X, Liu S, Tang Z (2011) J Electroanal Chem 656(1–2):167–173
73. Armstrong F (2000) Electrochim Acta 45(15–16):2623–2645
74. Murphy L (2006) Curr Opin Chem Biol 10(2):177–184
75. Lisdat F, Dronov R, Möhwald H, Scheller FW, Kurth DG (2009) Chem Commun (3):274–283
76. Pumera M, Sánchez S, Ichinose I, Tang J (2007) Sensor Actuator B Chem 123(2):1195–1205
77. Rivas G, Rubianes M, Rodriguez M, Ferrayra N, Luque G, Pedano M, Miscoria S, Parrado C (2007) Talanta 74(3):291–307
78. Shao Y, Wang J, Wu H, Liu J, Aksay IA, Lin Y (2010) Electroanal 22(10):1027–1036
79. Willner I, Baron R, Willner B (2007) Biosens Bioelectron 22(9–10):1841–1852
80. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Int J Biochem Cell B 39(1):44–84
81. Büttemeyer R, Philipp A, Schlenzka L, Mall J, Beissenhirtz M, Lisdat F (2003) Transplant P 35(8):3116–3120
82. Wegerich F, Turano P, Allegrozzi M, Möhwald H, Lisdat F (2009) Anal Chem 81(8):2976–2984
83. Tang L, Zhu Y, Yang X, Sun J, Li C (2008) Biosens Bioelectron 24(2):319–323
84. Yang X, Wang P, Zhu Y, Li C (2011) J Solid State Electro 15(4):731–736
85. Chen D, Zhang H, Li X, Li J (2010) Anal Chem 82(6):2253–2261
86. Kang Q, Yang L, Chen Y, Luo S, Wen L, Cai Q, Yao S (2010) Anal Chem 82(23):9749–9754
87. Wang G, Xu J, Chen H, Fu S (2009) Biosens Bioelectron 25(4):791–796
88. Zhao W, Wang J, Xu J, Chen H (2011) Chem Commun 47(39):10990–10992
89. Qian Z, Bai H, Wang G, Xu J, Chen H (2010) Biosens Bioelectron 25(9):2045–2050
90. Golub E, Pelossof G, Freeman R, Zhang H, Willner I (2009) Anal Chem 81(22):9291–9298
91. Gill R, Patolsky F, Katz E, Willner I (2005) Angew Chem Int Ed 44(29):4554–4557
92. Wang J, Liu G, Merkoçi A (2003) J Am Chem Soc 125(11):3214–3215
93. Kuçur E, Boldt FM, Cavaliere-Jaricot S, Ziegler J, Nann T (2007) Anal Chem 79(23):8987–8993
94. Frigerio C, Ribeiro DS, Rodrigues SSM, Abreu VL, Barbosa JA, Prior JA, Marques KL, Santos JL (2012) Anal Chim Acta 735:9–22
95. Mei Y, Wang H, Li Y, Pan Z, Jia W (2010) Electroanal 22(2):155–160
96. Liang G, Shen L, Zou G, Zhang X (2011) Chem Eur J 17(37):10213–10215
97. Ding S, Gao B, Shan D, Sun Y, Cosnier S (2012) Chem Eur J 18(6):1595–1598
98. Jiang H, Ju H (2007) Anal Chem 79(17):6690–6696
99. Jiang H, Ju H (2007) Chem Commun (4):404–406
100. Jie G, Liu P, Zhang S (2010) Chem Commun 46(8):1323–1325
101. Jie G, Zhang J, Wang D, Cheng C, Chen H, Zhu J (2008) Anal Chem 80(11):4033–4039
102. Han E, Ding L, Lian H, Ju H (2010) Chem Commun 46(30):5446–5448
103. Huang H, Zhu J (2009) Biosens Bioelectron 25(4):927–930
104. Huang H, Li J, Tan Y, Zhou J, Zhu J (2010) Analyst 135(7):1773–1778
105. Chen Y, Jiang B, Xiang Y, Chai Y, Yuan R (2011) Chem Commun 47(27):7758–7760
106. Jie G, Yuan J, Huang T, Zhao Y (2012) Electroanal 24(5):1220–1225
107. Jie G, Wang L, Yuan J, Zhang S (2011) Anal Chem 83(10):3873–3880
108. Wan F, Yu J, Yang P, Ge S, Yan M (2011) Anal Bioanal Chem 400(3):807–814
109. Shin I, Kim H, Bang JH (2011) J Electroanal Chem 663(1):24–29
110. Deng S, Lei J, Cheng L, Zhang Y, Ju H (2011) Biosens Bioelectron 26(11):4552–4558
111. Xu Y, Song C, Sun Y, Wang D (2011) Mater Lett 65(12):1762–1764
112. Wang J, Jiang X, Han H, Li N (2011) Electrochem Commun 13(4):359–362
113. Wang H, Zhang F, Tan Z, Chen Q, Wang L (2010) Electrochem Commun 12(5):650–652
114. Jie G, Wang L, Zhang S (2011) Chem Eur J 17(2):641–648
115. Guo Z, Hao T, Duan J, Wang S, Wei D (2012) Talanta 89:27–32
116. Li L, Liu K, Yang G, Wang C, Zhang J, Zhu J (2011) Adv Funct Mater 21(5):869–878
117. Jie G, Li L, Chen C, Xuan J, Zhu J (2009) Biosens Bioelectron 24(11):3352–3358
118. Shi C, Shan Y, Xu J, Chen H (2010) Electrochim Acta 55(27):8268–8272
119. Wang C, Yifeng E, Fan L, Yang S, Li Y (2009) J Mater Chem 19(23):3841–3846
120. Zhou H, Liu J, Xu J, Chen H (2011) Anal Chem 83(21):8320–8328
121. Liu X, Ju H (2008) Anal Chem 80(14):5377–5382
122. Zhang L, Zou X, Ying E, Dong S (2008) J Phys Chem C 112(12):4451–4454
123. Wang J, Han H, Jiang X, Huang L, Chen L, Li N (2012) Anal Chem 84(11):4893–4899
124. Li L, Hu X, Sun Y, Zhang X, Jin W (2011) Electrochem Commun 13(11):1174–1177
125. Wu M, Shi H, He L, Xu J, Chen H (2012) Anal Chem 84(9):4207–4213
126. Sun L, Chu H, Yan J, Tu Y (2012) Electrochem Commun 17:88–91

127. Zheng R, Fang Y, Qin S, Song J, Wu A, Sun J (2011) Sens Actuator B: Chem 157(2):488–493
128. Bao L, Sun L, Zhang Z, Jiang P, Wise FW, Abruña HD (2011) J Phys Chem 115(38):18822–18828
129. Wang H, Chen Q, Tan Z, Yin X, Wang L (2012) Electrochim Acta 72:28–31
130. Huang H, Tan Y, Shi J, Liang G, Zhu J (2010) Nanoscale 2(4):606–612
131. Liu Q, Han M, Bao J, Jiang X, Dai Z (2011) Analyst 136(24):5197–5203
132. Qian J, Zhang C, Cao X, Liu S (2010) Anal Chem 82(15):6422–6429
133. Han E, Ding L, Jin S, Ju H (2011) Biosens Bioelectron 26(5):2500–2505
134. Jie G, Huang H, Sun X, Zhu J (2008) Biosens Bioelectron 23(12):1896–1899