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Review

Trends in DNA biosensors

F.R.R. Teles, L.P. Fonseca*

IBB-Instituto de Biotecnologia e Bioengenharia, Centro de Engenharia Biológica e Química, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

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ABSTRACT

Biosensors have witnessed an escalating interest nowadays, both in the research and commercial fields. Deoxyribonucleic acid (DNA) biosensors (genosensors) have been exploited for their inherent physicochemical stability and suitability to discriminate different organism strains. The main principle of detection among genosensors relies on specific DNA hybridization, directly on the surface of a physical transducer. This review covers the main DNA immobilization techniques reported so far, new micro- and nanotechnological platforms for biosensing and the transduction mechanisms in genosensors. Clinical applications, in particular, demand large-scale and decentralized DNA testing. New schemes for DNA diagnosis include DNA chips and microfluidics, which couples DNA detection with sample pretreatment under in vivo-like hybridization conditions. Higher sensitivity and specificity may arise from nanoengineered structures, like carbon nanotubes (CNTs) and DNA/protein conjugates. A new platform for universal DNA biosensing is also presented, and its implications for the future of molecular diagnosis are argued.

Contents

* Corresponding author. Tel.: +351 218419139 65; fax: +351 218419062.
E-mail address: luis.fonseca@ist.utl.pt (L.P. Fonseca).

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1. Introduction

The enormous amount of genetic information brought by extensive genome sequencing has raised the need for simple, fast, cheap and high-throughput miniaturized and mass-producible analytical devices to attend the growing market of molecular diagnostics, thus accomplishing the basic criteria for decentralized DNA testing. Genome sequencing has allowed detecting, respectively, inherited disease-causing point mutations and human pathogens through their peculiar, specific nucleic acid sequences. Drug screening, monitoring of differential gene expression and forensic analysis have also benefited from the ongoing research in biosensor technology. Such analytical devices, known as biosensors, convert a biochemical reaction or interaction into an analytical signal that can be further amplified, processed and recorded. Among them, DNA biosensors consist of an immobilized DNA strand to detect the complimentary sequence by DNA–DNA hybridization. In a wider conception, DNA biosensors may still be conceived to detect other analytes, with the probe molecule usually in the form of an aptamer [1], but the study of these sensors is beyond the scope of this review. For their importance, large variety and widespread applications compared to other types of DNA biosensors, those based, for instance, on distinctive interactions of small analytes with single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) and in polymerase chain reaction (PCR) amplicon detection without hybridization are the subject of hybridization-based DNA biosensors justifies, by itself, a new and comprehensive overview, something that this paper intends to be. Compared to enzyme biosensors and immunosensors, there is still a scarcity of DNA biosensors available in the market and/or under research and development. Unlike enzymes or antibodies, DNA forms biological recognition layers easily synthesizable, highly stable and reusable after simple thermal melting of the DNA duplex [2]. In general, the underlying mechanism of quantitative DNA detection through DNA biosensors is the highly specific hybridization between two complimentary DNA chains which, unlike in conventional solid-state hybridization formats, occurs directly on the surface of a physical transducer. Conventional DNA microarrays also make use of sequence-specific DNA detection, but their efficiency is usually hampered by the typically large size of biological samples and by their complex treatment, which also makes it difficult to obtain real-time outputs. Moreover, their technology is still too expensive to turn them valuable in point-of-care diagnosis. In theory, DNA biosensors are able to surpass these handicaps, allowing easier, faster and cheaper results than in traditional hybridizing assays, while keeping high sensitivity and specificity of detection. A truly high performance biosensor with an immobilized DNA-probe should be able to discriminate as few as a single base-pair mismatch between different target DNA-strains. DNA multiplexed analysis of complex biological samples and related gene expression patterns have been performed with microarrays of multiple DNA biosensors, integrated with bioinformatics-processed data. In general, they are produced in the form of DNA biochips, inspired by the unending advances in planar silicon-based circuitry. The very high density of individual hybridization spots is a major highlight in microchip-based genetic analysis. However, this technology is highly costly and, unlike individual biosensors, biochip surfaces must be scanned for acquisition of full information about the genomic hybridization profile [3]. The newly developed concept of 'lab-on-a-chip' (or micro total analytical system, μTAS) integrates, in a single chip, modules for DNA extraction, purification, amplification and detection. Some advantages of these printable miniaturized devices for analyte detection include smaller sample and reagent requirements, lower cost and lower tendency for sample contamination than other detection schemes. Enhanced rapidity, high performance and high automation ability are also additional advantages. Disposability is also an advantage, especially when dealing with infectious agents. Innovative efforts have been assayed towards the development of electrical-driven microfluidic flow formats as advantageous alternatives to mechanical pumps and valves. The paper also covers some recent developments in nanotechnology, namely CNTs and DNA/protein conjugates, which are responsible for improved sensitivity and selectivity in DNA detection. Despite not being a hybridization-based platform for DNA detection – the ultimate subject of this paper – important applications of the mass-spectroscopy (MS)-based T5000 Universal Biosensor, from Ibis Biosciences, is also mentioned. This pioneering system, by using sets of broad-range primers, is able to amplify PCR products from a large number of closely related organisms without prior knowledge of their specific genomic sequences. By accurately determining the nucleotide composition (the amount of each nucleotide) of the unknown sequence through mass spectroscopy (MS), the identification of PCR products may be carried out almost instantaneously. The following text gives an overview of the DNA biosensors research and background, as well as current trends for the forthcoming future.

2. Fundamentals of hybridization DNA biosensors

Conventional methods for specific genomic sequence analysis include nucleic acid sequencing and hybridization, the later more routinely used in clinical laboratories due to its higher simplicity [4]. DNA hybridization usually occurs between a known DNA sequence (probe) and an unknown counterpart (target), but DNA–ribonucleic acid (RNA) and RNA–RNA hybridizations can also occur [5]. The duplex formation can be detected following the association of an appropriate hybridization indicator or through other changes accrued from the binding event. DNA probes may be produced by chemical methods or by molecular biology; in this case, a probe may be obtained by reverse-transcription (RT) of a previously isolated and specific messenger RNA (mRNA), or inferring its nucleotide sequence based on the amino acid sequence of the protein expressed by that DNA, despite the validity of this last strategy may be limited due to the genetic code degeneracy [6]. Conventional nucleic acid hybridization methods, like gel electrophoresis and Southern blotting, are usually lengthy and labor-intensive [7], and is also the intrinsic biomolecular recognizing event of most genosensors. However, in this case, it occurs directly on the surface of a physical-transducer [8]. In this way, the immobilized DNA-chain is a part of the biosensor itself. Both in vivo as onto a transducer surface (solid support), nucleic acid hybridization is stronger and more specific when the complimentarity degree between two DNA chains increases. The specificity and stability of the linkage reach a maximum in the case of full (100%) complimentarity. However, the molecular mechanisms of hybridization over solid supports are still greatly unknown and unpredictable, owing to the difficulty of accurately determine the concentration of the immobilized nucleic acid. Even so, it is commonly assumed that the relevant events in the solid/liquid interface are the analyte diffusion towards the surface of the sensor, bidimensional diffusion, adsorption and desorption [9]. Despite the similarity between the hybridization processes in solution and at an interface, the hybridization rate is typically dozens of times higher in the former case, assuming identical DNA sequences and conditions. This may be due to the partial unavailability of many linking groups in the immobilized chain, eventually involved in that immobilization process. The hybridization rate also decreases with the secondary structure level of one or both chains. This fact can be easily avoided with a proper selection of the probe-sequence. Moreover, the

hybridization mechanism between DNA chains with long-sized secondary structures is far more complex than that described by the traditional two-state model [10]. As in solution, the interfacial hybridization efficiency must be optimized in relation to environmental conditions (e.g.; ionic strength and temperature) and requires minimization of non-specific adsorption [11,12]. Among the most important factors affecting the hybridization efficiency is the surface coverage (Γ) of the transducing surface; above a certain density of attached DNA-probe molecules, steric effects between them become dominant, as well as repulsions between the incoming targets [13]. An ideal surface around 5×10^{12} molecules/cm^2 was estimated [14]. Repulsion of the target also depends on the ionic strength of the buffer solution and, in accordance, it was determined that, with 1 M phosphate buffer with a surface coverage up to 3×10^{12} molecules cm^{-2}, no significant electrostatic interference or steric effects between chains occur [15]. Unlike enzymes and antibodies, nucleic acids from biological recognition layers easily synthesizable in the laboratory, highly stable and readily reusable after thermal heating. Short, synthetic oligonucleotides are often preferred as sensing elements in view of the absence of complex conformational changes which decrease the hybridization speed, efficiency and selectivity. The diagnosis of infectious diseases with DNA biosensors permits to distinguish different strains of a pathogen by suitable choice of strain-specific DNA probes and to obtain an earlier diagnosis compared to immunosensors [6]. Despite the ultimate goal of autonomously determining DNA traces in clinical samples, it is still required, in general, previous PCR amplification until reaching detectable DNA levels [5]. It is convenient that hybridization does not require separation of unbound labeled probed from the matching probe–target complexes and, to achieve high detection sensitivity, amplification of the probe–target complex by PCR is usually required. Despite its ability to render, in principle, unlimited sensitivity and amplification, PCR settings are usually too complex, prone to easy contamination and require skilled manpower and bulky equipment for in-the-field applications [16]. PCR has been recently integrated in μTAS systems, a concept corresponding to the integration, in a single miniaturized device, of modules for DNA extraction, purification, amplification and detection, thus lowering contamination, reagent consumption and the time of detection. Additionally, the hybridization event occurs in a liquid instead of a solid phase, a similar condition to the in vivo microenvironment. Ongoing efforts with DNA chips, however, persecute the task of producing PCR-free DNA detection systems, despite this has not been fully achieved yet in commercially available devices [1]. The detection of the DNA duplex formation often makes use of a hybridization indicator (marker), but other changes in the system may be monitored [3]. Experimental findings showed that the interaction of cations with DNA is not affected by DNA immobilization onto a solid support [17]. In addition, the marker must not hinder significantly the formation of hydrogen bonds between the probe and the target, as well as the melting temperature (T_m) of the duplex [6]. In view of the biological hazard of the former radioactive markers, new kinds of DNA labeling have emerged. DNA immobilization is undoubtedly the key-step in genosensor development; the traditional lack of affinity and stability of DNA chains in solid surfaces has greatly benefited from recent advances in biocompatible polymer matrices production.

3. DNA-probe immobilization techniques

The use of polymeric supports in genosensors aims to overcome some traditional limitations of DNA detection in gold and glass surfaces, namely the low surface density of silanic groups – which hinders a high surface concentration of the immobilized oligonucleotide – and the high cost. Other goals include the maximization of sensitivity, surface functionality, DNA bonding density, DNA-layer stability and accessibility of interacting molecules, as well as minimization of non-specific linkages [18]. Hydrophilic surfaces are particularly amenable for nucleic acid hybridization because they facilitate exposure of hybridizing bases [19], despite being also proner to DNA detachment with increasing ionic strength [20]. Nonetheless, studies carried out with immobilized negatively charged human albumin in anionic latex showed that it is possible to link the two surfaces through a positive-potential barrier formed by the cations of the saline electrolyte [21]. However, the high hydrophobicity of silanic films and the surface tension of oligonucleotide solutions allow confining them in very small spots, thus preventing eventual mixing and cross-contamination in a microarray configuration [22]. Recently, conductive polymers have been used in biosensors for their unique advantageous electronic properties, including high electronic affinity. In general, the electrochemical response (current) with conductive cationic polymers decreases after DNA hybridization, presumably due to hindrance of the anionic exchange or to polymer reorganization [23]. On the other hand, the successful immobilization of DNA onto anionic polymers with cation exchanger-functional groups (e.g., quinine) may be attributed to the formation of a positive shield of solution cations around the DNA probe [24]. Nevertheless, the introduction of amino groups is still the main strategy to functionalize solid surfaces with biomolecules, like the covalent immobilization of oligonucleotides in bare silicon and onto polyethylenimine-coated nylon microspheres [25,26]. Several polymers may also be used simultaneously, like a polymeric gel of polyvinyl-alcohol crosslinked to polyallylamin chloride and poly(L-lysine) in polystyrene-modified surfaces [18,27]. Despite DNA attachment to solid surfaces is usually stronger via covalent linkages, adsorption may be preferred due to its slighter effect over DNA structure, which avoids its breakdown [28]. Chitosan, for example, is a natural cationic polymer which tightly binds the polyanionic DNA chains (both native and denatured), yielding a very stable immobilization [29]. However, maximization of detection sensitivity is usually achieved through one-point covalent immobilization of DNA which, by minimizing sterical hindrance, greatly favors formation of the DNA duplex. Previous works describe the use of polypyrrol in DNA biosensor build-up, by immobilization of the polymer itself or electropolymerization of the monomers directly on the surface of a transducer [30,31]. This technique exhibited high versatility since the sensor was able for reusing after simple rinsing, without altering the polymeric matrix of the immobilized polypyrrol. Bidan and coworkers used an electrocopolymerization process to immobilize an oligonucleotide [32]. A mixture of pyrrol and pyrrol covalently attached DNA was electrooxidized, resulting in irreversible immobilization of the oligonucleotides in a polypyrrolic copolymer onto an array of gold microelectrodes. The technique of self-assembled monolayers (SAMs), formed by a brief immersion of the transducer surface into a dilute solution of the polymer at room temperature, spontaneously generates an ultrathin and highly ordered layer, similar to the cell microenvironment. This monolayer strongly adsorbs to the solid surface and is thermodynamically very stable. The versatility of SAMs for several applications arises from the possibility of controlling the hydrophilicity degree and the chain length of the polymer [33]. The utilization of SAMs avoids conformational changes over the immobilized biocomponent, capable of affecting its activity. This assures higher homogeneity and reproducibility of the electrode surface. Furthermore, the molecular scale of the biolayer allows rapid diffusion of the electroactive species towards the electrode surface, in comparison with the slower kinetics observed with thin polymeric films or composites. SAMs also reduce drastically

non-Faradaic currents and electrode passivation [34]. SAMs of gold-attached aminoalcanothiols are undoubtedly the most studied and employed, taking advantage of the very high affinity in S–Au bonds. Bifunctional aminoalcanothiols allow straightforward one-point covalent immobilization of DNA through its 5′-phosphate end [35]. Nonetheless, the applicability of monolayers remains restricted owing to their poor stability and difficult synthesis.

4. New biosensor platforms

One of the major trendlines towards the research of novel diagnostic systems is the concept of DNA chips (or DNA microarrays), usually associated to microfabrication of diagnostic kits by screen-printing techniques, inspired by planar, silicon-based technologies. This aims to produce very high dense microband sensor arrays coated with different probes for simultaneous detection of multiple DNA-target sequences (with or without a label) printed on the chip by conventional photolithography [4]. DNA microarrays (DNA chips) for multiple and simultaneous target detection have been extensively used for studying genomic structure and gene expression. Despite microarrays should not be considered true biosensors – in the sense of simple, cheap and portable devices – they achieve high efficiency of analytical processing and use microfabrication techniques for highly selective immobilization of their recognition elements [36]. The ongoing progress from microarrays to biosensors will certainly be mediated by the production of disposable microchips, thus obviating the typical limitations of current microarrays. These include the difficulty of scaling-down the array and nucleotide densities, the limited resolution and the different optimal hybridization conditions between A–T and C–G linkages, which hinders the use of a single set of optimized parameters in the same chip and therefore a reliable DNA quantitative analysis, and strong sample concentration-dependence [37]. Despite the ability of DNA biochips to detect many genes in a single assay, detection at the cellular level without previous genomic sequence amplifications remains limited [38]. Bearing this task in mind, it is noteworthy the growing interest on the last few years for microfluidic analysis schemes and devices [39], essentially an adaptation of DNA chips to contain channels and chambers for flowing liquids. They integrate, in a single chip, modules for DNA extraction, purification, amplification and detection. Electroosmotic pumping is the most common technique to propagate the flow in these systems. Such flow is generated by the surface charge on the microchannel walls combined with an electric field along the microchannel. In these devices, chemical interferences arising from spatial confinement of the biorecognition and transduction elements may be avoided, thus allowing miniaturization with efficient signal transfer and highly sensitive detection. The high mass transfer rate thus achieved results from the low diffusional distance and high surface/volume ratio [40]. Gulliksen et al. detected herpes virus (HPV)-related synthetic sequences in a pioneer work with real-time nucleic acid sequence-based amplification (NASBA) for direct amplification of RNA, reaching a detection limit of 10^{-6} μM, similar to that of standard diagnostic procedures [41]. Conventional microarrays usually require relatively high volumes of reagent and solution. The diffusional rate of the reacting biomolecules is usually small, rendering long hybridization times. Wei et al., by integrating the concepts of μTAS and microarrays, was able to diminish the sample and reagent volumes to 1 μl, and the hybridization time for less than 10 min, reaching a detection limit of 19 attomole [42]. Electrokinetically driven separation schemes have been widely used to separate and detect desired analytes. They are based on the interaction of induced dipole in the bioparticles and electric fields, and are used for moving fluids through a channel network [43]. The method was implemented to detect single-nucleotide polymorphisms (SNPs) in a μTAS platform, with tight temperature control on the microarray interface, thus avoiding the need for common external temperature sensors [44]. Partial hybridization between non-complimentary chains may thus be avoided, thus enhancing the selectivity of the detection. An emergent topic in the development of new bioanalytical procedures, structures and systems is nanobiotechnology. A brand new range of electronic devices and biosensor platforms has emerged as a consequence of the inherent small size and unusual optical, magnetic, catalytic and mechanical properties of nanoparticles, unlike those of bulk materials. Moreover, with an appropriate transducing method, the selectivity of nanobiosensors may be tuned as a result of signal-dependence on nanoparticle morphology [45]. It is foreseeable, by technological and industrial reasons, the fabrication of future nanochips and nanofluidic systems as an extension of current mechanical methods for production of microsensing devices most often based on organic polymers and gels, especially PDMS frames, but the recent outburst of nanotechnology is creating a demand for a broader range of low-cost and easy fabrication methods. This may correspond essentially to shifting from top-down methods – which begin with a patterned, larger-scale layout, and reducing its dimensions to bottom-up methods, by building-up nanostructures from atoms or molecules. Such bottom-up methods are proner to cheap and easy production of small nanostructures. The sudden rise in the expected cost/benefit of miniaturizing photolitographically produced microsystems has pointed towards the assembly of micromechanical systems and functional biomimetic structures in the 5–100 nm range [46].

5. Transduction mechanisms in genosensors

5.1. Optical

5.1.1. Optical fibers

Many DNA optical biosensors use an optical fiber to propagate the signal emitted by a fluorescent label. In general, a DNA single-chain probe is placed in the end of the fiber and, after hybridization with the complimentary chain, changes in the fluorescence intensity resultant from the selective association between the DNA duplex and the label are measured. Piunno et al. used ethidium bromide as a hybridization indicator [47]. It strongly intercalates between base pairs and stacks into the major-grooves of a double-chain DNA. The hybridization event was detected by fluorescence, by measuring the total internal reflexion in the optical fiber, which is proportional to the total amount of intercalated ethidium bromide. Despite being regenerated even after prolonged storage and aggressive washings, the sensitivity was not very high compared to those of PCR and conventional nucleic acid hybridization. Another handicap is the biohazard concern of working with such a carcinogenic compound, which has triggered the search for substitutes [5]. Fergusson et al. developed a fiber-optical array biosensor for simultaneous detection of multiple oligonucleotide sequences, registering the fluorescence increase after hybridization [48]. An optical microarray-based biosensor with zeptomolar detection was developed by individually attaching the tips of a fiber-optic bundle to microspheres coated with different DNA probes, which were further identified by combinations of different fluorescent labels [49]. Biosensors based on optical fibers are suitable for miniaturization, due to the very small diameter of the fibers. By transmitting light for very long distances without signal lost, they allow remote detection of inaccessible or dangerous samples. The optical nature of the signal also avoids interference from electrical noises and, for being harmless, is appropriated for in vivo applications. These biosensors

have usually poor stability and are prone to interference from environmental light, apart the high cost of quartz optical fibers for UV light transmission.

5.1.2. SPR and evanescent waves

Until a few decades ago, the wide spreading of optical fibers motivated the consideration of photonic devices as natural substitutes for microelectronic circuits and chips, but their size and performance are constrained by the diffraction limit, resulting from interference between closely spaced light waves. However, optical transmission through minuscule structures gained a new impulse with the advent of the surface plasmon resonance (SPR) technique, by directing light waves to the interface between a metal and a dielectric. A real-time SPR system under continuous flow was applied to DNA detection, through an immobilized biotinylated probe in an avidin-coated chip, and further binding of the target-DNA [50]. Systems like these render high specificity, within 10 min, at room temperature [6]. SPR was also the basis for optimization of a DNA biosensor, in real-time, onto a gold surface previously functionalized with polypyrrol, aiming the future fabrication of DNA chips [51]. In this work, several hybridization events were detected simultaneously by an electrospotting technique, dispensing the use of thiolated reagents and several immobilization steps. Recently, Buhl and coworkers formulated a SPR biosensor chip to detect pathogenic dsDNA auto-antibodies produced by patients with systemic lupus erythematosus [52]. In accordance, an antigenic construct was formed by coupling a synthetic oligonucleotide with biotinylated human transferrin and hybridizing with the complimentary strand. The set was then linked to a human recombinant double-strand fragment and covalently immobilized in a flow-through cell; healthy and diseased sera were then compared. This format assured maximal stability for multiple serum injections and regeneration cycles, a benefic feature for clinical diagnosis and monitoring. Another well-known type of optical biosensor is the resonant mirror, an evanescent wave sensor that combines the simplicity of SPR devices with the enhanced sensitivity of wave-guiding devices [5]. These biosensors measure variations in the surface optical parameters caused by the biochemical reaction (e.g. DNA hybridization), namely the interfacial refractive index. They have found notable applications in detecting human genetic mutations and nanomolar levels of PCR products from genetically modified organisms [53,54]. The great interest of evanescent waves for biosensor applications emerges from unnecessary target-chain labeling, rapidity of the hybridization reaction (within a few minutes) and 100-fold or greater probe reutilization. A significant drawback is the somewhat low sensitivity, requiring up to 10 μg of DNA per milliliter [8].

5.1.3. Gold nanoparticles

Colorimetric biosensors have been traditionally used for DNA detection as an alternative to fluorescence tagging. Colorimetric systems are attractive for detection because they are harmless, simple and relatively inexpensive. As an example, it was proposed as a method for visual, qualitative and simultaneous detection of HIV, hepatitis C virus (HCV) and reverse-transcribed hepatitis B virus (HBV) genomes in infected blood samples with a DNA chip [55]. It was employed, in this work, a multiplex PCR for concomitant amplification and detection of all nucleic acid sequences present [56]. Detection was accomplished by color formation from an avidin-bound alkaline phosphatase reaction with a signal amplifier, and a detection limit of 1 pg of viral DNA fragments was achieved. An alternative to DNA labeling for optical detection may be performed by using functionalized gold nanoparticles (GNPs), having comparatively higher stability and lower background noise than fluorescence tagging [57]. Colorimetric biosensors were recently

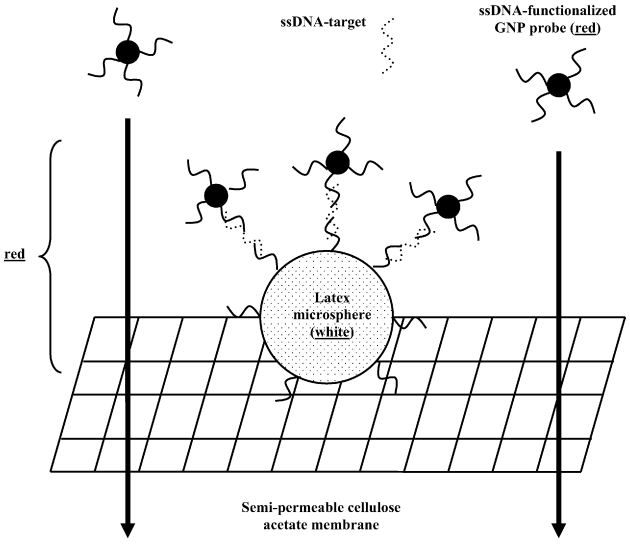

Fig. 1. Layout for DNA colorimetric detection with GNPs and latex microspheres. Free, red-colored ssDNA-functionalized GNPs freely move across the semi-permeable cellulose acetate membrane. White-colored latex microparticles, on the other hand, are too large to pass through that barrier. In the presence of an ssDNA-target, GNPs bind latex particles, generating large-size, red-colored conjugates, which become retained by the membrane.

built in conjunction with DNA/nanoparticle conjugates. An interesting scheme for drastic reduction of the background signal was developed [58] by coupling GNPs to latex microparticles. Both types of particles are linked to ssDNA-probes, which are complimentary of a given DNA-target strand. Typically, the red color of dispersed GNPs turns into blue when aggregated polymeric networks are formed upon extensive hybridization. This color variation can be measured by spectrophotometry or at naked eye, onto a solid silica-gel support. However, red and blue are not easily distinguishable at naked eye and the blue color is considerably less intense than the red of dispersed GNP probes. The use of white latex microspheres obviates such handicaps. Background signal from unbound GNP is significantly reduced by filtering the solution with the target and the probes through a size-selective cellulose acetate membrane. The unbound GNP probes pass through the membrane, while the larger latex particles are trapped (Fig. 1). An advantage of this system is that a large excess of GNP probes can be employed without interfering with signal interpretation. The method is rapid, sensitive, simple inexpensive and suitable for the utilization of other types of microspheres and nanoparticles. Metal nanoparticles, in general, are suitable for construction of high-density miniaturized DNA microarrays, taking advantage of their high signal-to-noise ratio. They are easily synthesizable and functionalized (by simple mixing at room temperature) and have a controlled, self-assembled surface structure [59]. Most GNP-based detection systems rely on the formation of aggregates of extended interconnected tridimensional networks by DNA hybridization. Most commonly, two sets of GNPs are used; each one binds a different DNA probe, and each probe binds one end of the target chain. Since each particle has multiple DNA-bound tentacles, the specificity of the target-sequence glues many particles together. Blueshifting of the emission spectrum thus occurs, and the resulting color change may then be observed at naked eye. In nanoparticle-based SPR systems, the complicated chemistry for the Au-SAM synthesis may produce structural defects in the DNA chips during mass-production, thus compromising the reproducibility and reliability of the detection. To overcome this setback, SPR was coupled to interferometry to detect picomolar amounts of synthetic and PCR-amplified DNA

sequences in a gold-deposited porous anodic alumina layer chip [60]. Gold deposition onto the chip formed a highly ordered cap-like layer on the top of the oxide nanostructures. The relative reflected intensity at the surface of the chip was strongly dependent on the effective thickness of the biomolecular layer. This format permits rapid detection of DNA in small volumes with disposable chips, which makes it amenable for miniaturization and mass-production. An interesting work [61] used a SPR imaging technique for monitoring selective deposition of GNPs modified with ssDNA and dsDNA, at high salt concentrations, in microchannel walls of a microchip formed by a surface-patterned polydimethylsiloxane (PDMS) plate bound to a gold thin film-deposited glass substrate. The detection limit thus achieved was 19 fmol and, in the form of a SPR portable device, is a promising tool for point-of-care analysis of SNPs. By avoiding the use of complex and expensive instrumentation, DNA/nanoparticle-based colorimetric biosensors seem quite promising for point-of-care diagnosis.

5.1.4. Quantum dots

One of the most important nanostructures generated by bottom-up approaches is the quantum dot, a type of nanoparticles for fluorescence tagging of probe biomolecules. They differ from conventional organic fluorophores for being much brighter (for having higher quantum yields) and photostable. In addition, their color can be directly correlated with their size, with emission of a single, well-defined wavelength after excitation (a higher size corresponding to a higher emitted wavelength); they have broad absorption spectra and narrow emission spectra with large emission shifts, which permits excitation at wavelengths far-removed from their emission peaks [62]. The fact of nearly all quantum dots of different emission peaks can be excited using a single, short-wavelength excitation source, is a powerful tool for monitoring several components in complex biological systems [63]. These properties make dots of different sizes able to be used as distinguishable labels for different targets [64]. An application of quantum dot-based DNA analysis is a surface plasmon-enhanced fluorescence microscopy detection scheme in a microarray format [65]. The resonant excitation of evanescent surface plasmons mode excites the dots chemically attached to the target molecules, giving rise to increased sensitivity for analyte monitoring. As a consequence of exciting several dot populations with a single light source, a single incidence angle for resonance surface plasmon is necessary. It has been demonstrated that quantum dots can undergo fluorescence resonance energy transfer (FRET) phenomena, basically a cascade energy transfer process from species with larger bandgaps to species with smaller ones [66]. This principle was used to build a biosensor with two target-specific probes-a fluorophore-tagged reporter and a biotinylated capture probes – and a quantum dot labeled with streptavidin molecules [67]. In the presence of DNA-target, an assembly is formed between all these structures. The result is fluorescence emission from the acceptor fluorophores by means of illumination of the quantum dot donor, thus indicating the presence of target. In this configuration, quantum dots thus serve as FRET energy donors as well as target concentrators. As the unhybridized probes do not participate in FRET and do not fluoresce, their removal is unnecessary. The detection limit of this system is 100-fold higher than that of a similar conventional FRET probe-based assay with confocal fluorescence spectroscopy, and therefore does not require target pre-amplification. Recently, Feng and colleagues reported the use of quantum dots as biomarkers to functionalize nanotubes for enhanced sensitivity of DNA detection [68]. The incorporation of the quantum dots in the nanotubes was carried out by the well-known layer-by-layer deposition approach. The key feature of this scheme is an efficient energy-transfer process that occurs from the larger bandgap quantum dots in the outer side of the nanotubes to the smaller bandgap quantum dots in the inner side, as an intrinsic energy ramp through the nanotube walls exists. The resulting sensitivity enhancement suggests a potential utility for detection of trace amounts of DNA [69].

5.1.5. Other systems

Electrochemiluminescence involves light generation near an electrode through species that undergo highly energetic electron-transfer reactions with solution reagents. It has a wide linear range of chemiluminescence and does not require the use of complex and expensive light sources and fluorescent dyes. Lee et al. [70] used the intercalator ruthenium bipyridine ($Ru(bpy)_3^{2+}$) and suitable detection of hybridization by electrochemiluminescence, with Ru complexes exhibiting excellent chemical stability and a relatively prolonged excited state. An interesting scheme to improve the detection of the hybridization signal is the utilization of DNA beacons as DNA probes [71]. A DNA beacon is a single-stranded oligonucleotide labeled with a fluorophore in one extremity and a quencher in the other; the close proximity between them, due to the stem-and-loop (hairpin) format, prevents emission of fluorescence. When the molecule becomes linearized as a consequence of hybridization with the complimentary chain, the system becomes fluorescent. It is possible, with this system, to achieve high specificity and single-base resolution in the picomolar range [72]. The major handicap of this technique, aiming the fabrication of DNA sensors and microarrays for real-time and simultaneous analysis of different targets, is the high cost and tedious preparation of hundreds of different probes, each one modified with the fluorophore and the quencher. Instead of the common methods that use an oligonucleotide probe with a covalently linked fluorophore, a new strategy employs a fluorescently labeled universal reporter strand, which binds a reporter-binding region in the hairpin [73]. This region has a base sequence common in all sequence-specific probes. By using a single sequence of fluorescently labeled universal reporter strand for all targets, a cheaper and simpler procedure is achieved. This scheme was also the basis for signal amplification with liposomes for detection and serotype-discrimination of dengue virus after amplification of the viral genome by NASBA [74]. This sandwich-system integrates a liposome-coupled reporter-probe (complimentary to a generic sequence added to RNA during the amplification step) and a membrane-immobilized biotinylated capture-probe. The number of DNA-tagged liposomes is proportional to the amount of RNA in the sample, being detected by electrochemiluminescence with a portable reflectometer within 15 min. A slight variation of this layout was the immobilization of a magnetic sphere-attached capture probe onto a permanent magnet in a detection region, built in a microfluidic format [75]. Liposomes were filled with a dye marker, thus yielding high signal amplification and sensitivity by fluorescence microscopy. Based upon the two layouts described above, a biosensor was built by integrating immobilization onto a polyethersulfone membrane with a dG-enriched universal reporter-probe and optical detection with a dye-filled liposome attached to a deoxycitosine (dC)-enriched universal probe [36]. The reporter and capture probes may be easily and quickly changed to become specific to the target-sequence. After optimization of this biosensor, previously amplified bacterial sequences were readily identified and quantified in less than 30 min. The same detection scheme was applied to the specific identification of the four dengue virus serotypes in a single, multianalyte assay, instead of four independent assays [76]. One of the most revolutionary achievements among DNA/protein conjugates is the one of single-molecule detectors; they mimetize the cellular protein machinery which reads and copies one single nucleic acid molecule at the time, with single-base resolution [8]. A common optical approach for single-molecule detectors utilizes

a SiO_2 surface-attached DNA polymerase, with real-time monitoring of the incorporation of fluorophore-labeled nucleotides into a growing DNA chain, by using a different fluorophore for each nucleotide [77]. A 'lab-on-a-chip' microsystem integrated with photolithographically patterned polymeric layers and interferometric detection was applied for real-time and label-free detection of DNA hybridization [78]. An ultrasensitive system was conceived to quantify nucleic acid traces using confocal fluorescence spectroscopy and a microfluidic reactor for molecular confinement of an ultrasmall volume of 1 fl [38]. By detecting single-molecule fluorescence, a further step for removing unbound probe molecules is avoided, with clear reduction of the overall hybridization assay cost; the detection limit was 14 zmol. Recently, a microfluidic sensor array for specific detection of ribosomal RNA-targets of several bacterial pathogens in human fluids was produced [79]. After RNA extraction, its detection was performed by immunofluorescence with labeled antibody-conjugated horseradish peroxidase (HRP); a detection limit of 2600 cultured bacterial cells was achieved within 45 min. It achieved 100% sensitivity for Gram-negative bacterial detection without previous RNA purification or amplification.

5.1.6. Commercial optical biosensors

It is noteworthy the commercial success of several optical sensing platforms in the last few years. It is unquestionable the advantage that the very high frequency of optical signals – compared, for instance, with electrical ones – may bring in terms of the enormous amount of information that can be carried by optical devices. Some well-succeeded commercial platforms include, for example, the GeneChip® high-density (high spatial resolution of individual probes) microarray from Affymetrix (Santa Clara, CA, USA), with fluorescence-based detection coupled to a confocal readout, which became the industry standard for molecular biology research. This microarray probably encloses the highest information capacity among similar chips, enabling a whole-genome approach in research studies. The GeneChip® can sequence some many thousand bases in a few days with almost very high accuracy, a clear advantage for pathogen subtyping. In addition, Affymetrix has largely benefited from its world leadership position as the first microarray ever commercialized and from resulting economical patent benefits. Moreover, this technology has exhibited improved performance and capabilities over other existing methods. Perhaps the most serious competitor for the GeneChip® is the fluorescence-detecting microbead-based BeadXpress® array system from Illumina (San Diego, CA, USA), also an industry-leading in genotyping. The high sensitivity of the BeadXpress® (owing to inherent stringency of code detection), wide multiplexing capability, assay versatility in a single platform (including a broad range of applications, e.g. nucleic acid and protein-based assays) and dual-color detection (through the industry-standard Infinium Whole-Genome Genotyping Assay) are claimed highlights [80]. BIAcore (real-time biospecific interaction analysis) SPR-based platforms have been also at the forefront of the commercial biosensor market; so far, they have been responsible for 90% of all published optical biosensor work. The BIAcore system of Pharmacia Biosensor AB (Uppsala, Sweden), is especially suitable for real-time monitoring of biological events under continuous flow [6]. A carboxymethyl dextran matrix-coated surface has been widely employed in these systems as a convenient way for applying numerous surface immobilization chemistries, for immobilized ligand stabilization and for reducing non-specific binding. It is envisaged that many of these systems may be applied to several biochemical assays, including DNA- and protein-arrays, and eventually replace the well-known enzyme immuno-assay (EIA). It seems clear that implementing sensor arrays with multiple-sample delivery, while being simple in concept, will require improved technological development fueled by strong commercial demands [81].

5.2. Piezoelectric (mass-sensitive)

A DNA sequence with a few hundred base pairs usually possesses a sufficiently high molecular weight so that the mass increase caused by hybridization of a DNA-chain with its complimentary counterpart previously immobilized on the surface of a piezoelectric quartz crystal may be specifically correlated with an increase in the fundamental resonance frequency of the crystal. This is the principle of the well-known quartz crystal microbalance (QCM). Campbell et al. used the quartz microbalance principle to detect the hybridization of DNA covalently bound to a polymer-modified piezoelectric crystal, thus achieving a near 100 Hz frequency increase compared to a control crystal to which a non-complimentary target was hybridized [82]. Similar results were obtained after DNA hybridization onto a polypyrrolic matrix [83]. Some studies reported the improvement of QCM hybridization efficiency and sensitivity by immobilizing biotin-labeled DNA multilayers in modified gold surfaces [84]. Detection of a cancer-causative mutation in the human TP 53 gene was reported with a piezoelectric transducer, by using a dextran–streptavidin surface-immobilized biotinylated probe [85]. The sensor was optimized with synthetic DNA sequences and successfully validated with PCR-amplified DNA samples, being amenable for application in routine analysis. An innovative approach was developed, with piezoelectric transduction, by designing and immobilizing a degenerate probe (chosen among a conserved genomic region) and two specific probes (chosen from less-conserved regions) for, respectively, simultaneous detection and genotyping of 16 strains of the human papilloma virus [86]. This is a straightforward method for detection, with highly specific probes, of microorganisms with high mutation rates. Besides QCM, other methods can be employed for mass detection with a piezoelectric crystal. In surface acoustic wave (SAW) devices, an electrode array in the material generates local deformations that are transmitted ahead as mechanical waves to a receiver electrode array. The interaction of these waves with a surface material changes the SAW speed and amplitude, thus enabling quantification of the deposited mass [87]. The classical SAW principle was recently applied to the fabrication of a DNA sensor in a microfluidic format [88]; the DNA-probe sequence modified with a thiol group was immobilized onto a gold surface and exhibited a sensitivity of 136 pg ml^{-1} Hz^{-1}. A similar effect can also occur in the inner transducing material with bulk acoustic waves (BAWs). Zhang's team built a BAW biosensor for on-line detection of damaged DNA, based on mass decreasing after DNA breakdown induced by UV radiation [89]. Some studies correlated resonance frequency changes with DNA concentration-dependent viscosity [90]. These biosensors can provide single-base resolution [91]. Bioanalytical applications require operation of the mass-sensing device in the liquid phase, a troublesome task owing to the typical sensitivity decrease and complex influence of multiple interfacial parameters, namely the viscosity of the surface and sample solution, the surface energy and roughness, the effect of compressional waves, the ionic strength and the dielectric constant of the electrolyte [92,93]. These effects are especially predominant when resonance frequency shifts are measured, but microcantilever platforms may circumvent this event. In a microcantilever, in addition to resonance frequency alterations, surface stress caused by the forces involved in the DNA adsorption process also occurs, and this parameter is less prone to environmental effects. When the adsorption is limited to a single surface of the cantilever, that surface becomes subjected to bending, an effect that can be amplified by making both surfaces of the cantilever chemically different [94]. A microcantilever

DNA biosensor using the micro-balancing technique was developed by incorporating a piezoelectric film, which acts both as a sensor and an actuator [95]. Unlike cantilever formats using optical detection schemes (e.g., light deflection after cantilever bending), the piezoelectric sensor does not need many external hardware and equipment, and hence is more suitable for the production of integrated analytical devices. Nevertheless, a recent report claims the development of a novel compact optical read-out scheme based on light transmission by single-mode waveguides through the cantilever structure (which also acts as a waveguide) [96]. As the cantilever deflects, less light can couple between the cantilever and the output waveguide, thus decreasing the optical output. Since a bulky external read-out detection system is not used, the production of a portable device can be envisaged. A good sensitivity was obtained, but improvements are expected by making longer and thinner cantilevers. Nanomechanical cantilevers are potentially useful for real-time monitoring, which is the basis for the description and characterization of dynamic interactions at sensor surfaces [97]. By fabricating devices with many nanocantilevers and coating each one with a different type of DNA, rapid screening of biological samples for the presence of specific genetic sequences can be performed without previous labeling. Nanocantilevers are emerging as the basic sensing-structures in array-based microsystems for sub-nanometer resolution of DNA sequences [98].

5.3. Electrochemical

In electrochemical biosensors, a single-chain of DNA is immobilized onto an electrically active surface (electrode), being measured changes in electrical parameters (e.g., current, potential, conductance, impedance and capacitance) caused by the hybridization reaction. The emergence of solid electrodes has improved enormously the applicability of electrochemical methods for nucleic acid analysis, as reviewed below.

5.3.1. Enzyme indirect detection

Enzymatic labels are commonly used to generate electrical signals for detection of DNA hybridization. The enzyme, previously bound to the DNA probe, triggers the catalysis of a redox reaction and further generates an electrochemical change due to the hybridization event. Lumley–Woodyear et al. monitored the duplex formation with a carbon fiber transducer, using a horseradish peroxidase-labeled DNA-target [99]. The resulting electrorreduction of H_2O_2 was followed by amperometry, with single base-pair resolution. An ingenious strategy was developed for detection of single-base DNA mutations by using a biotinylated nucleotide complimentary to a mutated residue in the DNA-target [100]; the subsequent binding of an avidin-bound alkalin phosphatase promotes a chemical reaction that generates a precipitate. By opposition, the non-mutated DNA-target molecule does not bind the enzyme conjugate and, therefore, does not generate the precipitate. In this study, electrochemical impedance spectroscopy (EIS) and QCM were employed for the analysis of the Tay-Sachs genetic disorder, reaching a detection limit of 10^{-14} M with no PCR pre-amplification. Efficient amplification schemes were achieved by labeling peroxidase with liposomes, with impedimetric detection, to detect PCR products with pulse techniques, as well as sequences related with human cytomegalovirus [101,102]. A dot-blot-based amperometric biosensor was produced for detection of a *Staphylococcus aureus* β-lactamase-producer oligonucleotide, using peroxidase and a graphite/epoxy electrode with a nylon membrane [103]. By using two biotinylated probes (in the 5′- and 3′-ends, respectively) instead of only one and previous PCR amplification of the bacterial DNA, a sensitivity of 10^5–10^6 bacteria was obtained, and a decrease in the overall assay time from 4–5 days to 36 h. Labeling enzymes are usually conjugated with a single DNA molecule [104]. However, a work reporting the conjugation of glucose oxidase with several oligonucleotide sequences showed significant signal amplification due to the various hybridization events and therefore an increased sensitivity [105]. Enzyme amplification schemes for DNA sensing are among the most successful for PCR-free detection in real, biological samples.

5.3.2. Label-based (indirect) detection

Electroactive hybridization indicators bind single-chain DNA and double-chain DNA with different affinities, thus resulting in unequal concentrations near the electrode surface and therefore in a variation of the electrochemical response. The ways of interaction with DNA include electrostatic attraction (to the sugar-phosphate backbone), intercalation and groove-binding (within the double-helix). Heterocyclic dyes (e.g., methylene blue and ethidium bromide), ferrocene derivatives and organometallic complexes are among the most widely used redox indicators. Pioneering studies to detect deoxyguanosine (dG)-elongated polynucleotides by cyclic voltammetry (CV), using the metallointercalators $Co(bpy)_3^{3+}$ (bpy = bipyridin) and $Co(phen)_3^{3+}$ (phen = phenanthroline), immobilized DNA onto a glassy carbon electrode (GCE) through covalent attachment with *N*-hydroxysuccinimide and a carbodiimide, and an octadecylamine/stearic acid-modified carbon paste electrode (CPE) for cystic fibrosis [106,107]. Electrochemical adsorption after positive polarization (pretreatment) of carbon paste and screen-printed electrodes was accomplished in order to increase their affinity for human immunodeficiency virus (HIV) and HBV-related DNA sequences [108,109]. Detection was performed by chronopotentiometry, using $Co(phen)_3^{3+}$ as the hybridization indicator. It was also reported the covalent immobilization of DNA onto a SAM using methylene blue and daunomycin as labels [110,111]. Jin's team reported the self-assembled immobilization of a thiolated hairpin DNA-probe sequence onto a gold electrode and specific discrimination of a complimentary target DNA from both a single-base mutation and a random oligonucleotide [112]. Detection was carried out by CV, using methylene blue as electroactive hybridization indicator. Results showed that the greatest effect on the hybridization with a hairpin DNA probe is achieved when the mutation occurs in the center of the DNA-target sequence. Ferrocene, a dsDNA electrostatic- and groove-binder, was used to electrochemically detect yeast DNA covalently attached to a SAM-functionalized gold electrode [113] and a dengue-related oligonucleotide sequence with a chitosan-coated GCE [114]. Xu et al. reported the use of ferrocene derivatives as electrochemical hybridization labels, suitable for covalent labeling of DNA [115]. This type of DNA labeling is more costly, labor-intensive and complex than non-covalent binding (due to the synthesis, labeling and product separation steps), but provides a stronger and more stable attachment to the DNA probe. However, new intercalators with higher electrochemical sensitivity are under investigation. An example is ferrocenylnaphtalene-diimide (*N*,*N*c-Bis[[4-(3-aminopropyl)-piperazinyl]propyl]-naphthalene-1,4,5,8-tetracarboxylic acid diimide), which displays higher affinity for a double-chain and negligible affinity for a single-chain DNA than classical intercalators, with sensitivities in the zmol range [116]. Traditional schemes of DNA biochips with fluorescent detection are well known, but Hashimoto et al. developed an analogue system with electrochemical detection (Fig. 2), allowing to avoid the complexity of fluorescent labeling and an expensive laser device for excitation [117,118]. In particular, screen-printed electrodes are not only prone to mass-production, but also more mechanically resistant than traditional carbon-paste electrodes; these, in addition, also suffer from poorly reproducible manufacture. With standard photolithographic components, electrochemical

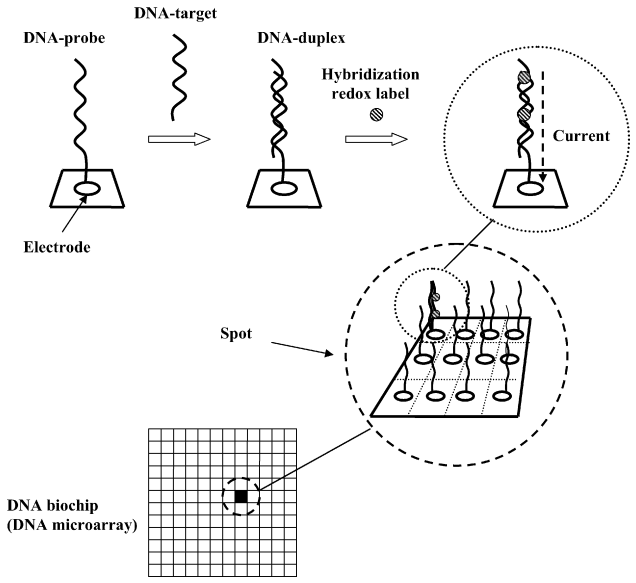

Fig. 2. DNA biochip with electrochemical detection. An unlabeled DNA-target chain hybridizes with a known electrode-bound DNA-probe chain. Further attachment of a hybridization redox label to the DNA duplex generates a current level that can be assigned to a known, specific DNA-probe sequence. The resulting current pattern in each spot corresponds to a certain level of gene expression.

detection in 20 μm gaps in a microelectrode deposited onto a silicon chip was performed with a sandwich technique, using a reporter-probe, a DNA-target and a GNP-bound capture-probe [184]. After 6–15 h of hybridization, probe-bound nanoparticles remained on the gaps, facilitating the formation of conductive silver bridges after a treatment with film-forming reagents. This system achieved single-base resolution in the femtomolar range. A disposable electrochemical printed chip for the detection of SNPs, with PCR products being analyzed directly on the chip by differential pulse voltammetry (DPV) without prior purification [119]. One notable peculiarity of high-density DNA microarrays is the need for physical addressing, by microjet deposition techniques, of picoliter volumes in discrete, highly specific spots on the chip. Fixe et al. used a pixel-addressed technique, by covalent immobilization and hybridization of DNA sequences onto a plastic-deposited thin film after the application of a very short 4.5 ns potential pulse, below 1V, which is compatible with standard, silicon-based microelectronic circuitry [120]. The enormous 10^9-fold raise in immobilization and hybridization efficiencies over an analogous system without the potential pulse may be attributed to the rapid spatial reorientation of adsorbed single-chain DNA due to the rapid change of the electrical field, which lowers sterical hindrances and accelerates both reactions. Similar works were reported earlier [121]; nevertheless, the too long 5 min pulses thus applied may trigger DNA-damaging electrochemical reactions. This highly dense hybridization spot technique may reduce both the time and cost of microarray fabrication, while increasing the data acquisition speed. Electrochemical transduction is easier to perform, simpler, faster and more suitable for miniaturization than fluorescence or MS [3]. Nowadays, chip nucleic acid immobilization still lacks optimization over sensitivity, specificity and hybridization efficiency, as well as minimization of cross-hybridizations [122]. In parallel with quantum dots, the newly discovered CNTs constitute a major class among bottom-up methods in nanobiotechnology. They exhibit a notable range of unique electronic properties and enlarged surface area for DNA immobilization, making them excellent elements for chemical sensing. Their electrical conductivity is comparable to that of copper and several orders of magnitude higher than that of polymers. In addition, they are physically robust and inert towards most chemicals [123]. Nie et al. used CNTs to modify a CPE in which an oligonucleotide was immobilized via streptavidin/biotin coupling [124]. After hybridization with a GNP-labeled DNA probe, a second hybridization between this system and additional GNPs was carried out, and total GNPs were monitored by DPV. The resulting signal was enhanced in comparison with that of a pure CPE and was about one order of magnitude higher than that with one-layer hybridization; clear distinction with one-base mismatched DNA was obtained. The high sensitivity performance of GNPs and CNTs was recently challenged by a polyaniline (PANI) nanotube array-immobilized DNA sensor; the array was built-up onto a graphite electrode, using a thin nanoporous layer as the template [125]. Some advantages of the conducting PANI biosensor include low temperature synthesis and no need for catalytic enhancement, purification or end-opening processing. In addition, the uniform orientation of the individual nanotubes on the array and the enhanced conductivity of arrayed PANI obviate the common limitations of conventional PANI. The biosensor exhibited an ultralow detection limit of DNA (1 fM) and good discrimination of one-nucleotide mismatches down to 38 fM, with obvious application for SNP analysis. Despite the undeniable advantages of CNTs, their manufacture is troublesome. A direct consequence is the high variability of shapes between different single CNTs, which renders unreproducible electrical properties, unless many average-in units are used together. Arrays of nanotubes bound to different DNA-probe molecules may be built to cheaply detect specific genes for diagnostic purposes [123].

5.3.3. *Label-free (direct) detection*

Variations on the intrinsic DNA electroactivity after the occurrence of hybridization are especially envisaged due to their simplicity [122]. This has obvious advantages in terms of simplicity and rapidity of the experimental procedures, and avoids signal lost caused by gradual liberation of the indicator from the immobilized DNA. Essentially, the adenine and guanine residues become oxidized in carbon electrodes and (in addition to cytosine) reduced in mercury electrodes [126]. The hybridization reaction generally causes a decrease in the redox current peak because the redox points in the DNA molecule are compromised with the hydrogen bonds that keep both chains together. In addition, the higher rigidity of a double-chain compared to that of a single-chain hinders the former to completely cover the rough microscopic surface of a solid electrode, thus decreasing the number of DNA/electrode attaching points and the overall electron transfer rate [127]. The emergence of solid electrodes has improved enormously the applicability of electrochemical methods for nucleic acid analysis. Stripping methods have the lowest detection limits among all voltammetric techniques [128]. The technique of adsorptive transfer stripping voltammetry (AdTSV) was used to detect DNA amounts below 1 pg, in sample volumes as small as 5–10 μl, without the need for a special voltammetric cell [126]. Compared to stripping voltammetry, stripping potentiometry devices exhibit smaller background noise [127]. This technique was applied to high-sensitive detection of DNA in electrochemically pretreated carbon paste and in screen-printed electrodes [11,129]. Changes in interfacial electrochemical parameters have also been used for DNA analysis, despite the molecular mechanisms underlying interfacial electrical changes as a result of affinity interactions are only fairly understood [130]. Capacitive transducers' operation relies on the decrease in capacitance caused by thickening of the electrode/solution dielectric layer as a result of displacement of water and electrolyte molecules due to the immobilization and further hybridization events. A genosensor was reported for capacitive detection of short oligonucleotides with thiolated and SAM-immobilized probes in gold electrodes

[131]. DNA detection through impedimetry is based on the fact that single-chain DNA desorption from a solid electrode corresponds to a higher dielectric loss compared to a double-chain, owing to the higher structural flexibility of the last [132]. At 100 Hz, impedance measurements on a label-free DNA gold electrode sensor increase about 25% upon hybridization, but it was reported an increase of up to 160% with an enzymatic amplification scheme [133]. The surface potential variation upon hybridization of a silicon nitride gate insulator-immobilized peptide nucleic acid (PNA) with its negatively charged DNA counterpart was used to specifically detect the hybridization event with a FET sensor [134]. The PNA, a DNA/protein hybrid, is particularly advantageous for this purpose, because it enables highly specific and selective binding at low ionic strength. PNAs are nucleic acid analogues with the sugar–phosphate backbone replaced by a peptide structure, and have received considerable attention as new recognition probes for DNA detection; they significantly improve the sensitivity and the discriminatory ability between DNA sequences differing in as few as one base-pair [3]. Other advantages include high sensitivity, low dependence on the ionic strength and high thermal stability [135]. An electrochemical detection scheme was recently developed by using a PNA probe and polythiophene which, for being a water-soluble electroactive cationic polymer, avoids the strong electrical interferences caused by the hydrophobic polymers in permanent contact with the electrodes [136]. The neutral character of the PNA probe permits its binding to polythiophene only after the hybridization reaction with the negatively charged DNA-target. Field-effect transistor (FET) biosensors operate by interaction between external charges with carriers in a nearby semiconductor [130]. FET biosensors have been used by direct immobilization of DNA strands on the gate surface of a DNA chip by chemical modification [137,138]. These silicon-based devices monitor the increase in surface charge after DNA hybridization on the sensor surface [139]. Wang et al. developed an electrochemical biosensor for detection of short Mycobacterium tuberculosis-related DNA sequences by adsorptive stripping chronopotentiometry with a redox marker [140]. The performance of this microfabricated screen-printed carbon-strip biosensor was similar to that of a carbon paste biosensor, with short detection times, in the range of 5–15 min. They also used this methodology to detect the oxidation peak current of guanine with a thick-film sensor incorporated into a battery-operated portable device, as required for in situ DNA diagnosis [141]. Low or moderate sensitivity is usually a problem in FET sensors due to significant fluctuations of the interface potential in an aqueous environment, but a recent strategy was implemented with a gold electrode-based FET DNA sensor to stabilize the sensor, by superimposing a 1 kHz high-frequency voltage to the reference electrode [142]. In addition, the stabilization time was reduced from 1 h to 5 min. An interesting innovation to increase the sensitivity and performance of genosensors are nucleic acid dendrimers, branched supermolecular structures able to be used as DNA probes. Upon hybridization of these spherical, tree-like structures with multiple target-strain molecules, the response is greatly amplified [3]. In addition, DNA dendrimers have structural homogeneity and controlled composition, making them valuable candidates for biosensing applications. Amino-terminated dendrimers were used as building blocks to form multilayer thin films and as linkers for immobilization of amino-modified DNA probes [143]. Besides the high sensitivity and selectivity thus achieved by EIS, the multilayer biosensor is very stable and regenerable. Morosity limits the utilization of impedimetry and capacitance for biosensor construction [132]. However, these biosensors may find a market where low cost, portability and analysis speed are required and moderate sensitivity is sufficient [130]. A well-succeeded diagnostic may not only require detection but also quantification of the disease-causing substance, since pathological states are usually associated with high serological levels of these compounds. Nucleic acids are usually quantified through the adenine/guanine ratio, but a method was proposed to simultaneously detect the individual levels of both nucleotides with a GCE after DNA adsorptive stripping [12]. Well-defined oxidation peaks were produced above 4 ng ml^{-1}. A label-free X-ray photoelectron microscopy detection system was recently used to detect the nitrogen content and the DNA nitrogen/sulfur ratio from alkanethiols introduced for surface immobilization to confirm the occurrence of hybridization [144]. The method was shown to be a good alternative to fluorescence radioisotope detection. The potential of photoelectrochemistry for unlabeled DNA detection was demonstrated by a 10^4-fold sensitivity enhancement over voltammetry in PCR-free biological samples [145]. Instead of electrochemical changes in nucleobases, direct electrooxidation of sugar residues in cupper electrodes may also be monitored [146]. In this case, however, the response tends to increase after hybridization, reaching detection limits in the picomolar range, since more sugar residues are accessible in an outer double-helix than in a single-chain. As an application of CNTs, multi-wall nanotubes (MWNTs) were used to improve direct detection of guanine and adenine oxidation currents in an MWNT-modified GCE [147]. The detection was highly sensitive, simple, reproducible and rapid. The surface area of MWNTs may be further enhanced by coupling end- and lateral-functionalization in CPEs, with a detection limit of 10 pg ml^{-1}, compatible with genetic testing requirements [148]. Pointing towards nanotechnology, DNA/protein conjugates have emerged has valuable tools for biosensor construction. Investigations about the interaction between DNA and DNA-binding proteins are pertinent due to the importance of DNA–protein interactions in many cellular processes (e.g., transcription) [13]. Semi-synthetic DNA–protein conjugates go far beyond the typical avidin-biotin-streptavidin affinity systems for DNA-probe labeling, and may help to solve some basic constraints of currently available biosensors. An example is a thermostable probe for DNA hybridization assays, formed by an oligonucleotide and a fungal lipase [149]. In this study, working with screen-printed carbon electrodes modified with single-wall nanotubes (SWNTs), the interaction of the SSB protein from *Escherichia coli* with ssDNA (for which the protein has high affinity) immobilized on the SWNTs was evaluated through the voltammetric oxidation peaks of the DNA-probe. There is a competition process between the SSB protein and the target DNA-chain for binding the DNA-probe; in this way, the oxidation signal of the DNA guanine and adenine residues increases after hybridization, while the oxidation signals of the tyrosine and tryptophan residues from the protein vanish, as a result of protein displacement. Thus, the electron transfer rate increases. The SWNT-amplified signal reached a detection limit of 0.15 mg ml^{-1} of target-DNA. An important topic in electrochemical biosensing with DNA/protein conjugates is the detection of individual molecules with DNA nanopores, which are usually formed by covalently immobilizing a single DNA-chain into the lumen of a *Staphylococcus aureus* α-hemolysin nanomeric pore. This strategy is based in former works, according to which nucleic acid molecules produce ionic current 'signatures' while crossing the α-hemolysin ionic channels [150]. The conjugated is able to detect, with single-base resolution, DNA target-chains that eventually bind the immobilized probe, by measuring the variation of the ionic current flowing through the nanopore [151]. This system, however, is still limited for nucleotide sequencing, since several nucleotides occupy the transmembrane pore and all contribute to the overall resistance, thus 'darkening' the effect of any individual nucleotide [152]. Moreover, high frequencies of about 10 MHz are needed to reduce the experimental noise, but they can also mislead nucleotide sequence-dependent signature currents. It is thus very likely that a future

nanopore-based sequencing device includes an enzyme to regulate the DNA translocation velocity across the nanopore from microseconds to milliseconds per nucleotide. Being an alternative to the extense DNA-probe microarrays, nanopores may constitute a next generation of DNA biosensors. A newly developed system to detect the duplex formation is based on the reduction of the ionic conductivity, after DNA hybridization, through an immobilized probe bound to a bilayer lipid membrane (BLM); this is due to alterations in the ionic permeability of the BLM as a consequence of structural changes caused by the hybridization event. An ion-channel sensor for highly specific DNA detection was built-up, making use of the electrostatic repulsion between ferrocianide (negatively charged hybridization marker) and a negatively charged DNA target-strain bound to a neutral PNA probe, on a gold electrode [153]. A similar approach is the use of negatively charged liposomes that bind the immobilized probe, thus creating a giant negatively charged surface that repels the target-DNA [154].

In general, electrochemical biosensors are relatively simple, rapid, less costly and amenable for miniaturization and mass-production [1]. Together with the compatibility with microfabrication techniques, the low power demands and the portable nature, electrochemical transduction seems to be quite appropriated for decentralized DNA diagnosis of many infectious and inherited diseases [11,12].

5.4. Magnetic particles

Labels used in bioassays are very often molecular, nanoscale-sized, in order to match the size of molecular recognition bioprobes and analyte targets. For this reason, microscale-labels, including magnetic microbeads, are usually discarded as true labels [155]. Nonetheless, when compared to smaller labels, microbeads offer two important advantages that far outweigh the disadvantages of size mismatch: the easiness of detecting a low amount of microbeds than much more abundant nanolabels (e.g., fluorophores or nanoparticles) by routine optical microscopy or magnetic detection and the possibility of applying fluidic drag forces to microbeads for, under controlled laminar flow at the capture surface, remove non-specifically bound labels, thus improving dramatically the assay performance [156]. The sensitivity, specificity and reproducibility of label-based systems is much more dependent on non-specific background signals than on the ability for label detection [157]. Very often, magnetic beads in biomolecular analysis schemes are used for target preconcentration rather than for the detection step itself, together with the additional label (e.g., metal nanoparticle or fluorophore) for detection. Sandwich models with metal nanoparticles, DNA and magnetic microbeads have been increasingly used for high-sensitivity DNA detection [158]. When magnetic nanoparticle labeled-DNA probe molecules bind their target sequences on a surface, under brief exposure to a strong magnetic field, their magnetic moments align collectively and yield a measurable signal. Meanwhile, there is no net signal from the randomly oriented DNA sequences, which permits to eliminate the common and tedious washing steps [63]. The use of magnetic micro- or nanoparticle for specific detection of low-abundant DNA analytes has drastically increased in recent years. An example recently reported was the development of a magnetically assisted DNA detection platform based on magnetic particle preconcentration [159]. Alternative schemes for detection and quantification of nucleic acids rely on the magnetic bead-based sandwich hybridization (BBSH) assay, in which the DNA-target simultaneously binds a magnetic bead-tagged DNA and the labeled DNA-probe. An application of this method was carried out with an electrochemical readout system based on interdigitated microelectrode arrays on silicon chips [160]. Another example was the sandwiching of a target-DNA between DNA probe-functionalized magnetic microparticles and DNA-modified GNPs to separate the target from the sample matrix and amplify the signal, with suitable detection with a chip-based silver metallization technique [161]. A common feature of these schemes is the so-called 'capture and release' strategy, by which a target-DNA firstly binds the DNA probe-functionalized magnetic particle and is then released (dehybridized) for final detection. This multi-step process, however, tends to lengthen the analysis time and dilute the sample. Aiming to reduce the analysis time, some modifications were introduced in the BBSH assay in a recent work of an electrical biochip for analysis of messenger RNA (mRNA) levels and gene expression [162]. The layout consisted of one biotin-labeled capture probe and two digoxigenin (DG)-labeled detection probes. After hybridization of these probes with the target, the

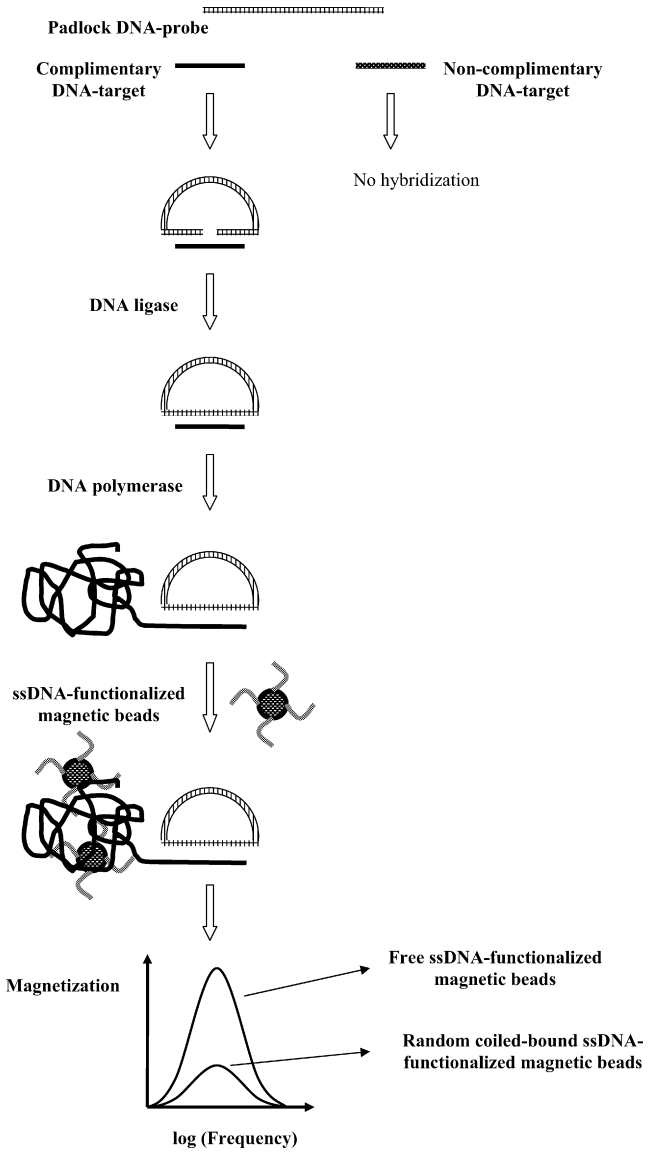

Fig. 3. Scheme of the VAM-NAD assay. The ends of the padlock DNA-probe hybridize with the complimentary DNA-target chain, forming a circularized structure. Such ends are then joined together by a DNA ligase. Afterwards, a DNA polymerase triggers the RCA process for a certain period of time. The addition of magnetic beads functionalized with ssDNA chains complimentary to a sequence in the random-coil repeating motif originates magnetic bead attachment to the macromolecular DNA structure by base-pair hybridization. This bead incorporation results in a considerable downsizing in the magnetization spectrum curve, when compared to that of unbound ssDNA-functionalized magnetic beads.

Table 1
Transduction mechanism, DNA detection assay characterization and limit of detection (LOD) for several bibliographic references on DNA biosensing

Transduction mechanism	DNA detection assay characterization	LOD [#ref.]
1. Optical		
1.1 Label-free (reagentless) detection		
SPR	Capture- and target- oligos (11–100 mer)	10 pM [189], 10 pM [60], 100 pM [190], 0.68 pM [191]
	Capture-oligo (11–25 mer) and target-DNA fragment (143–570 mer)	0.06 pg [192], 0.25 μM [193]
Fluorescence and chemiluminescence	Capture- and target-oligos (18–123 mer)	90 pM [194], 0.3 nM [72], 70 nM [195], 1.9 pM [196], 2 nM [197]
1.2 Label (dye or intercalator)-based detection		
SPR	Capture- (18 mer), target- (24–0 mer) and signaling- (18–21 mer) oligos	1.8 pM [198], 500 nM [199]
	Capture- and target- oligos (11–80 mer)	1.22 nM [200], 3.0 nM [201], 19 pM [42], 30 fM [202], 0.2 nM [203]
Fluorescence	Capture- (27 mer), target- (40 mer) and signaling- (19 mer) oligos	5 fmol [204], 0.6 fmol [205]
	Capture-oligo (15 mer) and target-DNA fragment (196 mer)	500 fmol [206]
2. Mass-change		
Resonance frequency	Capture- (20–30 mer), target- (40 mer) (or DNA fragment (104 mer)) and signaling- (20–30 mer) oligos	120 CFU/ml [207], 0.7 μM [208]
	Capture- and target- oligos (11–42 mer)	50 nM [86], −3.6 Hz/pg [209]
	Capture-oligo (26–42 mer) and target-DNA fragment (104–244 mer)	1.0 μM [210], 0.12 μM [85], 10 nM [211], 200 μg/ml [212]
SAW	Capture- and target- oligos (15–20 mer)	0.01 μM [88,213,214], 1 ng/ml [215]
3. Electrochemical		
3.1 Label-free (reagentless) detection		
Voltammetry	Capture- and target- oligos (20–38 mer)	100 pM [216], 0.01 fmol [217], 400 pM [218], 10 pM [219], 10 pg/ml [148]
	Capture-oligo (18–25 mer) and target-DNA fragment (244–300 mer)	1.8 zM [220], 30 μg/ml [221], <1000 DNA-target amplicons [222]
EIS	Capture- and target- oligos (15–30 mer)	10 fM [223], 0.5 nM [224], 5 nM [225,226], 100 pmol [227], 100 nM [228]
FET	Capture- and target- oligos (20–30 mer)	79 nM [229], 10 fM [230]
3.2 Enzyme-based detection (sandwich-assay mode)		
Streptavidin-alkaline phosphatase conjugate	Capture- and biotinylated target- oligos (13–30 mer)	8 pM [231], 6 pM [232], 1 nM [233], 30 fM [234]
	Capture- (13–35 mer), target- (23–52 mer) and signaling- (12–35 mer) oligos	340 pM [235], 0.3 fM [79], 1.2 pM [236], 50 fM [237]
Other conjugates	Capture- and biotin- or 5′-fluorescein isothiocyanate (FITC)-labeled oligos (20–50 mer)	0.1 nM [238], 0.5 fM [239], 100 pmol [103]
	Capture-oligo (20 mer) and labeled-DNA fragment (322 mer)	10 isolated genomes [240]
	Capture-oligo (20–50 mer), DNA fragment (87–362 mer) and signaling-probe (12–35 mer)	1 fmol [241], 1 fM [242], 1 fM [243]
3.3 Label (redox marker)-based detection		
Voltammetry	Capture- and target- oligos (10–35 mer)	1 nM [244], 0.3 pM [245], 0.12 pM [246], 0.54 ag/ml [247], 7 nM [248], 8.3 μM [249], 0.59 nM [250], 0.5 nM [251], 1 fM [125], 10 pM [252], 0.3 pM [253], 0.51 nM [254], 90 pM [255], 0.5 nM [256], 10 zmol [116]
	Capture-oligo (23 mer), target-DNA fragment (256 mer) and signaling-probe (23 mer)	0.78 fmol [257]
4. Magnetic particles and other oligo probe-supporting beads		
4.1 Optical	Capture- (20–30 mer), target- (20–41 mer) and signaling-probe (25–41 mer)	10 fM [258], 10 pM [75]
	Capture- and target- oligos (15–37 mer)	32 μM [61], 50 pM [259], 100 pM [49]
	Target-DNA fragment (600 mer) and signaling-probe (180 mer)	1.4 pM [260]
4.2 Electrochemical	Capture- and target- oligos (20–32 mer)	2 pM [261], 2 nM [262]
	Capture-oligo (20 mer) and target-DNA fragment (437 mer)	74.8 pM [263]
4.3 Magneto-resistive	Capture- and target-oligo (30 mer)	100 fM [264]

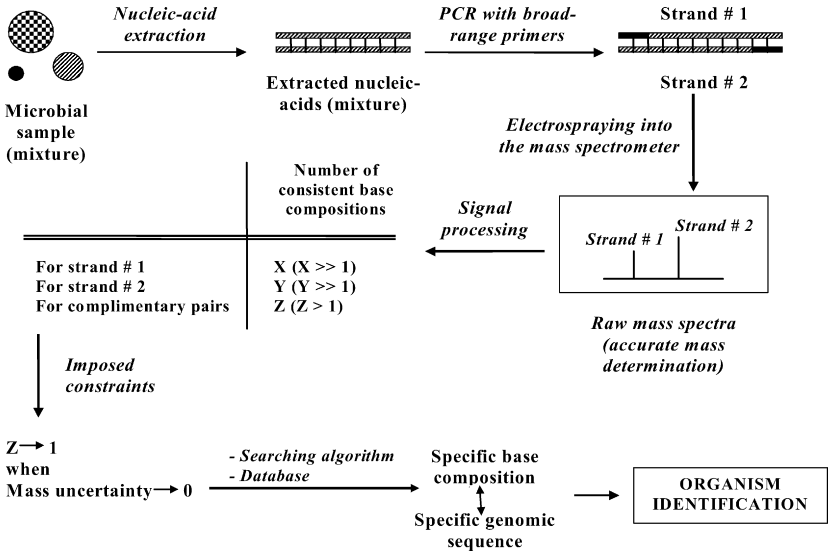

Fig. 4. Identification of microorganism species in biological mixtures by the universal MS biosensor. All nucleic acids in the sample are firstly extracted and amplified with different sets of universal, broad-range primers. The PCR products are then electrosprayed into a mass spectrometer and resulting raw mass spectra are recorded. Very accurate mass measurements of each DNA strand allow determining the number of consistent base compositions for each one, a number which is greatly reduced by imposing the condition of complimentarity between them. This number is further reduced towards unity when the uncertainties of mass measurements for both strands reach zero. Finally, an internal algorithm searches for a database that assigns a specific genomic sequence to the unique, specific base composition determined above, thus identifying the organism in the sample mixture.

sandwich-hybrid is bound to paramagnetic streptavidin-coated particles (which allow separating the sandwich-hybrid from the reaction solution by external magnets) and labeled with anti-DG alkaline phosphatase conjugates. The redox reaction catalyzed by the enzyme generates an electrical current which correlates with the level of hybridized mRNA. In contrast to former protocols in which probes were targeted to regions of the mRNA-target separated by a few hundred nucleotides, DNA-probes were, with this approach, adjacently bound to their target regions in the same mRNA molecule. The resulting enhancement of the hybridization efficiency may result from a cooperative effect of the adjacently bound probes (by modifications on the secondary structures of the mRNA molecule) [163] or from prevention of breaking or hydrolysis points that, otherwise, may occur along the structure of a mRNA strand in the case of separated probes. By using two instead of only one DG-labeled detection probe, further signal amplification was achieved, thus resulting in a substantial time reduction of the detection protocol. In addition, the expression profile exhibited by this optimized protocol is comparable with those of real-time RT-PCR assays. An alternative method proposed by Dubus et al., with optical detection, does not require the release of the hybridized target-DNA prior to its detection [164]; instead, a polymeric polythiophene derivative was attached to a magnetic microbead-grafting ssDNA followed by hybridization with the DNA-analyte, with formation of triplex-branched beads. The detection principle relies on the different conformations adopted by the polymer molecule when electrostatically bound to either ssDNA or dsDNA, giving rise to distinct fluorescence properties (chromism). The other novelty of this work was the confinement of the particle-bound target-DNA in a small volume of a microelectromagnetic trap, which allows performing the preconcentration and the detection steps simultaneously on the same support, thus decreasing the final sample volume and increasing the signal-to-noise ratio. This scheme not only avoids the hybridized DNA-releasing step, but also renders results in only 5 min, with detection limits similar to those of 'capture and release' methods. The above combination is able to extend the application of this ultrasensitive biosensor to biological samples with complex matrices and integration in lab-on-a-chip platforms.

Martins et al. developed a magnetoresistive biochip for real-time monitoring of pathogens in water [165]. With this system, DNA-target molecules may be magnetically labelled before or after the recognition process, by paramagnetic bead-driven transport and manipulation across a chip surface onto a microfluidic platform. The detection was carried out by measuring the variation of the sensor resistance with the label-borne magnetic fields. The system exhibited a fast response with high sensitivity, specificity and ease of integration and automation, thus constituting an attractive option to fluorescent labelling and allowing tight stringency control. In addition, since biomaterials usually are not magnetic, background signal subtraction is greatly simplified [166]. Such magnetic field sensors can be miniaturized to match the size of a single magnetic bead, thereby increasing the sensitivity of the detection. In another work, a magnetic splitter was used to separate, within a microchannel, two types of magnetic microspheres bound to different DNA-probes, resulting in single-base resolution of the target-DNA [167]. Recently, a non-fluorescent volume-amplified magnetic nanobead assay scheme for DNA detection was developed [16]. This method involves circularizing, 'padlock' oligonucleotide probes designed with two terminal target-complimentary segments. Upon specific hybridization with the target, the ends are joined by a ligase, creating a circular, target-catenated DNA-probe molecule, which provides highly specific and sensitive detection [168]. The DNA-target is recognized and volume-amplified to large coils by circularization of the linear padlock probes through probe hybridization and ligation, followed by rolling circle amplification (RCA) of the probes by a DNA polymerase. This generates a DNA strand consisting of a large number of tandem copies of the complement to the circularized probe, collapsing into a random-coil DNA macromolecule in solution. After nanobead binding in the RCA coils, the nanobead magnetization spectrum changes considerably, induced by the attached volume-amplified target molecules (Fig. 3). Although having been used for single-target detection, this method can be easily generalized for multi-target detection by using several nanobeads with different sizes, one for each target. The method was already applied for single-molecule DNA detection with fluorophore-tagged probes [169]. The resulting confined cluster of fluorophores

was detected and quantified with a microfluidic device mounted onto a confocal fluorescence microscope. This layout could also be applied for the simultaneous amplification of different probe and target complexes for multiplexed target analysis with fluorescence probes with different colors. The detection limit can be greatly improved by optimizing, for instance, RCA time, bead size and bead surface coverage of oligonucleotides. Since bead incorporation in the coils is diffusion-controlled, it can be accelerated by sample incubation at higher temperatures. A multiplexed technique was implemented for simultaneous and label-free detection of three short HBV-related DNA fragments in a single vessel, with detection of a chemiluminescent product of a reaction between the labeling reagent 3,4,5-trimethoxylphenylglyoxal (TMPG) and the guanine-enriched regions within the DNA-target [170]. In this work, thermosensitive poly(*N*-isopropylacrylamide), polystyrene beads and magnetic beads were employed as different labels in DNA capture-probe conjugates, which were split apart under different thermal conditions. In contrast with most of the current multiplexing detection methods, which usually require complex instrumentation (e.g., flow cytometer or imaging system) instead of a simple chemiluminescence setup, every hybridization signal for the corresponding DNA-target in this work is uniquely immobilized onto one carrier with a unique and intrinsic physico-chemical pattern. Despite not employing any extra label (e.g., organic fluorophore) unless TMPG – with potential advantages in terms of detection speed, cost and simplicity – it is possible to further enhance the detection sensitivity by increasing the amount of DNA-target strands or introducing other labels (e.g., enzymes or colloidal gold). Lee et al. [171] coupled antibody-conjugated magnetic beads and μRT-PCR on a microfluidic chip for detection of RNA viruses after thermal lysis of extracted RNA. The targeted virus in the sample was captured by the specific antibody-conjugated magnetic beads for viral pretreatment and RNA enrichment, thus avoiding the effect of interferents and inhibitors usually present when direct thermolysis of virus-containing samples is performed. After capturing the virus, magnetic beads are trapped in a magnetic field and can be then easily manipulated for further processing. This format exhibited sensitivity similar to that of a commercial RNA extraction kit and a large-scale RT-PCR apparatus. Multiplexed analysis in complex biological samples (including whole blood, serum, plasma and milk) was coupled to magnetic microbead labeling for femtomolar detection of DNA and proteins [157]. Non-specific binding of bead labels was minimized by applying fluidic force discrimination, in which a controlled laminar flow promotes chip-captured microbead labeling. The density of beads that remain bound is proportional to analyte concentration and can be determined either by optical counting or magnetoelectonic detection. Compared to a previous nanowire-based FET for multiplexed and label-free protein detection [172], this method exhibited similar sensitivity and additional ability for analysis of untreated clinical matrices onto a simple microscope slide. By combining simple optical or magnetic bead counting with microfluidics, straightforward hand-held and higher throughput analysis can be performed in a few minutes. The main advantage of using an on-chip transport system is that diffusion constraints are overcome by the attraction between a magnetic field and functionalized magnetic particles. Its combination with a magnetoresistive transducer enables the detection of minute amounts of target biomolecules in a reasonable time frame. However, biosensing applications still require improvement of field sensitivity and reduction of the sensor noise background [173].

5.5. Bibliographic revision on limit of detection of DNA biosensors

We have reviewed a large number of different systems on the literature for DNA detection, mainly concerning the transducer surface and transducing mode of optical, mass-sensitive, electrochemical and nanoparticle bead-based biosensors (Table 1). A particular focus has been given to: transducer systems; different detection methods: label-free/reagentless (direct), label- (with dye or intercalator) and enzyme-based (both indirect); characterization of the DNA detection assay; and detection limit (a measure of sensitivity). Increased attention has been given recently to direct, label-free electrochemical detection schemes and to the development of high-sensitivity DNA biosensing devices for detection of DNA targets and SNPs without the need for target amplification. The limit of detection of a given quantification method is the sample concentration or quantity that yields a signal equal to the blank signal plus a multiple of the standard deviation of the blank [174]. For practical purposes, it is usually assumed that it corresponds to signal(s)/noise(n) = 3. From the reviewed publications, it becomes clear that, despite significant differences registered for nucleic acid detection limit values among the various applications, recent published works have reported increasingly small detection limits. Nevertheless, further sensitivity enhancement is still needed in order to achieve the extremely small detection limits required for diagnostic assays with real, non-amplified biological samples – in the attomolar range [175] – which is often beyond the fundamental limits of common sensing devices.

6. A new paradigm: MS DNA sensing and the universal biosensor

A common assumption in the conception of conventional biosensors is the necessary prior knowledge of the specific genomic sequence from a given target-pathogen. Unlike nucleic acid probes or arrays, MS does not require anticipation of the analyzed products, rather measuring the masses of the nucleic acids in a sample. The commercial T5000 Biosensor System, from Ibis Biosciences, is an integrated platform for analysis of complex samples, and relies in that essential common features are encoded in all the genomes among living organisms. In practice, broad-range ('intelligent') primers are used to amplify PCR products from large groups of organisms, but more specific, division-wide primers may be used to enhance species resolution [176]. Since the exact mass of each DNA base is accurately known, a high precision measurement is able to derive a constrained list of base compositions of each DNA strand [177]. An internal detection algorithm searches a database that assigns a specific base composition to a given genomic sequence, thus identifying the corresponding organism (Fig. 4). This strategy successfully led to include the human severe acute respiratory syndrome (SARS) virus in the coronavirus family [178]. It was also tested as a rapid and inexpensive method for global surveillance of emerging influenza virus genotypes [179]. Their main advantages are high resolution speed (above one sample per minute), high degree of automation and software control, no need for specialized manpower and possibility of performing strain typing and antibiotic resistance studies. As main disadvantages, the intrinsic difficulty MS device miniaturization, the need for continuous enrichment of databases with new genomic sequences, the requirement for high-power instrumentation for unambiguous compositional assignment and the need for signal processing enhancement, in order to detect a single sequence among thousands of others, can be mentioned. It is expected, however, that future technological improvements may ally the extraordinary analytical powerfulness of universal primer-based technologies with developments in the production and ongoing miniaturization of DNA chips and flow systems.

7. Conclusions

It can be anticipated that, in a near future, the advanced level of medical diagnosis will be largely dependent on the successful development and implementation of new materials and technology envisaging the fabrication of state-of-the-art biosensors. Common drawbacks of current biosensors have already been extensively summarized in the literature. However, the still limited availability of commercialized biosensors may be due mainly to a lack in the appropriate technology for their manufacture at a competitive cost rather than a lack of fundamental knowledge [180]. In vitro diagnostic devices will likely be expensive purchase over a one-test basis, although the overall cost may be lower due to the minimal requirement for laboratory manpower. In addition, the production and commercialization of high-throughput devices, as the GeneChip® from Affimetrix, may require investments in the order of several hundred million dollars, a serious obstacle for small start-up companies. Biosensors represent a quite disruptive technology for being very different from those currently used in clinical facilities worldwide. In addition, very often they face difficulties for obtaining regulatory approvals for testing and commercialization [181]. A more technical obstacle hindering their wide acceptance by the clinical community may be the controversial usefulness of monitoring one-single analyte as a disease-biomarker. The need for point-of-care nucleic acid testing, especially in resource-limited settings, requires simpler and cheaper instrumentation. As efforts for improving the amplification and detection of nucleic acids have been a major concern, the sample preparation and the nucleic acid extraction steps remain relatively underestimated towards the development of a true point-of-care diagnostic device. In addition, many of these current systems also require off-line sample preparation and reagent handling, being therefore unable for routine home testing [182]. It seems likely that, among the vast diversity of available approaches, none will fulfill all needs for a given application, but instead the choice will depend on the particular conditions and requirements. DNA analysis has been considered lengthy since standard filter hybridization protocols last no less than 20 h [183]. Some current biosensors already yield outputs in less than 1 h, but a truly simple, rapid and low-cost biosensor for routine analysis is still missing, which is particularly limiting for clinical purposes [184]. Clinical applications still face the problem of the very low levels of nucleic acids in biological fluids, otherwise undetectable if previous PCR amplification is not performed [180]. In the case of blood infections, the amount of human genomic DNA can be 10^{14} times higher than pathogen target-DNA, an important challenge in terms of selectivity [185]. Both in solution and at an interface, nucleic acids exhibit strong salt-dependent electrostatic effects over its structure, stability and reactivity [186]. Moreover, research about the effects of interfering substances has been carried out in pure, synthetic model-DNA sequences rather than in complex real samples, while prior knowledge of the selective molecular recognition processes is needed [187]. The lack of robustness in real samples may be attributed to the usually low operational and/or long-term stability of the biological receptor and/or the physical transducer [188]. Nonetheless, DNA is a particularly suitable material for nanosystem fabrication owing to unique peculiarities, including the ability of highly specific, mutual recognition between a very short oligonucleotide and a complex, long-sized eukaryotic genome, and high physico-chemical stability. Enhanced manipulation and processing precision at the atomic, ångstrom range by very specific molecular tools such as ligases, nucleases and other DNA-processing enzymes is another remarkable feature [46]. DNA has also higher chemical stability compared with other biorrecognition elements (e.g., enzymes and antibodies) and a superior ability to distinguish different strains from the same organism, especially when isolated from different geographical locations. Nucleic acid arrays are also proner than protein counterparts for direct synthesis onto a chip surface, without the need to produce and purify the ligands [82]. Taken together, these facts hold great promise for a future outburst of DNA biosensors for clinical and other purposes.

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References

[1] K. Kerman, M. Kobayashi, E. Tamiya, Meas. Sci. Technol. 15 (2004) R1.
[2] S.R. Mikkelsen, Electroanalysis 8 (1996) 15.
[3] J. Wang, Nucleic Acids Res. 28 (2000) 3011.
[4] M.I. Pividori, A. Merkoçi, S. Alegret, Biosens. Bioelectron. 15 (2000) 291.
[5] Z. Junhui, C. Hong, Y. Ruifu, Biotechnol. Adv. 15 (1997) 43.
[6] T. McCormack, G. Keating, A. Killard, B.M. Manning, R. O'Kennedy, in: D. Diamond (Ed.), Principles of Chemical and Biological Sensors, John Wiley & Sons, Toronto, 1998, pp. 133–194.
[7] J. Wang, Anal. Chim. Acta 469 (2002) 63.
[8] W. Vercoutere, M. Akeson, Curr. Opin. Chem. Biol. 6 (2002) 816.
[9] V. Chan, D.J. Graves, P. Fortina, S.E. McKenzie, Langmuir 13 (1997) 320.
[10] Y. Gao, L.K. Wolf, R.M. Georgiadis, Nucleic Acids Res. 34 (2006) 3370.
[11] J. Wang, P. Gründler, G.-U. Fleichsig, M. Jasinski, G. Rivas, E. Sahlin, J.L.L. Paz, Anal. Chem. 72 (2000) 3752.
[12] H.-S. Wang, H.-X. Ju, H.-Y. Chen, Anal. Chim. Acta 461 (2002) 243.
[13] K.J. Odenthal, J.J. Gooding, Analyst 132 (2007) 603.
[14] K.A. Peterlinz, R.M. Georgiadis, T.M. Herne, M.J. Tarlov, J. Am. Chem. Soc. 119 (1997) 3401.
[15] R. Levicki, T.M. Herne, M.J. Tarlov, S.K. Satija, J. Am. Chem. Soc. 120 (1998) 9787.
[16] M. Strömberg, J. Göransson, K. Gunnarsson, M. Nilsson, P. Svedlindh, M. Strømme, Nano Lett. 8 (2008) 816.
[17] A.B. Steel, T.M. Herne, M.J. Tarlov, Bioconjugate Chem. 10 (1999) 419.
[18] C. Preininger, P. Chiarelli, Talanta 55 (2001) 973.
[19] S. Chen, L. Li, C.L. Boozer, C. Jiang, Langmuir 16 (2002) 9287.
[20] T. Delair, F. Meunier, A. Elaïssari, M.-H. Charles, C. Pichot, Colloids Surf. A 153 (1999) 341.
[21] T.R. Gingeras, D.Y. Kwoh, G.R. Davis, Nucleic Acids Res. 15 (1987) 5373.
[22] Y.-H. Rogers, P.J.-B. Com, Z.-J. Huang, V. Bogdanov, S. Anderson, M.T. Boyce-Jacino, Anal. Biochem. 266 (1999) 23.
[23] J. Cha, J.I. Han, Y. Choi, D.S. Yoon, K.W. Oh, G. Lim, Biosens. Bioelectron. 18 (2003) 1241.
[24] S. Reisberg, B. Piro, V. Noël, M.C. Pham, Anal. Chem. 77 (2005) 3351.
[25] T. Strother, J. Hamers, L.M. Smith, Nucleic Acids Res. 28 (2000) 3535.
[26] J.V. Ness, S. Kalbfleisch, C.R. Petrie, M.W. Reed, J.C. Tabone, N.M.J. Vermeulen, Nucleic Acids Res. 19 (1991) 3345.
[27] E.P. Ivanova, D.K. Pham, N. Brack, P. Piagram, D.V. Nicolau, Biosens. Bioelectron. 19 (2004) 1363.
[28] G.B. Sukhorukov, M.M. Montrel, A.I. Petrov, L.I. Shabarchina, B.I. Sukhorukov, Biosens. Bioelectron. 11 (1996) 913.
[29] H. Hayatsu, Y. Tanaka, K. Negishi, Nucleic Acids Symp. Ser. 37 (1997) 139.
[30] N. Lassalle, P. Mailley, E. Vieil, T. Livache, A. Roget, J.P. Correia, L.M. Abrantes, J. Electroanal. Chem. 509 (2001) 48.
[31] A. Dupont-Filliard, A. Roget, T. Livache, M. Billon, Anal. Chim. Acta 449 (2001) 45.
[32] G. Bidan, M. Billon, K. Galasso, T. Limache, G. Mathis, A. Roget, L.M. Torres-Rodriguez, E. Vieil, Appl. Biochem. Biotechnol. 89 (2000) 183.
[33] N.K. Chaki, K. Vijayamohanan, Biosens. Bioelectron. 17 (2002) 1.
[34] R.S. Freire, C.A. Pessoa, L.T. Kubota, Quim. Nova 26 (2003) 381.
[35] Y.-D. Zhao, D.-W. Pang, S. Hu, Z.-L. Wang, J.-K. Cheng, H.-P. Dai, Talanta 49 (1999) 751.
[36] A.J. Baeumner, J. Pretz, S. Fang, Anal. Chem. 76 (2004) 888.
[37] F.F. Bier, F. Kleinjung, Fresen. J. Anal. Chem. 371 (2001) 151.
[38] T.-H. Wang, Y. Peng, C. Zhang, P.K. Wong, C.-M. Ho, J. Am. Chem. Soc. 127 (2005) 5354.
[39] P.S. Dittrich, K. Tachikawa, A. Manz, Anal. Chem. 78 (2006) 3887.
[40] H.H. Ruf, T. Knoll, K. Misiakos, R.B. Haupt, M. Denninger, L.B. Larsen, P.S. Petrou, S.E. Kakabakos, E. Ehrentreich-Förster, F. Bier, Microelectron. Eng. 83 (2006) 1677.
[41] A. Gulliksen, L.A. Solli, K.S. Drese, O. Sörensen, F. Karlsen, H. Rogne, E. Hovig, R. Sirevåg, Lab Chip 5 (2005) 416.
[42] C.-W. Wei, J.-Y. Cheng, C.-T. Huang, M.-H. Yen, T.-H. Young, Nucleic Acids Res. 33 (2005) e78.
[43] D. Ivnitski, D.J. O'Neil, A. Gattuso, R. Schlicht, M. Calidonna, R. Fisher, BioTechniques 35 (2003) 862.
[44] D. Erickson, X. Liu, R. Venditti, D. Li, U.J. Krull, Anal. Chem. 77 (2005) 4000.

[45] C.R. Yonzon, D.A. Stuart, X. Zhang, A.D. McFarland, C.L. Haynes, R.P. van Duyne, Talanta 67 (2005) 438.
[46] C.M. Niemeyer, Rev. Mol. Biotechnol. 82 (2001) 47.
[47] P.A.E. Piunno, U.J. Krull, R.H.E. Hudson, M.J. Damha, H. Cohen, Anal. Chem. 67 (1995) 2635.
[48] J.A. Ferguson, T. Boles, C. Adams, D. Walt, Nat. Biotechnol. 14 (1996) 1681.
[49] F.J. Steemers, J.A. Ferguson, D.R. Walt, Nat. Biotechnol. 18 (2000) 91.
[50] S.J. Wood, Microchem. J. 47 (1993) 330.
[51] P. Guedon, T. Livache, F. Martin, F. Lesbre, A. Roget, G. Bidan, Y. Levy, Anal. Chem. 72 (2000) 6003.
[52] A. Buhl, J.H. Metzger, N.H.H. Heegaard, P. von Landenberg, M. Fleck, P.B. Luppa, Clin. Chem. 53 (2007) 334.
[53] G. Feriotto, A. Ferlini, A. Ravani, E. Calzolari, C. Mischiati, N. Bianchi, R. Gambari, Hum. Mutat. 18 (2001) 70.
[54] E. Mariotti, M. Minunni, M. Mascini, Anal. Chim. Acta 453 (2002) 165.
[55] J.-K. Wen, X.-E. Zhang, Z. Cheng, H. Liu, Y.-F. Zhou, Z.-P. Zhang, J.-H. Yang, J.-Y. Deng, Biosens. Bioelectron. 19 (2004) 685.
[56] J.S. Chamberlain, R.A. Gibbs, J.E. Ranier, P.N. Nguyen, C.T. Caskey, Nucleic Acids Res. 16 (1988) 11141.
[57] W. Fritzsche, Rev. Mol. Biotechnol. 82 (2001) 37.
[58] R.A. Reynolds III, C.A. Mirkin, R.L. Letsinger, Pure Appl. Chem. 72 (2000) 229.
[59] K. Tamada, F. Nakamura, M. Ito, X. Li, A. Baba, Plasmonics 2 (2007) 185.
[60] D.-K. Kim, K. Kerman, M. Saito, R.R. Sathuluri, T. Endo, S. Yamamura, Y.-S. Kwon, E. Tamiya, Anal. Chem. 79 (2007) 1855.
[61] Y. Sato, K. Sato, K. Hosokawa, M. Maeda, Anal. Biochem. 355 (2006) 125.
[62] S. Hohng, T. Ha, Chem. Phys. Chem. 6 (2005) 956.
[63] A.P. Alivisatos, Sci. Am. 23 (2007) 73.
[64] G.M. Whitesides, J.C. Love, Sci. Am. 17 (2007) 13.
[65] R. Robelek, L. Niu, E.L. Schmid, W. Knoll, Anal. Chem. 76 (2004) 6160.
[66] S.F. Wuister, R. Koole, C.M. Donegá, A. Meijerink, J. Phys. Chem. B 109 (2005) 5504.
[67] C.-Y. Zhang, H.-C. Yeh, M.T. Kuroki, T.-W. Wang, Nat. Mater. 4 (2005) 826.
[68] C.-L. Feng, X. Zhong, M. Steinhart, A.-M. Caminade, J.-P. Majoral, W. Knoll, Adv. Mater. 19 (2007) 1933.
[69] P. Alivisatos, Nat. Biotechnol. 22 (2004) 47.
[70] J.-G. Lee, K. Yun, G.-S. Lim, S.E. Lee, S. Kim, J.-K. Park, Bioelectrochemistry 70 (2007) 228.
[71] X. Fang, X. Liu, S. Schuster, W. Tan, J. Am. Chem. Soc. 121 (1999) 2921.
[72] X. Liu, W. Farmerie, S. Schuster, W. Tan, Anal. Biochem. 283 (2000) 56.
[73] S.-W. Tam-Chang, T.D. Carson, L. Huang, N.G. Publicover, K.W. Hunter Jr., Anal. Biochem. 366 (2007) 126.
[74] A.J. Baeumner, N.A. Schlesinger, N.S. Slutzki, J. Romano, E.M. Lee, R.A. Montagna, Anal. Chem. 74 (2004) 1442.
[75] S. Kwakye, A. Baeumner, Anal. Bioanal. Chem. 376 (2003) 1062.
[76] N.V. Zaytseva, R.A. Montagna, E.M. Lee, A.J. Baeumner, Anal. Bioanal. Chem. 380 (2004) 46.
[77] J. Korlach, M. Levene, S.W. Turner, H.G. Craighhead, W.W. Webb, Biophys. J. 82 (2002) 507.
[78] B. Sepúlveda, J.S. del Río, M. Moreno, F.J. Blanco, K. Mayora, C. Domínguez, L.M. Lechuga, J. Opt. A: Pure Appl. Opt. 8 (2006) S561.
[79] J.C. Liao, M. Mastali, V. Gau, M.A. Suchard, A.K. Moller, D.A. Bruckner, J.T. Babbitt, Y. Li, J. Gornbein, E.M. Landaw, E.R.B. McCabe, B.M. Churchill, D.A. Haake, J. Clin. Microbiol. 44 (2006) 561.
[80] http://www.illumina.com.
[81] C.L. Baird, D.G. Myszka, J. Mol. Recognit. 14 (2001) 261.
[82] N.F. Campbell, J.A. Evans, N.C. Fawcett, Biochem. Biophys. Res. Commun. 196 (1993) 858.
[83] N. Lassalle, E. Vieil, J.P. Correia, L.M. Abrantes, Biosens. Bioelectron. 16 (2001) 295.
[84] X.C. Zhou, L.Q. Huang, S.F.Y. Li, Biosens. Bioelectron. 16 (2001) 85.
[85] D. Dell'Atti, S. Tombelli, M. Minunni, M. Mascini, Biosens. Bioelectron. 21 (2006) 1876.
[86] D. Dell'Atti, M. Zavaglia, S. Tombelli, G. Bertacca, A.O. Cavazzana, G. Bevilacqua, M. Minunni, M. Mascini, Clin. Chim. Acta 383 (2007) 140.
[87] M. Thompson, A.L. Kipling, Analyst 116 (1991) 881.
[88] J. Sakong, H. Roh, Y. Roh, Jpn. J. Appl. Phys. 46 (2007) 4729.
[89] H. Zhang, H. Tan, R. Wang, W. Wei, S. Yao, Anal. Chim. Acta 374 (1998) 31.
[90] Y. Okahata, Y. Matsunobu, I. Kunihara, M. Masayuki, A. Murukani, K. Makino, J. Am. Chem. Soc. 114 (1992) 8229.
[91] L.M. Furtado, M. Thompson, Analyst 123 (1998) 1937.
[92] A. Janshoff, H.-J. Galla, C. Steinem, Angew. Chem. Int. Ed. 39 (2000) 4004.
[93] M. Yang, W.C. Duncan-Hewitt, M. Thompson, Langmuir 9 (1993) 802.
[94] K.M. Hansen, T. Thundat, Methods 37 (2005) 57.
[95] Y. Lee, G. Lim, W. Moon, Sens. Actuator 130–131 (2006) 105.
[96] M. Nordström, D.A. Zauner, M. Calleja, J. Hübner, A. Boisen, Eleventh International Conference on Miniaturized Systems for Chemistry and Life Sciences, Paris, France, October 7–11, 2007.
[97] J. Koeser, P. Shahgaldian, M. Bammerlin, F.M. Battiston, U. Pieles, J. Phys.: Conf. Ser. 61 (2007) 612.
[98] L.M. Lechuga, J. Tamayo, M. Álvarez, L.G. Carrascosa, A. Yufera, R. Doldán, E. Peralías, A. Rueda, J.A. Plaza, K. Zinoviev, C. Domínguez, A. Zaballos, M. Moreno, C. Martínez-A, D. Wenn, N. Harris, C. Bringer, V. Bardinal, T. Camps, C. Vergnenègre, C. Fontaine, V. Díaz, A. Bernad, Sens. Actuator B 118 (2006) 2.
[99] T. de Lumley-Woodyear, C. Campbell, A. Heller, J. Am. Chem. Soc. 118 (1996) 5504.
[100] F. Patolsky, A. Lichtenstein, I. Willner, Nat. Biotechnol. 19 (2001) 253.
[101] L. Alfonta, A.K. Singh, Anal. Chem. 73 (2001) 91.
[102] F. Azek, C. Grossiord, M. Joannes, B. Limoges, P. Brossier, Anal. Biochem. 284 (2000) 107.
[103] M.I. Pividori, A. Merkoçi, S. Alegret, Analyst 126 (2001) 1551.
[104] E. Bakker, Y. Qin, Anal. Chem. 78 (2006) 3965.
[105] E. Domínguez, O. Rincón, A. Narváez, Anal. Chem. 76 (2004) 3132.
[106] K.M. Millan, S.R. Mikkelsen, Anal. Chem. 65 (1993) 2317.
[107] K. Millan, A. Saraullo, S.R. Mikkelsen, Anal. Chem. 66 (1994) 2943.
[108] J. Wang, X. Cai, G. Rivas, H. Shiraishi, P.A.M. Farias, N. Dontha, Anal. Chem. 68 (1996) 2629.
[109] A. Erdem, K. Kerman, B. Meric, U.S. Akarca, M. Ozsoz, Electroanalysis 11 (1999) 586.
[110] K. Kerman, D. Ozkan, P. Kara, B. Meric, J.J. Gooding, M. Ozsoz, Anal. Chim. Acta 462 (2002) 39.
[111] K. Hashimoto, K. Ito, Y. Ishimori, Anal. Chim. Acta 286 (1994) 219.
[112] Y. Jin, X. Yao, Q. Liu, J. Li, Biosens. Bioelectron. 22 (2007) 1126.
[113] H. Ju, B. Ye, J. Gu, Sensors 4 (2004) 71.
[114] F.R.R. Teles, D.M.F. Prazeres, J.L. Lima-Filho, Sensors 7 (2007) 2510.
[115] C. Xu, H. Cai, P. He, Y. Fang, Analyst 126 (2001) 62.
[116] S. Takenaka, K. Yamashita, M. Takagi, Y. Uto, H. Kondo, Anal. Chem. 72 (2000) 1334.
[117] K. Hashimoto, K. Ito, Y. Ishimori, U.S. Patent 5,972,692 (1999).
[118] K. Hashimoto, K. Ito, Y. Ishimori, Anal. Chem. 66 (1994) 3830.
[119] M.U. Ahmed, K. Idegami, M. Chikae, K. Kerman, P. Chaumpluk, S. Yamamura, E. Tamiya, Analyst 132 (2007) 431.
[120] F. Fixe, H.M. Branz, N. Louro, V. Chu, D.M.F. Prazeres, J.P. Conde, Biosens. Bioelectron. 19 (2004) 1591.
[121] C.F. Edman, D.E. Raymond, D.J. Wu, E. Tu, R.G. Sosnowski, W.F. Butler, M. Nerenberg, M.J. Heller, Nucleic Acids Res. 25 (1997) 4907.
[122] P. Bean, Am. Clin. Lab. (2001) 11.
[123] G. Gruner, Sci. Am. 17 (2007) 48.
[124] L. Nie, H. Guo, Q. He, J. Chen, Y. Miao, J. Nanosci. Nanotechnol. 7 (2007) 560.
[125] H. Chang, Y. Yuan, N. Shi, Y. Guan, Anal. Chem. 79 (2007) 5111.
[126] E. Paleček, Electroanalysis 8 (1996) 7.
[127] M.A. La-Scalea, S.H.P. Serrano, I.G.R. Gutz, Quim. Nova 22 (1999) 417.
[128] D.A. Skoog, F.J. Holler, T.A. Nieman, Principles of Instrumental Analysis, 5th ed., Harcourt College Publishers, USA, 1998, p. 666.
[129] J. Wang, X. Cai, C. Jonsson, M. Balakrishnan, Electroanalysis 8 (1996) 20.
[130] J.S. Daniels, N. Pourmand, Electroanalysis 19 (2007) 1239.
[131] C. Guiducci, C. Stagni, G. Zuccheri, A. Bogliolo, L. Benini, B. Samorì, B. Riccò, Euro. Solid-State Dev. Res. Conf. (2002) 479.
[132] J.-G. Guan, Y.-Q. Miao, Q.-J. Zhang, J. Biosci. Bioeng. 97 (2004) 219.
[133] K.S. Ma, H. Zhou, J. Zoval, M. Madou, Sens. Actuator B 11 (114) (2006) 58.
[134] T. Uno, H. Tabata, T. Hawai, Anal. Chem. 79 (2007) 52.
[135] J. Wang, E. Paleček, P.E. Nielsen, G. Rivas, X. Cai, H. Shiraishi, N. Dontha, D. Luo, P.A.M. Farias, J. Am. Chem. Soc. 118 (1996) 7667.
[136] F. Le-Floch, H.-A. Ho, P. Harding-Lepage, M. Bedard, R. Neagu-Plesu, M. Leclerc, Adv. Mater. 17 (2005) 1251.
[137] D.-S. Kim, Y.-T. Jeong, H.-J. Park, J.-K. Shin, P. Choi, J.-H. Lee, G. Lim, Biosens. Bioelectron. 20 (2004) 69.
[138] T. Sakata, Y. Miyahara, ChemBioChem 6 (2005) 703.
[139] J. Fritz, E.B. Cooper, S. Gaudet, P.K. Sorger, S.R. Manalis, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 14142.
[140] J. Wang, G. Rivas, X. Cai, N. Dontha, H. Shiraishi, D. Luo, F.S. Valera, Anal. Chim. Acta 337 (1997) 41.
[141] J. Wang, X. Cai, B. Tian, H. Shiraishi, Analyst 121 (1996) 965.
[142] M. Kamahori, Y. Ishige, M. Shimoda, Anal. Sci. 23 (2007) 75.
[143] A. Li, F. Yang, Y. Ma, X. Yang, Biosens. Bioelectron. 22 (2007) 1716.
[144] A. Lapicki, F. Sakamoto, A. Sandhu, Jpn. J. Appl. Phys. 46 (2007) L49.
[145] Z. Gao, N.C. Tansil, Nucleic Acids Res. 33 (2005) e123.
[146] P. Singhal, W.G. Kuhr, Anal. Chem. 69 (1997) 4828.
[147] K. Wu, J. Fei, W. Bai, S. Hu, Anal. Bioanal. Chem. 376 (2003) 205.
[148] K. Kerman, Y. Morita, Y. Takamura, M. Ozsoz, E. Tamiya, Electroanalysis 16 (2004) 1667.
[149] K. Kerman, Y. Morita, Y. Takamura, M. Ozsoz, E. Tamiya, Anal. Bioanal. Chem. 381 (2005) 1114.
[150] S.E. Henrickson, M. Misakian, B. Robertson, J.J. Kasianowicz, Phys. Rev. Lett. 85 (2000) 3057.
[151] S. Howorka, S. Cheley, H. Bayley, Nat. Biotechnol. 19 (2001) 636.
[152] A. Meller, L. Nivon, D. Branton, Phys. Rev. Lett. 86 (2001) 3435.
[153] H. Aoki, P. Bühlmann, Y. Umezawa, Electroanalysis 12 (2000) 1272.
[154] F. Patolsky, A. Lichtenstein, I. Willner, Angew. Chem. Int. Ed. 39 (2000) 940.
[155] D.L. Graham, H.A. Ferreira, P.P. Freitas, Trends Biotechnol. 22 (2004) 455.
[156] J.C. Rife, L.J. Whitman, US Patent Application Publication 2004-0253744.
[157] S.P. Mulvaney, C.L. Cole, M.D. Kniller, M. Malito, C.R. Tamanaha, J.C. Rife, M.W. Stanton, L.J. Whitman, Biosens. Bioelectron. 23 (2007) 191.
[158] A. Merkoçi, M. Aldavert, S. Marin, S. Alegret, Trends Anal. Chem. 24 (2005) 341.
[159] H. Xu, H. Wu, F. Huang, S. Song, W. Li, Y. Cao, C. Fan, Nucleic Acids Res. 33 (2005) e83/81.
[160] M. Gabig-Ciminska, A. Holmgren, H. Andresen, K.B. Barken, M. Wümpelmann, J. Albers, R. Hintsche, A. Breitenstein, P. Neubauer, M. Los, A. Czyz, G. Wegrzyn,

G. Silfversperre, B. Jürgen, T. Schweder, S.O. Enfors, Biosens. Bioelectron. 19 (2004) 537.
[161] J.-M. Nam, S.I. Stoeva, C.A. Mirkin, J. Am. Chem. Soc. 126 (2004) 5932.
[162] D. Pioch, B. Jürgen, S. Evers, K.-H. Maurer, M. Hecker, T. Schweder, Appl. Microbiol. Biotechnol. 78 (2008) 719.
[163] P. Yakovchuk, E. Protozanova, M.D. Frank-Kamenetskii, Nucleic Acids Res. 34 (2006) 564.
[164] S. Dubus, J.-F. Gravel, B. Le Drogoff, P. Norbert, T. Veres, D. Boudreau, Anal. Chem. 78 (2006) 4457.
[165] V. Martins, L.P. Fonseca, H.A. Ferreira, D.L. Graham, P.P. Freitas, J.S. Cabral, Technical Proceedings of the 2005 NSTI-Nanotech., vol. 1, 2005, p. 493.
[166] P.P. Freitas, H. Ferreira, D. Graham, L. Clarke, M. Amaral, V. Martins, L. Fonseca, J.S. Cabral, Europhys. News 34 (2003) 224.
[167] K. Smistrup, B.G. Kjeldsen, J.L. Reimers, M. Dufva, J. Petersen, M.F. Hansen, Lab Chip 5 (2005) 1315.
[168] M. Nilsson, H. Malmgren, M. Samiotaki, M. Kwiatkowski, B.P. Chowdhari, U. Landegren, Science 265 (1994) 2085.
[169] J. Jarvius, J. Melin, J. Göransson, J. Stenberg, S. Fredriksson, C. Gonzalez-Rey, S. Bertilsson, M. Nilsson, Nat. Methods 3 (2006) 725.
[170] J. Miao, Z. Cao, Y. Zhou, C. Lau, J. Lu, Anal. Chem. 80 (2008) 1606.
[171] W.-C. Lee, K.-Y. Lien, G.-B. Lee, H.-Y. Lei, Diag. Microbiol. Infect. Dis. 60 (2008) 51.
[172] G. Zheng, F. Patolsky, Y. Cui, W.U. Wang, C.M. Lieber, Nat. Biotechnol. 23 (2005) 1294.
[173] P.P. Freitas, H.A. Ferreira, F. Cardoso, S. Cardoso, R. Ferreira, J. Almeida, A. Guedes, V. Chu, J.P. Conde, V. Martins, L. Fonseca, J.S. Cabral, J. Germano, L. Sousa, M. Piedade, B. Silva, J.M. Lemos, L.A. Clarke, M.D. Amaral, in: M.S. Pereira (Ed.), Nanotechnology and the Detection of Biomolecular Recognition Using Magnetoresistive Transducers. A Portrait of State-of-the-art Research at the Technical University of Lisbon, Springer, Dordrecht, The Netherlands, 2007, pp. 3–22.
[174] IUPAC website (http://old.iupac.org/goldbook/L03540.pdf).
[175] F. Caruso, E. Rodda, D.N. Furlong, Anal. Chem. 69 (1997) 2043.
[176] S.A. Hofstadler, R. Sampath, L.B. Blyn, M.W. Eshoo, T.A. Hall, Y. Jiang, J.J. Drader, J.C. Hannis, K.A. Sannes-Lowery, L.L. Cummins, B. Libby, D.J. Walcott, A. Schink, C. Massire, R. Ranken, J. Gutierrez, S. Manalili, C. Ivy, R. Melton, H. Levene, G. Barrett-Wilt, F. Li, V. Zapp, N. White, V. Samant, J.A. McNeil, D. Knize, D. Robbins, K. Rudnick, A. Desai, E. Moradi, D.J. Ecker, Int. J. Mass Spectrom. 242 (2005) 23.
[177] D.C. Muddiman, G.A. Anderson, S.A. Hofstadler, R.D. Smith, Anal. Chem. 69 (1997) 1543.
[178] R. Sampath, S.A. Hofstadler, L. Blyn, M. Eshoo, T. Hall, C. Massire, H. Levene, J. Hannis, P.M. Harrell, B. Neuman, M.J. Buchmeier, Y. Jiang, R. Ranken, J. Drader, V. Samant, R.H. Griffey, J.A. McNeil, S.T. Crooke, D.J. Ecker, Emerg. Infect. Dis. 11 (2005) 373.
[179] R. Sampath, K.L. Russell, C. Massire, M.W. Eshoo, V. Harpin, L.B. Blyn, R. Melton, C. Ivy, T. Pennella, F. Li, H. Levene, T.A. Hall, B. Libby, N. Fan, D.J. Walcott, R. Ranken, M. Pear, A. Schink, J. Gutierrez, J. Drader, D. Moore, D. Metzgar, L. Addington, R. Rothman, C.A. Gaydos, S. Yang, K.S. George, M.E. Fuschino, A.B. Dean, D.E. Stallknecht, G. Goekjian, S. Yingst, M. Monteville, M.D. Saad, C.A. Whitehouse, C. Baldwin, K.H. Rudnick, S.A. Hofstadler, S.M. Lemon, D.J. Ecker, PLoS ONE 2 (2007) e489.
[180] F.R.R. Teles, D.M.F. Prazeres, J.L. Lima-Filho, Rev. Med. Virol. 15 (2005) 287.
[181] K.M. Black, N. Vats, Analyst 132 (2007) 1183.
[182] M.A. Dineva, L. Mahilum-Tapay, H. Lee, Analyst 132 (2007) 1193.
[183] M.J. Wolcott, Clin. Microbiol. Rev. 5 (1992) 370.
[184] P. Fortina, S. Surrey, L.J. Kricka, Trends Mol. Med. 8 (2002) 264.
[185] R.H.P. Peters, M.A. van Agtmael, S.A. Danner, P.H.M. Savelkoul, C.M.J.E. Vandenbroucke-Grauls, Lancet Infect. Dis. 4 (2004) 751.
[186] D. Griffiths, G. Hall, Trends Biotechnol. 11 (1993) 122.
[187] K. Cammann, U. Lemke, A. Rohen, J. Sander, H. Wilken, B. Winter, Angew. Chem. Int. Ed. 30 (1991) 516.
[188] S. Andreescu, O.A. Sadik, Pure Appl. Chem. 76 (2004) 861.
[189] J. Suter, M. White, H. Zhu, H. Shi, C.W. Caldwell, X. Fan, Biosens. Bioelectron. 23 (2008) 1003.
[190] M. Piliarik, H. Vaisocherová, J. Homila, Sens. Actuator B 121 (2007) 187.
[191] T. Endo, K. Kerman, N. Nagati, Y. Takamura, E. Tamiya, Anal. Chem. 77 (2005) 6976.
[192] F. Zezza, M. Pascale, G. Mule, A. Visconti, J. Microbiol. Methods 66 (2006) 529.
[193] S. Sawata, E. Kai, K. Ikebukuro, T. Iida, T. Honda, I. Karube, Biosens. Bioelectron. 14 (1999) 397.
[194] J. Zhang, H. Qi, Y. Li, J. Yang, Q. Gao, C. Zhang, Anal. Chem. 80 (2008) 2888.
[195] M. Cunha, D.L. Stokes, G.D. Griffin, T. Vo-Dinh, Biosens. Bioelectron. 19 (2004) 1007.
[196] T.H. Wang, S. Masset, C.M. Ho, 14th IEEE International Conference on Microelectro-Mechanical Systems, Technical Digest (MEMS 2001), 2001, p. 431.
[197] J. Li, W. Tan, K. Wang, D. Xiao, X. Yang, X. He, Z. Tang, Anal. Sci. 28 (2000) 3011.
[198] Q. Wang, X. Yang, K. Wang, Sens. Actuator B 123 (2007) 227.
[199] Y. Sato, A. Okumura, K. Suzuki, H. Kawaguchi, J. Biomater. Sci. Polym. Ed. 15 (2004) 297.
[200] K.S. Rao, S.U. Rani, D.K. Charyulu, K.N. Kumar, B.-K. Lee, H.-Y. Lee, Y. Kawai, Anal. Chim. Acta 576 (2006) 177.
[201] D. Caputo, G. de Cesare, A. Nascetti, R. Negri, J. Non-Cryst. Solids 352 (2006) 2004.
[202] F. Kleinjung, F.F. Bier, A. Warsinke, F.W. Seheller, Anal. Chim. Acta 350 (1997) 51.
[203] B.G. Healey, R.S. Matson, D.R. Walt, Anal. Biochem. 251 (1997) 270.
[204] A.J. Baeumner, R.N. Cohen, V. Miksic, J.H. Min, Biosens. Bioelectron. 18 (2003) 405.
[205] E.L. McCarthy, L.E. Bickerstaff, M.P. da Cunha, P.J. Millard, Biosens. Bioelectron. 22 (2007) 1236.
[206] D.F. Yao, F. Yu, J.Y. Kim, J. Scholz, P.E. Nielsen, E.K. Sinner, W. Knoll, Nucleic Acids Res. 32 (2004) e177.
[207] S.H. Chen, V.C.H. Wu, Y.C. Chuang, C.S. Lin, J. Microbiol. Methods 73 (2008) 7.
[208] M. Lazerges, H. Perrot, N. Zeghib, E. Antoine, C. Compere, Sens. Actuator B 120 (2006) 329.
[209] L. Nicu, M. Guirardel, F. Chambosse, P. Rougerie, S. Sinh, E. Trevisiol, J.M. Francois, J.P. Maioral, A.M. Caminade, E. Cattan, C. Bergaud, Sens. Actuator B 110 (2005) 125.
[210] V.C.H. Wu, S.H. Chen, C.S. Lin, Biosens. Bioelectron. 22 (2007) 2967.
[211] F. Mannelli, A. Minunni, S. Tombelli, R.H. Wang, M.M. Spiriti, M. Mascini, Bioelectrochemistry 66 (2005) 129.
[212] P. Skladal, C.D. Riccardi, H. Yamanaka, P.I. da Costa, J. Virol. Methods 117 (2004) 145.
[213] Y. Roh, J. Woo, Y. Hur, Y.E. Pak, Smart Structures and Materials 2005: Smart Electronics, MEMS, BIOMEMS, and Nanotechnology Conference, Proceedings of the Society of Photo-Optical Instrumentation Engineers (SPIE) 5763 (2005) 291.
[214] Y. Hur, J. Han, J. Seon, Y.E. Park, Y. Roh, Sens. Actuator A 120 (2005) 462.
[215] M. Duman, R. Saber, E. Piskin, Biosens. Bioelectron. 18 (2003) 1355.
[216] A. Bouchet, C. Chaix, C.A. Marquette, L.J. Blumb, B. Mandrand, Biosens. Bioelectron. 23 (2007) 735.
[217] K. Arora, N. Prabhakar, S. Chand, B.D. Malhotra, Sens. Actuator B 126 (2007) 655.
[218] Y. Xiao, A.A. Lubin, B.R. Baker, K.W. Plaxco, A.J. Heeger, Proceedings of the National Academy of Sciences of the USA 103, 2006, p. 16677.
[219] H. Korri-Youssoufi, A. Yassar, Biomacromolecules 2 (2001) 58.
[220] C.D.S. Riccardi, C. Kranz, J. Kowalik, H. Yamanaka, B. Mizaikoff, M. Josowicz, Anal. Chem. 80 (2008) 237.
[221] M. Mascini, M. Del Carlo, M. Minunni, B.N. Chen, D. Compagnone, Bioelectrochemistry 67 (2005) 163.
[222] J. Koehne, H. Chen, J. Li, A.M. Cassell, Q. Ye, H.T. Ng, J. Han, M. Meyyappan, Nanotechnology 14 (2003) 1239.
[223] G.J. Zhang, J.H. Chua, R.-E. Chee, A. Agarwal, S.M. Wong, K.D. Buddharaju, N. Balasubramanian, Biosens. Bioelectron. 23 (2008) 1701.
[224] Y. Fu, R. Yuan, Y. Chai, L. Zhou, Y. Zhang, Anal. Lett. 39 (2006) 467.
[225] F. Fu, R. Yuan, L. Xu, Y. Chai, X. Zhang, D. Tang, Biochem. Eng. J. 23 (2005) 37.
[226] Y. Fu, R. Yuan, L. Xu, Y. Chai, Y. Liu, D. Tang, Y. Zhang, J. Biochem. Biophys. Methods 62 (2005) 163.
[227] H. Berney, J. West, E. Haefele, J. Alderman, W. Lane, J.K. Collins, Sens. Actuator B 68 (2000) 100.
[228] T. Hianik, M. Fajkus, B. Sivak, I. Rosenberg, P. Kois, J. Wang, Electroanalysis 12 (2000) 495.
[229] M. Kamahori, Y. Ishige, M. Shimoda, Biosens. Bioelectron. 23 (2008) 1046.
[230] Z.Q. Gao, A. Agarwal, A.D. Trigg, N. Singh, C. Fang, C.H. Tung, Y. Fan, K.D. Buddharaju, J. Kong, Anal. Chem. 79 (2007) 3291.
[231] M. Díaz-González, A. Escosura-Muñiz, M.B. González-García, A. Costa-Garcia, Biosens. Bioelectron. 23 (2008) 1340.
[232] P. Abad-Valle, M.T. Fernandez-Abedul, A. Costa-Garcia, Biosens. Bioelectron. 20 (2005) 2251.
[233] G. Carpini, F. Lucarelli, G. Marrazza, M. Mascini, Biosens. Bioelectron. 20 (2004) 167.
[234] D.K. Xu, K. Huang, Z.H. Liu, Y.Q. Liu, L.R. Ma, Electroanalysis 13 (2001) 882.
[235] R. Miranda-Castro, P. De-Los-Santos-Alvarez, M.J. Lobo-Castanon, A.J. Miranda-Ordieres, P. Tunon-Blanco, Anal. Chem. 79 (2007) 4050.
[236] F. Lucarelli, G. Marrazza, M. Mascini, Biosens. Bioelectron. 20 (2005) 2001.
[237] F. Patolsky, A. Lichtenstein, I. Willner, Chem. A Euro. J. 9 (2003) 1137.
[238] X. Mao, J. Jiang, X. Xu, X. Chu, Y. Luo, G. Shen, R. Yu, Biosens. Bioelectron. 23 (2008) 1555.
[239] Y.C. Zhang, H.H. Kim, A. Heller, Anal. Chem. 75 (2003) 3267.
[240] M.J. LaGier, J.W. Fell, K.D. Goodwin, Mar. Pollut. Bull. 54 (2007) 757.
[241] S. Suye, T. Matsuura, T. Kimura, H. Zheng, T. hori, Y. Amano, H. Katavama, Microelectron. Eng. 81 (2005) 441.
[242] H. Xie, C.Y. Zhang, Z.Q. Gao, Anal. Chem. 76 (2004) 1611.
[243] M.I. Pividori, A. Merkoci, S. Alegret, Biosens. Bioelectron. 19 (2003) 473.
[244] X.Y. Du, M.Q. Wang, L.Y. Liu, W.L. Gonga, B.Z. Wang, Sensor Lett. 6 (2008) 226.
[245] C. Jiang, T. Yang, K. Jiao, H. Gao, Electrochim. Acta 53 (2008) 2917.
[246] Z. Chang, H. Pan, K. Zhao, K.M. Chen, P. He, Y. Fang, Electroanalysis 20 (2008) 131.
[247] K. Arora, N. Prabhakar, S. Chand, B.D. Malhotra, Biosens. Bioelectron. 23 (2007) 613.
[248] I.O. K'Owino, S.K. Mwilu, O.A. Sadik, Anal. Biochem. 369 (2007) 8.
[249] M. Revenga-Parra, T. Garcia, E. Lorenzo, F. Pariente, Biosens. Bioelectron. 22 (2008) 2675.
[250] X.-H. Lin, P. Wu, W. Chen, Y.-F. Zhang, X.-H. Xia, Talanta 72 (2007) 468.
[251] H. Peng, C. Soeller, J. Travas-Sejdic, Macromolecules 40 (2007) 909.
[252] N.N. Zhu, Z. Chang, P.G. He, Y.Z. Fang, Anal. Chim. Acta 545 (2005) 21.

[253] N.N. Zhu, A.P. Zhang, Q.J. Wang, P.G. He, Y.Z. Fang, Electroanalysis 16 (2004) 577.
[254] H. Aoki, Y. Umezawa, Electroanalysis 14 (2002) 1405.
[255] F. Yan, A. Erdem, B. Meric, K. Kerman, M. Ozsoz, O.A. Sadik, Electrochem. Commun. 3 (2001) 224.
[256] H. Cai, C. Xu, P. He, Y. Fang, J. Electroanal. Chem. 510 (2001) 78.
[257] M. Ozsoz, A. Erdem, K. Kerman, D. Ozkan, B. Tugrul, N. Topcuoglu, H. Ekren, M. Taylan, Anal. Chem. 75 (2003) 2181.
[258] D. Dell'Atti, M. Zavaglia, S. Tombelli, G. Bertacca, A.O. Cavazzana, G. Bevilacqua, M. Minunni, M. Mascini, Anal. Chem. 383 (2007) 140.
[259] C.A. Marquette, D. Thomas, A. Degiuli, L.J. Blum, Anal. Bioanal. Chem. 377 (2003) 922.
[260] S.A.M. Martins, G.A. Monteiro, L.P. Fonseca, D.M.F. Prazeres, Anal. Bioanal. Chem. 391 (2008) 2179.
[261] K. Kerman, Y. Matsubara, Y. Morita, Y. Takamura, E. Tamiya, Sci. Technol. Adv. Mater. 5 (2004) 351.
[262] J. Wang, G.D. Liu, R. Polsky, A. Merkoci, Electrochem. Commun. 4 (2002)722.
[263] A. Erdem, D.O. Ariksoysal, H. Karadeniz, P. Kara, A. Sengonul, A.A. Saviner, M. Ozsoz, Electrochem. Commun. 7 (2005) 815.
[264] V.C. Martins, F.A. Cardoso, J. Loureiro, M. Mercier, J. Germano, S. Cardoso, R. Ferreira, L.P. Fonseca, L. Sousa, M.S. Piedade, P.P. Freitas, in: Proceedings of the Biomagnetism and Magnetic Biosystems Based on Molecular Recognition Processes, San Feliu de Guixols, Spain, September 22–27, AIP Conference Proceedings 1025 (2008) 150.