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Chem. Rev., **2008**, 108 (1), 109-139 • DOI: 10.1021/cr0684467

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Received June 21, 2007

Contents

1. Introduction

Development of DNA biosensors and DNA microarrays has increased tremendously over the past few years as demonstrated by the large number of scientific publications in this area (Figure 1).

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Recent progress in the development of DNA biosensors and microarrays is summarized in this review. It is important to point out that reviews dealing with DNA biosensors mainly focus on electrochemical transduction.[2−10] Very few papers have been written describing DNA biosensors including not only electrochemical but also optical and piezoelectric transduction modes.[11] Moreover, the recent evolution of DNA microarrays is also studied in this work. DNA probe immobilization and hybridization detection are largely reported.

In recent years, the interest for DNA-based diagnostic tests has been growing. The development of systems allowing DNA detection is motivated by applications in many fields: DNA diagnostics, gene analysis, fast detection of biological warfare agents, and forensic applications. Detection of genetic mutations at the molecular level opens up the possibility of performing reliable diagnostics even before any symptom of a disease appears.

Numerous DNA detection systems based on the hybridization between a DNA target and its complementary probe, which is present either in solution or on a solid support, have been described. Homogeneous assays allowing the determination of DNA sequences have been developed. These systems can be based on optical[12−15] or electrochemical[16,17] detection. However, they do not allow easily continuous monitoring and miniaturization. DNA biosensors and DNA microarrays offer promising alternatives to these methods. They allow continuous, fast, sensitive, and selective detection of DNA hybridization, and they also can be reused.

DNA biosensors (also called genosensors) and DNA microarrays (commonly called gene chips, DNA chips, or biochips) exploit the preferential binding of complementary single-stranded nucleic acid sequences. This system usually relies on the immobilization of a single-stranded DNA (ssDNA) probe onto a surface to recognize its complementary DNA target sequence by hybridization (Figure 2). Transduction of hybridization of DNA can be measured optically, electrochemically, or using mass-sensitive devices.

In the case of DNA biosensors and contrary to DNA microarrays, the immobilization of a DNA probe is achieved directly onto a transducer surface. DNA microarrays are made from glass, plastic, or silicon supports and are constituted of tens to thousands of 10−100 μm reaction zones onto which individual oligonucleotide sequences have been immobilized.[18,19] The exact number of DNA probes varies in accordance with the application. Contrary to DNA biosensors that allow single-shot measurements, DNA microarrays allow multiple parallel detection and analysis of the patterns of expression of thousands of genes in a single experiment.[20−24]

10.1021/cr0684467 CCC: $71.00
Published on Web 12/21/2007

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A DNA probe can be synthesized before immobilization, or alternatively, each base can be deposited successively on the sensor surface.[25] Another strategy is the use of peptide nucleic acids (PNAs) instead of DNA as probe sequences.[26−35]

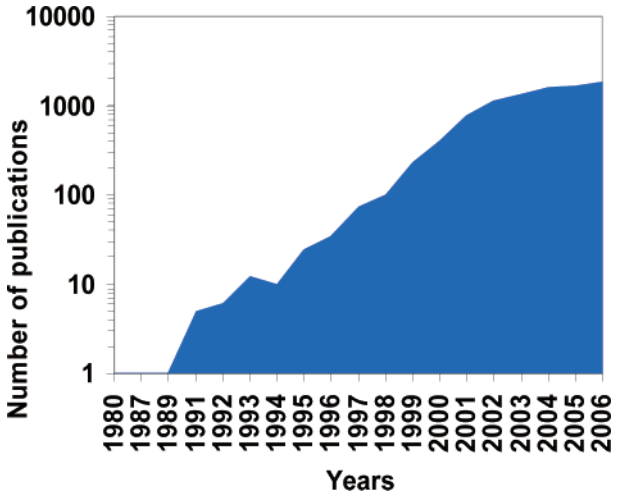

Figure 1. Annual trends in the number of publications for DNA biosensors and microarrays. The terms "DNA microarray*", "DNA chip*", "DNA biochip*", "genosensor*", "DNA sensor*", "DNA array*", "DNA biosensor*", "gene chip*", and "gene array*" have been considered. The literature search was done using ISI's Web of Science.[1]

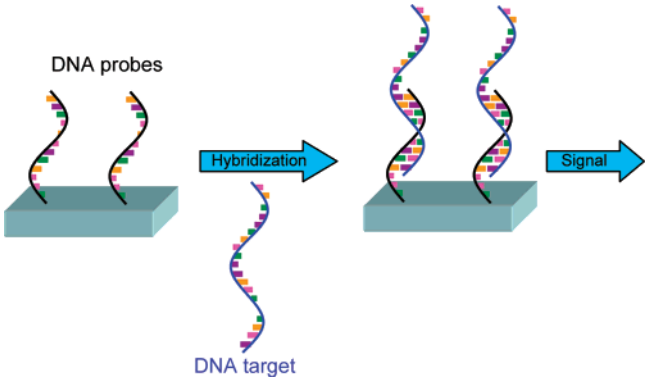

Figure 2. Steps involved in the detection of a DNA sequence. In this review, DNA probes refer to immobilized sequences and DNA targets refer to sequences in the sample being captured.

PNA is a DNA mimic where the negatively charged sugar phosphate backbone is replaced by a neutral pseudopeptidic chain consisting of repeated *N*-(2-aminoethyl)glycine units linked by amide bonds.[36] PNA can form Watson−Crick complementary duplexes with DNA.[30] In comparison to DNA duplexes, PNA hybrids show higher thermal stability that is strongly affected by the presence of imperfect matches.[35] Moreover, PNA hybrids can be formed at low ionic strengths, and they present a greater resistance to both nuclease and protease digestion.[37] The dendritic nucleic acid structures also can be used as DNA probes. They are highly branched arborescent structures assembled by sequential hybridization of ssDNA.[38] Dendrimers possess numerous single-stranded oligonucleotide (ODN) arms able to hybridize with a complementary nucleic acid sequence with a high sensitivity. Thus, they are used as recognition elements in DNA biosensors.[39,40] Another interesting DNA probe family can be pointed out: molecular beacons, stem-and-loop structures responsible for mismatch discrimination.

Depending on the application, the choice of the immobilization technique is extremely important. To develop microarrays, immobilization techniques are based on two main strategies: either the deposition of ready-made DNA probes onto chemically activated surfaces or the in situ synthesis of ODNs directly on the support.[41,42] Moreover, many immobilization methods can be employed to develop

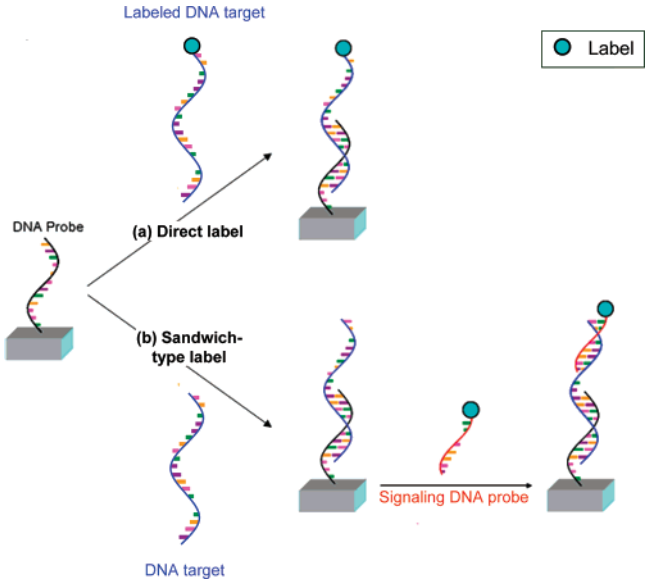

Figure 3. Main types of labeled DNA biosensors and microarrays: (a) direct label between immobilized DNA probe and labeled DNA target; (b) sandwich-type system. A sandwich-type ternary complex is formed between immobilized DNA probe, target, and signaling DNA probe

both DNA microarrays and DNA biosensors: adsorption, covalent immobilization, and (strept-)avidin−biotin interaction.[43,44]

The traditional method of labeling is radioactivity.[45,46] Although this method is one of the most sensitive, the use of radioisotopes, such as ^{32}P or ^{125}I, presents serious disadvantages. However, optical and electrochemical techniques have been developed based on various labels. For example, fluorescent dyes, and more recently quantum dots (QDs), are largely used for optical detection.[47] Electrochemistry has received considerable attention recently[2−5,48−53] for the detection of DNA hybridization. These systems offer some advantages, such as low cost, simple design, or small dimensions. The label can be an enzyme, an electroactive indicator, such as ferrocene (Fc), cationic metal complexes or intercalating organic compounds (e.g., methylene blue), or a nanoparticle. A direct labeling strategy can be used where the immobilized DNA probe hybridizes with the labeled DNA target (Figure 3a). However, a sandwich-type ternary complex can be formed. The immobilized DNA probe hybridizes to a part of the target whereas the other part of the target is complementary to a signaling DNA sequence that serves to label the target upon hybridization (Figure 3b). In a few cases, a competitive system can be used with a competition between the target and a labeled sequence both complementary to a DNA probe.[54]

Label-free electrochemical detection of hybridization represents an attractive alternative approach for detecting DNA sequences. In this case, the detection is based on modifications of properties, such as capacitance, or an intrinsic electrochemical response due to DNA (e.g., oxidation of guanines). Gravimetric DNA biosensors are able to detect label-free ODNs.

It can be highlighted that regeneration of the surface-immobilized probe is possible, allowing the reuse of the DNA biosensor or the microarray without a significant loss of hybridization activity. For that, a thermal or chemical regeneration step is necessary. Biotinylated ODN probes have been immobilized on the core surface of a multimode optical fiber.[55] After the hybridization reaction, the two types of regeneration have been tested. For thermal separation of probe/target duplexes, the surface has been washed with hybridization buffer at 70 °C for 2 min whereas the chemical regeneration has been done by pumping a 4 M urea solution through the flow cell for 2 min. Regeneration of an optical biosensor system has also been achieved based on fluorescence excitation and detection in the evanescent field of a quartz fiber by thermal and chemical treatment.[56] In this case, the sensor surface has been heated to a temperature of 68.5 °C or treated by 50% (w/w) aqueous urea solution. Regeneration has also been achieved by a flash of 50 mM NaOH.[57] More than 60 hybridization−regeneration cycles have been performed with a 10% loss of reproducibility. In the same way, strand regeneration of the DNA hybrid has been described by the addition of 10 mM NaOH for 1 min.[58] Microarray surfaces also have been regenerated by a treatment with 50 mM NaOH/0.1% sodium dodecyl sulfate (SDS).[59] HCl also has been reported at 1[60] or 10 mM.[61]

Two aspects are essential when developing hybridization biosensors and microarrays: the sensitivity and the selectivity. It is important to be able to detect low DNA concentrations and to detect a point mutation. Thus, two types of systems can be developed: systems for DNA hybridization and systems for detection of DNA damage.[62] A perfect match in the target sequence produces very stable double-stranded DNA (dsDNA), whereas one or more base mismatches decreases the stability, causing a signal modification.

2. DNA Immobilization

DNA probes are short ODNs (12−40-mer) able to hybridize with specific target sequences.

The immobilization step for the DNA probe is essential to develop a whole range of biosensors and microarrays. The achievement of high sensitivity and selectivity requires minimization of nonspecific adsorption and stability of immobilized biomolecules. The control of this step is essential to ensure high reactivity, orientation, accessibility, and stability of the surface-confined probe and to avoid nonspecific binding.

2.1. Immobilization Techniques Used To Develop DNA Biosensors and Microarrays

DNA can be immobilized on sensor surfaces with methods similar to those used for enzyme-based biosensors: adsorption, covalent immobilization, and avidin (or streptavidin)−biotin interaction.[63] These immobilization techniques also can be used to develop DNA microarrays.[41]

2.1.1. Adsorption

Adsorption is the simplest immobilization method because it does not require any nucleic acid modification.

Immobilization has been reported based on ionic interactions occurring between the negatively charged groups present on the DNA probe and positive charges covering the surface. For instance, a chitosan film was used for the immobilization of ssDNA on a glassy carbon electrode (GCE).[64,65] Chitosan is a cationic polymer that can form a stable complex with the negatively charged phosphate groups of the DNA. A DNA-based diagnostic quartz crystal microbalance (QCM) sensor was also developed.[66] The sensing layer was prepared according to different methods, among which was adsorption by electrostatic interactions. DNA

probes were adsorbed on the outer layer of poly(allylamine)-hydrochloride (PAAH)/sodium poly(styrenesulfonate) (PSS)/PAAH film. The coupling between the negatively charged phosphate backbone of the DNA probe and a positively charged film surface also allowed the development of DNA microarrays.[67] For example, short ODN probes were immobilized to a positively charged amino-silanized glass surface.[68,69]

DNA also can be linked by physical adsorption. For example, a screen-printed electrode (SPE) was immersed overnight in a DNA-containing solution before rinsing it to remove unadsorbed DNA.[70] The electrochemical DNA biosensor can detect 6×10^{-16} M of target. The DNA probe was adsorbed on a polished basal plane pyrolytic graphite (BPPG) electrode.[71] DNA was also immobilized on gold microelectrodes,[72] which were modified by dropping a small volume of DNA on their sensor surface before an overnight air-drying. More recently, the probe was immobilized onto preoxidized GCE by physical adsorption.[73]

ssDNA is often immobilized by applying a potential to an electrode. The electrode surface is sometimes electrochemically pretreated to increase its hydrophobicity and its roughness. Then, the controlled-potential adsorption of the ssDNA is achieved.[74−78] This potential applied during immobilization (generally +0.5 V vs Ag/AgCl) enhances the stability of the probe through the electrostatic attraction between the positively charge surface and the negatively charged sugar−phosphate backbone of DNA.

2.1.2. Covalent Immobilization

DNA immobilization by covalent attachment is often used.[79−83] Many different methods for covalent immobilization of DNA probes on different supports have been reported in the literature and are briefly described below.

2.1.2.1. Chemisorption

Thiol−metal interactions are frequently used to bind biomolecules covalently onto gold surfaces. The strong affinity of the thiol groups for noble metal surfaces enables the formation of covalent bonds between the sulfur and gold atoms.

$$R{-}SH + Au \rightarrow R{-}S{-}Au + e^- + H^+$$

On the basis of this principle (chemisorption), biosensors have been developed using thiol-modified DNA probes.[81,84−87] In the same way, DNA probes were immobilized onto gold-interdigitated ultramicroelecrode arrays by self-assembly of thiol-modified ODNs.[81] DNA strands also were attached to gold micropads deposited on a silicon surface.[86]

2.1.2.2. Covalent Attachment of a Modified Probe on Functionalized Surfaces

Covalent reactions often use carbodiimide as a reagent, with or without *N*-hydroxysuccinimide (NHS). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) is the most frequently used activation coupling reagent (Figure 4).

For example, self-assembled carbon nanotube (CNT) layers were formed on gold substrates.[88] Carboxylic acid groups were introduced to CNTs that formed covalent bonds with amino groups at the 5′ ends of DNA probes in the presence of EDC. Different covalent immobilization techniques also were tested.[82] According to the authors, the one-step EDC reaction was the most efficient for ODN immobilization. A DNA probe was immobilized onto a carboxylate-terminated 4-aminobenzoic acid monolayer via EDC and

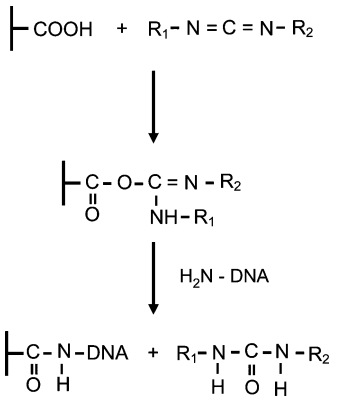

Figure 4. Schematic representation of DNA immobilization using EDC coupling.

NHS.[89] Aminated or carboxylated DNA were also immobilized to the respective carboxylated or aminated single-walled carbon nanotube (SWNT) multilayer films using EDC.[90] DNA was also attached to an aminosilane film.[91] For that purpose, the phosphorylated DNA reacted with EDC to form an *O*-phosphoryl isourea intermediate that reacted with the aminated surface to produce a phosphoramidate linkage.

Covalent immobilization of DNA probes on electrodes modified with a conducting polymer also has been described. For example, an amino-substituted ODN was immobilized onto the surface of the conducting copolymer of pyrrole and 4-(3-pyrrolyl)butanoic acid (PBA) via EDC.[92] Another sort of conductive polymer was used by polymerization of terthiophene monomers having a carboxyl group.[93] DNA probes were immobilized on the polymer using an EDC-mediated reaction. The immobilization of probes also was reported on a film of a polyacrylamide-based electron conductive redox hydrogel on a vitreous carbon electrode.[94] Probes were covalently attached to hydrazide functions of the hydrogel by carbodiimide coupling. EDC was also used to immobilize aminated probes onto polyaniline/polyacrylate (PAn/PAA)-modified boron-doped diamond electrodes possessing a high density of carboxylic groups.[95]

Situma and co-workers produced functional scaffolds on polymers for the covalent immobilization of ODN probes for DNA microarray applications.[96,97] They developed a UV photomodification protocol using poly(methyl methacrylate) and polycarbonate, producing surface-confined carboxylate functional groups that allow covalent immobilization of amino probes to these surfaces through carbodiimide coupling.

The optimization of covalent immobilization of dsDNA was reported on self-assembled monolayer (SAM)-modified gold electrodes.[98] dsDNA was covalently immobilized on amino-, hydroxyl-, or carboxyl-terminated SAM/Au surfaces obtained under the activation of EDC.

Other covalent methods for DNA immobilization also have been described. For example, Fuentes and co-workers proposed the use of a polyaldehyde−aspartic acid dextran to covalently immobilize probes. First, electrostatic adsorption of functional dextran on aminated plates[99] or on superparamagnetic nanoparticles[100] containing amino groups was realized. Aminated probes were then covalently attached to the support using a reaction between the aldehyde groups in the dextran and the amino groups on the surface.

ODN probes were grafted on a copolymer of 3-acetic acid pyrrole and 3-*N*-hydroxyphthalimide pyrrole.[101−103] A direct

chemical substitution of the *N*-hydroxyphthalimide leaving group by an ODN bearing a terminal amino group on its 5′ phosphorylated position allowed the covalent immobilization of probes onto the copolymer.

ssDNA was covalently immobilized onto cantilevers using glutaraldehyde to develop an atomic force microscopy (AFM)-based DNA sensor.[104] After silanization of the surface, glutaraldehyde allows reaction between amino groups of both the solid support and ssDNA. This bridging agent also has been used to develop DNA chips.[105]

A method was also described for the preparation of DNA microarrays based on disulfide-modified ODNs immobilized onto a mercaptosilane-modified glass surface.[106]

The 20-mer probes also were attached on microbeads that were adsorbed as arrays on glass surfaces that were first silanized with 3-glycidoxypropyltrimethoxysilane (GOPS).[107] The DNA immobilization relied on a reaction between the epoxy group of GOPS and an amino linker of ODNs.

Recently, a new immobilization strategy based on aniline derivative electroaddressing has been investigated, creating a covalent linkage with a conducting material surface.[108,109] First, the diazotation reaction of an aniline derivative easily leads to the formation of an aryl diazonium. Then, the electrochemical reduction of this latter species generates an aryl radical, which attacks the surface and forms an X−C bond (where X is the electrode material, namely, Au, C, Cu, Si) between the aryl group and the electrode material. This strategy has been used to develop an ODN-functionalized biochip.[110] A 20-mer sequence from a "hot spot" of exon 8 of the p53 tumor suppressor gene was functionalized at its 5′ end with a 4-aminobenzylamine aniline derivative to provide oriented grafting. It was electroaddressed to be used as a stationary-phase probe sequence for hybridization testing of a biotinylated target sequence.

2.1.3. Avidin (or Streptavidin)–Biotin Interactions

The formation of avidin (or streptavidin)−biotin complexes is useful in a wide variety of applications.[111−113] This specific binding is largely used to immobilize enzymes, antibodies, or DNA. Biotin is a small molecule that binds with a very high affinity to the avidin or streptavidin binding sites ($K_a = 10^{15}$ M^{-1}). Moreover, avidin and streptavidin are tetrameric proteins that have four identical binding sites for biotin. Streptavidin with an isoelectric point (pI) equal to 5 is thus preferably used over avidin, which has a pI of 10.5, to avoid nonspecific interactions.

The avidin (or streptavidin)−biotin interaction is often used to develop DNA biosensors. For example, ODNs were bound to a SAM of 2-mercaptoethanol and 11-mercaptoundecanoic acid through streptavidin−biotin interactions.[114] Avidin was also adsorbed onto a silica surface before immobilizing a biotinylated molecular beacon (MB).[115] A system based on biotin covalently linked to pyrrole monomers also was described.[116] Polypyrrole (PPy) was formed on the electrode, and the biotin units attached to the film were used as anchoring points for the avidin immobilization. Three sites still remained free on the avidin to react with biotinylated DNA probes.

Photobiotin was used as a biotin derivative.[117] An activation step based on photolithographic techniques was necessary to initiate attachment of a photoactive biotin molecule to a poly(dimethylsiloxane) (PDMS) chip. Then, avidin binding enabled immobilization of the biotinylated DNA probe to the surface. Microelectrode-based DNA chips were also produced using streptavidin−biotin interaction.[118] Streptavidin was immobilized on the surface of carbon or gold metal electrodes within an electrodeposited polymer of 7-hydroxy-6-methoxy-coumarin (scopoletin). Biotinylated DNA probes were then immobilized on top of the modified electrodes.

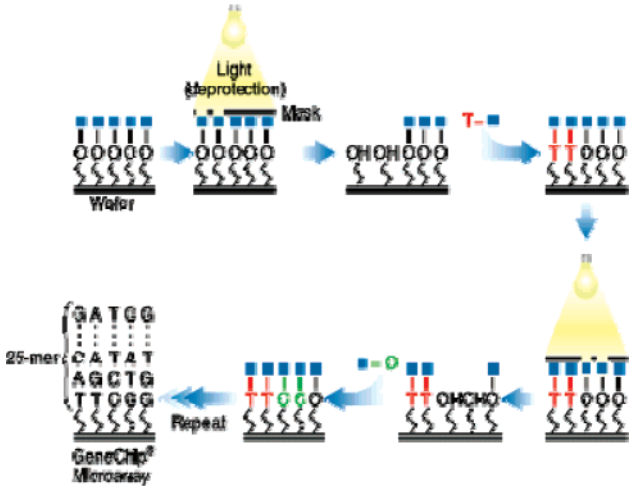

Figure 5. Photolithographic process for on-chip oligonucleotide synthesis.[120] Affymetrix arrays use light to convert a protective group on the terminal nucleotide into a hydroxyl group to which further bases can be added. Masks are used to direct light to the appropriate region so that the base is added to the correct position. Reprinted with permission from Affymetrix. Copyright 2007 Affymetrix.

2.2. DNA Microarrays

Microarrays can be categorized as either: complementary DNA (cDNA) arrays, usually using probes constructed with polymerase chain reaction (PCR) products of up a few thousands base pairs, or ODN arrays, using either short (25−30-mer) or long ODN (60−70-mer) probes.

Three methods can be considered for ODN probe deposition on microarray surfaces.[25,41,43,119] In the first case, oligonucleotides are built up base-by-base on the surface of the array. This takes place between the 5′ hydroxyl group of the sugar of the last nucleotide to be attached and the phosphate group of the next nucleotide. Each nucleotide added to the ODN on the support has a protective group on its 5′ position to prevent the addition of more than one base during each round of synthesis. Different methods for deprotection can be considered. Affymetrix[120,121] (Figure 5) technology is based on photodeprotection using masks, whereas NimbleGen[122] uses a Maskless Array Synthesizer technology (Figure 6). Agilent[123] employs a synthesis via ink-jet technology based on chemical deprotection.

In the second case, the DNA probe is presynthesized and then spotted on surfaces by contact printing or noncontact printing.

In the third case, an electronic current is used to address negatively charged DNA probes to positively charged specific sites.[124]

2.2.1. In Situ Synthesis of DNA Microarrays

Affymetrix[120] uses lithographic masks to define in which areas of the array the photolabile protective group on the phosphoramidite group is decomposed (Figure 5). A solution containing adenine, guanine, thymine, or cytosine is deposited on this surface. Coupling occurs only in the regions that have been deprotected through illumination.[125] The coupled

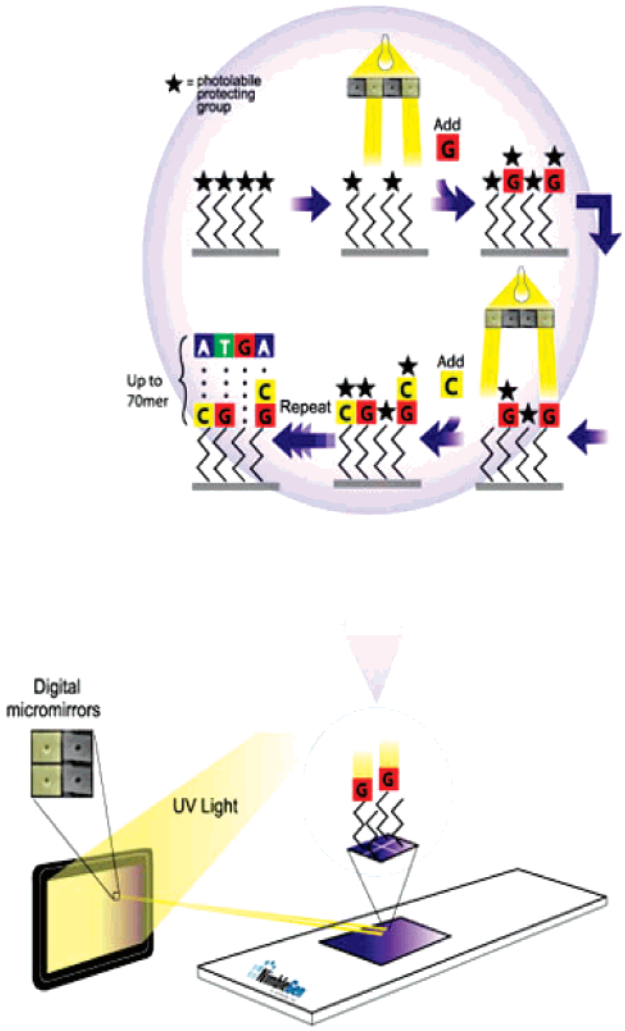

Figure 6. Synthesis of a microarray using NimbleGen's Maskless Array Synthesizer Technology.[122] This system uses light-mediated deprotection. The light is directed via micromirror arrays. Each mirror can move between two positions: one position to reflect light and the other to block light. At each step, the mirrors direct light to the appropriate parts of the arrays to cleave the UV-labile protecting group at a precise location where the next nucleotide will be coupled. Reprinted with permission from NimbleGen. Copyright 2007 NimbleGen Systems, Inc.

nucleotide also bears a light-sensitive protecting group so that the cycle can be repeated. In this way, the microarray is built as the probes are synthesized through repeated cycles of deprotection and coupling. The process is repeated until the probes reach their full length, usually 25 nucleotides.

In the same way, NimbleGen[122] (Figure 6) synthesizes ODNs in situ using maskless photolithography.[126] Individually addressable aluminum mirrors, controlled by a computer, reflect the desired pattern of UV light, which cleaves a UV-labile protecting group at the precise location where the next nucleotide will be coupled. The only commercially available complete system for on-chip synthesis is the FEBIT Biotech Geniome One. This system contains in situ DNA synthesis, hybridization, and detection units all within one instrument. Microarrays are synthesized using NimbleGen's Maskless Array Technology. Geniome One can synthesize arrays with up to 48 000 features on one chip. However, one chip can be divided into eight subarrays each consisting of 6000 features, allowing eight separate experiments to be analyzed on the same chip.[41]

The ink-jet method also allows the precise synthesis of an ODN directly on a slide.[42] Instead of using light to convert the protective group, Agilent[123] uses chemical deprotection. However, this technique is less efficient than the methods used by Affymetrix or NimbleGen for the design of large numbers of identical arrays.

2.2.2. Spotted Microarrays

In this case, the probes are presynthesized and then immobilized in precise locations on the support. These spotted microarrays can be produced by either contact or noncontact printing.

Microarray manufacture based on contact printing requires high-definition pins that deposit a small amount of probe solution after contact with the microarray surface. Various types of pin tools can be used: tweezers, microspotting pins, microspotting pins with reservoirs, or pin rings.[43]

Noncontact printing allows DNA probe deposition without direct contact on the surface. In this case, small dispensing systems are mounted on the robotic arm instead of pins. Ink-jet, bubble-jet, or piezo-actuation technologies are capable of dispersing single drops in the range extending from 100 pL to 2 μL[43].

Synthesized probes are spotted on the surface, and then they are immobilized through various methods. DNA probes can be attached to the surface either covalently or noncovalently (cf. section 2.1).

2.2.3. Nanogen Technology

Nanogen[124] uses the natural positive or negative charges of biological molecules. By application of a positive electric current to individual test sites on the microarray slides, sequences of negatively charged DNA probes are electronically placed at specific sites on the microarray.[127−129] For example, Nanogen developed a 1 cm^2 silicon chip comprising 25 microelectrodes arranged in a five-by-five array. An agarose permeation layer containing streptavidin coats the chips, separates the biological materials from the harsh electrochemical environment near the electrode, and allows the binding of biotinylated DNA samples. Each electrode may be individually biased positively or negatively or remain neutral to move and concentrate molecules on the test site. Molecular binding on the NanoChip microarray is up to 1000 times faster than traditional passive methods.

2.3. Immobilization Technique Specific to DNA Biosensors: Entrapment

Direct DNA immobilization in polymeric matrixes also has been described to develop DNA biosensors rather than DNA microarrays. Entrapment in electropolymerized films remains a popular electrochemical approach for biomolecule immobilization. This simple one-step method involves the application of an appropriate potential to the working electrode soaked in an aqueous solution containing a biomolecule (e.g., DNA probe) and an electropolymerizable monomer. Biomolecules present in the immediate vicinity of the electrode surface are physically incorporated in the growing polymer during its formation.[130]

PPy is the most often cited polymer for the design of DNA sensors.[131,132] It has been demonstrated that ODN probes can serve as the sole counteranion during the polymer formation.[133] A reagentless direct electrochemical DNA sensor has been developed to detect a biowarfare pathogen (*Variola*

Table 1. Performances of Optical DNA Systems

detection technique	immobilization technique	type of assay	linearity range (M)	detection limit (M)	references
fluorescence (fluorescein)	avidin−biotin interaction	direct	10^{-13}−10^{-8}	2×10^{-13}	56
fluorescence (fluorescein)	avidin−biotin interaction	competitive	10^{-9}−10^{-7}	1.1×10^{-9}	56
fluorescence (FITC)	avidin−biotin interaction	direct	up to 10^{-8} M	$\sim 10^{-10}$	141
fluorescence (FITC)	covalence	direct	2×10^{-10}−1.96×10^{-7}	2×10^{-10}	142
fluorescence	avidin−biotin interaction	direct	10^{-13}−10^{-6}	10^{-13}	143
fluorescence (*R*-phycoerythrin)	electro-copolymerization of a Py-modified ODN and Py	direct	ND	10^{-12}	144
fluorescence (fluorescein)	covalence	direct	ND	10^{-8}	145
fluorescence (Cy3)	covalence	direct	ND	10^{-14}	146
fluorescence (Cy3)	covalence	direct	ND	10^{-17}	147
fluorescence (Cy3−5)	covalence	sandwich	5×10^{-12}−10^{-8}	10^{-12}	148
fluorescence (MB)	avidin−biotin interaction	direct	ND	$\sim 10^{-9}$	115
fluorescence (MB)	streptavidin−biotin interaction	direct	5×10^{-9}−10^{-7}	2×10^{-9}	149
fluorescence (MB)	chemisorption	direct	10^{-11}−10^{-5}	10^{-11}	150
fluorescence (MB)	streptavidin−biotin interaction	direct	up to 10^{-7}	1.1×10^{-9}	151
CL	chemisorption	sandwich	ND	10^{-9}	84
CL	chemisorption	sandwich	ND	10^{-10}	204
CL	entrapment of beads bearing DNA (PDMS)	direct	5.8×10^{-11}−$2.3\ 10^{-8}$	5.8×10^{-11}	205
CL	covalence	direct	10^{-12}−10^{-9}	5×10^{-13}	206
CL	entrapment of beads bearing DNA (PVA-SbQ)	direct	2.5×10^{-10}−5×10^{-8}	2.5×10^{-10}	207
CL	chemisorption	direct	ND	1.5×10^{-11}	208
ECL	adsorption	direct	9.6×10^{-11}−9.6×10^{-8}	3×10^{-11}	209
ECL	inclusion of beads bearing nucleic acid at the surface of PDMS	direct	up to 2×10^{-6}	10^{-8}	210
SPR	chemisorption	direct	up to 2.5×10^{-8}	2.5×10^{-9}	226
SPR	covalence	sandwich	ND	1.38×10^{-15}	227

major virus) using ultrathin films of the conducting PPy doped with an ODN probe.[134] ssDNA was entrapped within PPy formed on a platinum electrode.[135] In the same way, oligonucleotide probes were immobilized during the electropolymerization of PPy onto multiwalled CNT-modified electrodes.[136] Electropolymerization by cyclic voltammetry (CV) also was reported to entrap DNA sequences into a PPy film.[137,138]

More rarely, other electrically conductive films have been used to entrap DNA probes. Polyaniline, polydiaminobenzene, and poly(3,4-ethylenedioxythiophene)[139,140] allowed the immobilization of DNA probes to develop electrochemical biosensors.

3. Nucleic Acid Hybridization Detection

Tables 1−3 present the performances obtained with different DNA biosensors or microarrays. Nucleic acid hybridization can be detected according to different techniques, based on optical (Table 1), electrochemical (Table 2), or gravimetric (QCM) (Table 3) detection.

3.1. Optical DNA Biosensors and DNA Microarrays

DNA hybridization can be optically detected using fluorescence, surface plasmon resonance (SPR), chemiluminescence, colorimetry, interferometry, or surface-enhanced Raman scattering (SERS) spectroscopy (Table 1).

3.1.1. Fluorescence Detection

Table 1 presents the performances obtained with some fluorescent-based biosensors or microarrays.[56,115,141−151]

When the DNA target is labeled with a fluorophore, such as fluorescein isothiocyanate (FITC), its hybridization with a probe can be easily measured with an imaging fluorescence apparatus. p(dA) probes (18-mer) or H-ras wild-type probes (10-mer) were immobilized with succinimidyl ester residues in acrylamide-based polymer matrixes deposited on an optical fiber.[142] This sensor also was able to identify one point mutation in the Ras oncogene PCR products. It can detect point mutations at DNA concentrations of 2×10^{-10}−1.96×10^{-7} M following a 20 min hybridization (Table 1). Probes were immobilized on self-assembled DNA-conjugated polymer of PAA.[152] The complementary target was labeled with FITC. A biotinylated probe also was immobilized on an optical fiber via avidin.[56] The hybridization with a fluorescein-labeled complementary target (16-mer) was monitored in real time by fluorescence detection. In this case, the detection limit was 2×10^{-13} M (Table 1). A multiplexed DNA hybridization system also was developed using encoded Ni microparticles.[141] These particles were coated with a SAM of 16-mercaptohexadecanoic acid and were treated with avidin. Biotinylated DNA probes were immobilized on the particle having a specific code via avidin−biotin interaction. The detection limit was estimated to be around 1×10^{-10} M (Table 1). Recently, organic-dye-doped silica nanoparticles were used to detect DNA.[153] A large number of fluorophores was encapsulated inside a single nanoparticle, which produced a strong fluorescence signal when it was properly excited. Therefore, when one probe DNA was labeled with one dye-doped silica nanoparticle, the signal was greatly amplified as compared to that with one fluorophore. Through the use of this strategy, DNA target molecules could be detected at a concentration as low as 8×10^{-13} M.[154]

Fluorescence detection using a fluorophore as a label also has been employed in DNA chip technology. Amino-modified ODNs were immobilized on an aldehyde-functionalized polyacrylamide gel.[155] A chip also was developed based on the incorporation of biotinylated DNA probes into bead microreactors.[143] In both cases, a fluorophore-conjugated DNA target was complementary to the capture strand. Ali et al.[143] used pyramidal wells to confine the sensor beads in a central position. It seems that the porous beads' internal microenvironment was more suitable for DNA hybridization.

Table 2. Performances of Electrochemical DNA Systems

detection technique	immobilization technique	type of assay	linearity range (M)	detection limit (M)	references
		A. Enzyme Label (Section 3.2.1)			
SWV (HRP + aniline)	chemisorption	sandwich	2×10^{-15}−10^{-12}	10^{-15}	34
DPV (HRP)	adsorption	direct	ND	6×10^{-16}	70
DPV (HRP)	adsorption	direct	1.5×10^{-10}−9.5×10^{-9}	5×10^{-11}	247
impedance (HRP)	chemisorption	sandwich	ND	6.5×10^{-13}	248
impedance (HRP)	chemisorption	sandwich	ND	2.3×10^{-9}	249
amperometry (GOD)	covalent binding of the DNA probe over a polymer	sandwich	ND	10^{-9}	250
amperometry (GOD)	chemisorption	sandwich	up to 8×10^{-13}	10^{-15}	251
amperometry (GOD)	chemisorption	direct	10^{-9}−2×10^{-6}	2×10^{-10}	252
DPV (PAL)	covalence	direct	3×10^{-9}−3×10^{-7}	10^{-9}	49
DPV (PAL)	chemisorption	direct	1.5×10^{-9}−5×10^{-8}	2.2×10^{-10}	253
DPV (PAL)	adsorption	sandwich	up to 1.5×10^{-7}	3×10^{-10}	254
DPV (PAL)	chemisorption	sandwich	2×10^{-10}−2×10^{-6}	3.4×10^{-10}	255
DPV (PAL)	chemisorption	direct	up to 2.5×10^{-8}	2.5×10^{-10}	256
impedance (PAL)	chemisorption	sandwich	1.2×10^{-11}−1.2×10^{-8}	1.2×10^{-12}	257
CV (PAL)	covalence	sandwich	3.4×10^{-7}−3.4×10^{-6}	1.46×10^{-7}	258
CV (PAL)	streptavidin−biotin interaction	direct	ND	1.63×10^{-11}	259
amperometry (G6PDH)	streptavidin−biotin interaction	sandwich	2.63×10^{-12}−2.63×10^{-8}	3.3×10^{-13}	260
amperometry ((PQQ)GDH)	avidin−biotin interaction	direct	5×10^{-8}−1.1×10^{-5}	ND	261
		B. Ferrocene (Section 3.2.2)			
CV (ferrocene)	chemisorption	sandwich	6.9×10^{-12}−1.5×10^{-10}	2×10^{-12}	277
CV (ferrocene)	chemisorption	direct	ND	2.5×10^{-13}	278
SWV (ferrocene)	chemisorption	direct	ND	5×10^{-10}	279
ACV (ferrocene)	chemisorption	sandwich	3.4×10^{-12}−1.4×10^{-7}	10^{-12}	280
DPV (FCA)	chemisorption	direct	10^{-9}−5×10^{-7}	5×10^{-10}	281
CV/DPV (AFc)	adsorption	direct	10^{-8}−6×10^{-6}	2×10^{-9}	282
CV/DPV (naphthalene diimide derivative)	chemisorption	direct	10^{-14}−10^{-12} mol	10^{-14} mol	283
		C. Interacting Electroactive Substance (Section 3.2.3)			
		a. Groove Binders (Section 3.2.3.1)			
admittance $[Ru(NH_3)_6]^{3+}$	chemisorption	direct	10^{-10}−10^{-8}	8×10^{-11}	31
CV $(Ru(bpy)_3^{2+})$	adsorption	direct	1.8×10^{-10}−9×10^{-8}	9×10^{-11}	298
LSV (Hoechst 33258)	chemisorption	direct	10^{-10}−10^{-7}	ND	297
		b. Intercalators (Section 3.2.3.2)			
DPV/PSA (daunomycine)	adsorption at fixed potential	direct	ND	2.9×10^{-8}	309
LSV (daunomycine)	covalence	direct	1.26×10^{-11}−1.26×10^{-8}	3.8×10^{-12}	310
impedance (doxorubicin)	chemisorption	direct	ND	$\sim 10^{-10}$	311
ethidium bromide	covalence	direct	1.26×10^{-10}−1.26×10^{-8}	2.52×10^{-11}	312
SWV (methylene blue)	covalence	direct	ND	7.2×10^{-8}	89
SWV (methylene blue)	adsorption	direct	ND	1.7×10^{-7}	313
CV (methylene blue)	chemisorption	direct	2×10^{-8}−10^{-7}	2×10^{-10}	314
CV (methylene blue)	chemisorption	direct	ND	10^{-11}	315
CV/DPV (methylene blue)	adsorption	direct	5×10^{-10}−2×10^{-8}	2.52×10^{-10}	316
DPV (methylene blue)	covalent binding of the DNA probe over a PAn film	direct	2.25×10^{-12}−2.25×10^{-10}	10^{-12}	317
DPV (methylene blue)	affinity (zirconia − oxygen)	direct	2.25×10^{-10}−2.25×10^{-8}	10^{-10}	318
DPV (methylene blue)	covalence	direct	1.25×10^{-7}−6.75×10^{-7}	5.9×10^{-8}	319
DPV (methylene blue)	adsorption	direct	10^{-7}−10^{-4}	2.25×10^{-8}	320
		D. Metal Nanoparticles for DNA Labeling (Section 3.2.4)			
DPV (gold nanoparticles)	adsorption	direct	10^{-10}−10^{-8}	5×10^{-11}	64
impedance (CdS nanoparticles)	entrapment	direct	3.7×10^{-9}−3.7×10^{-7}	10^{-9}	138
chronopotentiometry (gold nanoparticles)	streptavidin−biotin interaction	direct	1.5×10^{-11}−7.6×10^{-8}	$\sim 6 \times 10^{-12}$	341
chronopotentiometry (gold nanoparticles)	covalent binding of the DNA probe over a polymer	direct	10^{-11}−10^{-8}	ND	342
capacitance (gold nanoparticles)	covalence	direct	ND	2×10^{-10}	343
capacitance (gold nanoparticles)	covalence	sandwich	ND	5×10^{-11}	344
conductivity (gold nanoparticles)	chemisorption	sandwich	ND	10^{-9}	345
conductivity (gold nanoparticles)	chemisorption	direct	5×10^{-13}− 5×10^{-8}	5×10^{-13}	346
CV (gold nanoparticles)	covalence	direct	ND	10^{-11}	347
CV (aurothiomalate)	adsorption	direct	10^{-9}−2×10^{-8}	5×10^{-10}	73
ASV (silver nanoparticles)	chemisorption	direct	10^{-11}−8×10^{-10}	5×10^{-12}	348
cathodic stripping voltammetry (iron-containing magnetic beads)	adsorption	direct	8.2×10^{-9}−4.9×10^{-8}	1.6×10^{-9}	349
		E. Label-Free Electrochemical Detection (Section 3.2.5)			
chronopotentiometry	adsorption	direct	up to 3.1×10^{-7}	$\sim 1.25 \times 10^{-8}$	75
CV	covalent binding of the DNA probe over a polymer	direct	ND	2×10^{-10}	101
chronoamperometry	entrapment	direct	ND	1.6×10^{-11}	134
impedance	entrapment	direct	3×10^{-8}−10^{-5}	10^{-8}	136

Table 2 (Continued)

detection technique	immobilization technique	type of assay	linearity range (M)	detection limit (M)	references
		E. Label-Free Electrochemical Detection (Section 3.2.5)			
EIS	chemisorption	direct	8×10^{-9}–10^{-6}	4×10^{-9}	366
EIS	covalence	direct	ND	10^{-9}	367
EIS	covalent binding of the DNA probe over a PPy film	direct	10^{-7}–5.5×10^{-6}	2×10^{-10}	102
EIS	covalence	direct	2×10^{-9}–2×10^{-7}	9.8×10^{-10}	368
EIS	covalence	direct	3×10^{-9}–2×10^{-7}	5×10^{-10}	369
EIS	entrapment	direct	10^{-10}–10^{-6}	5×10^{-11}	370
EIS	chemisorption	direct	10^{-9}–10^{-6}	5×10^{-10}	371
SWV	covalence	direct	ND	1.25×10^{-9}	372
DPV	covalence	direct	ND	10^{-10}	373
DPV	adsorption	direct	ND	1.2×10^{-8}	374
DPV	adsorption	direct	ND	2.7×10^{-10}	375
DPV	adsorption	direct	3.95×10^{-6}–1.98×10^{-5}	1.327×10^{-11}	376
DPV	streptavidin–biotin interaction	direct	ND	9.68×10^{-12}	377

Table 3. QCM DNA Biosensors

immobilization techniques	type of assay	linearity range (M)	detection limit (M)	references
adsorption/entrapment	direct	up to 8×10^{-6}	8×10^{-8}	39
chemisorption	direct	ND	3×10^{-16}	391
chemisorption	sandwich	ND	10^{-13}	392
chemisorption	direct	ND	10^{-12}	393
chemisorption	direct	ND	10^{-12}	394
streptavidin–biotin interaction	direct	ND	10^{-9}	395
streptavidin–biotin interaction	direct	up to 1.5×10^{-7}	$\sim 2 \times 10^{-8}$	396
streptavidin–biotin interaction	direct	8.4×10^{-6}–1.2×10^{-5}	ND	66

A cDNA array can allow the simultaneous analysis of the expression levels of numerous genes in a single experiment.[156] Different approaches can be considered. One popular system relies on measuring the absolute intensity of the labeled cDNA probe from each sample. First, mRNAs from one type of cells or tissues are isolated. To prevent mRNA degradation, they are reverse-transcribed in cDNA while fluorescently labeled nucleotides are incorporated. After labeling, cDNAs are hybridized on a microarray, which is then analyzed by a laser scanner or a charge-coupled device (CCD) camera, and the intensity of each cDNA "spot" is determined. This one-color strategy was used by manufacturers to develop DNA microarrays.[120,123,157] The second approach is based on comparative intensities between two cDNA samples. In this technique, the cDNA from one sample is labeled with one fluorescent dye and the cDNA from the sample to be compared is labeled with a dye that fluoresces at a distinct and non-overlapping wavelength[156] (Figure 7). The cDNAs are mixed and then hybridized to the array. For example, if expression levels are similar, then both red and green cDNA hybridized, resulting in a yellow color. If expression levels from red-labeled cDNAs are higher, then a red spot will appear. Conversely, if expression levels from green-labeled cDNAs are higher, then a green spot will be observed. This two-color system was used by Agilent.[123]

Fluorescence detection was often employed to detect a specific DNA sequence. Manufacturers also used this technique to develop platforms allowing the detection of mutations.[158–160] Asper's genotyping platform[158] is based on arrayed primer extension (APEX). This method combines the accuracy of primer extension with the high-throughput capacity of a microarray (Figure 8). The method is based upon a two-dimensional array of ODNs, immobilized via the 5′ terminal amino group onto an epoxysilanized glass support. A patient DNA is amplified by PCR, enzymatically digested, and hybridized to the immobilized primers, promoting sites for template-dependent DNA polymerase extension reactions using four unique fluorescently labeled di-

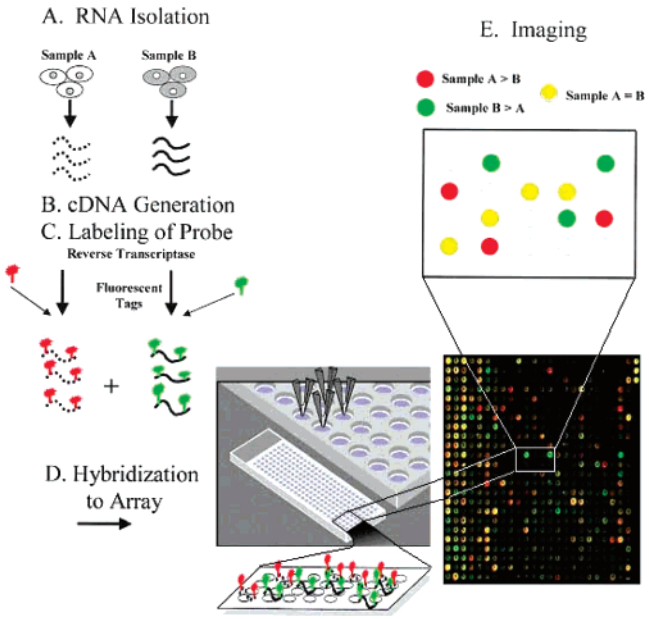

Figure 7. Comparative hybridization experiment. A second commonly used technique to measure gene expression involves RNA isolation from two separate samples (A). The RNA from each sample is treated with reverse transcriptase (B) and labeled with a distinct fluorescent tag (C). The two pools of labeled RNA are mixed, hybridized to the array, and washed (D). The array is imaged using a specialized fluorimeter, and the color of each spot is determined (E). In this example, genes only expressed in sample A would be red in color, genes only expressed in sample B would be green, and those genes expressed equally in both samples would be yellow. Reprinted with permission from ref 156. Copyright 2000 American Thoracic Society.

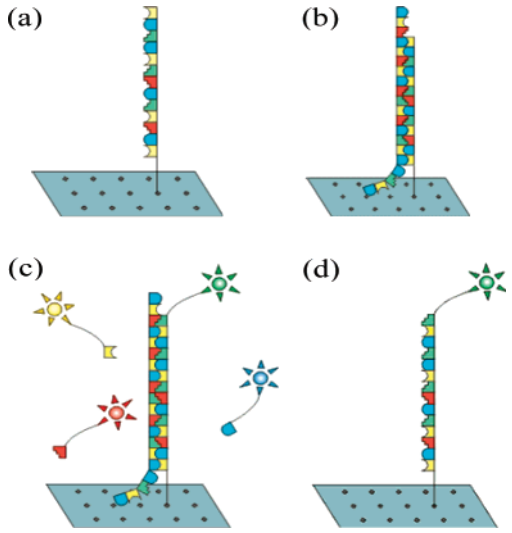

Figure 8. Arrayed Primer Extension of Asper Biotech.[158] (a) Up to 6000 known 25-mer oligonucleotides are immobilized via their 5′ ends on a coated glass surface. (b) Hybridization of a complementary fragment of a PCR amplified sample DNA with ODNs. (c) Template-dependent single-nucleotide extension by DNA polymerase. Terminator nucleotides are labeled with four different fluorescent dyes. (d) DNA fragments and unused dye terminators are washed off. Reprinted with permission from Asper Biotech. Copyright 2007 Asper Biotech.

deoxynucleotides. A mutation is detected by a change in the color code of the primer sites. The APEX method can be applied to any DNA target for efficient analysis of mutations and polymorphisms. The APEX technology was used to present the results of a β-thalassemia mutation.[161] This technique was also used for the identification of mutations in the tumor suppressor gene TP53.[162]

Nanogen[124] uses the natural positive or negative charge of biological molecules to detect single-nucleotide polymorphisms and mutations.[163] The NanoChip array technology (Figure 9) involves recognition of the electronically addressed biotinylated reference DNA with a known sequence to an individual test site and then addressing of a cyanine 3 (Cy3)-labeled complementary test DNA from a patient sample. If the test DNA contains a mutation when compared to the reference DNA, then the mismatch can be detectable by a fluorescence-labeled protein involved in DNA repair: cyanine5−Mut S (Figure 9b). Balogh et al.[159] also used microarray technology for forensic single-nucleotide polymorphism (SNP) analysis. They used a microchip for the electronic addressing of capture probes to specific array sites followed by electronic hybridization of single-stranded PCR products and passive hybridization of fluorescently labeled reporter probes. Discrimination was achieved by applying thermal stringency to denature the mismatched reporter probes.

Next Generation Screening Technology,[160] developed by TeleChem International, Inc., is a universal microarray analysis platform for nucleic-acid-based genetic screening, testing, diagnostics, genotyping, and SNP analysis. Patient DNA is amplified and immobilized on a chip. Normal, carrier, and disease genotypes are easily distinguished because complementary fluorescent ODNs hybridized with different efficiencies to the samples produce different fluorescence intensities according to the type of DNA from patients. A multicolor strategy also can be considered for improving the readability of the assays. In this approach, different fluorescently labeled oligonucleotides whose colors represent normal or disease conditions are hybridized to the chip.

The BioMérieux FoodExpert-ID microarray, powered by Affymetrix GeneChip technology, allows the presence of unwanted or unknown animal species to be genetically identified. The BioMérieux FoodExpert-ID array can be used to detect DNA sequences specific to an animal, allowing the species composition to be determined and the purity of food products to be confirmed. Biomérieux is also working on a high-density DNA probe array based on 16S rRNA to identify *Staphylococcus* species,[164] such as *S. aureus* and *S. intermedius*, which are considered as dangerous. They use a set of probe pairs per gene or target sequence. The first probe is complementary to the target sequence whereas the other contains a mismatch at a central position within the probe. The fluorescent signal emitted by the target bound to the DNA chip is detected by a GeneArray scanner (Agilent). The complementary sequence is considered to be present in the sample if the signal intensity for the perfect match probe is superior to that of the mismatched one.

Walt and co-workers and Illumina developed a particular strategy based on DNA probes bound to microspheres.[47,165−174] In earlier work, they observed that when a solution of latex microspheres was dripped onto the array of etched microwells the beads positioned themselves into each microwell if the sizes of the microspheres and wells matched (Figure 10). In a more recent work, microspheres were modified with different ODN probes and were tagged with a unique combination of fluorescent dyes either before or after probe attachment. This "optical bar code" is simply a combination of fluorescent dyes with different excitation and emission wavelengths and intensities that allow each bead to be independently identified. For example, the dyes could be incor-

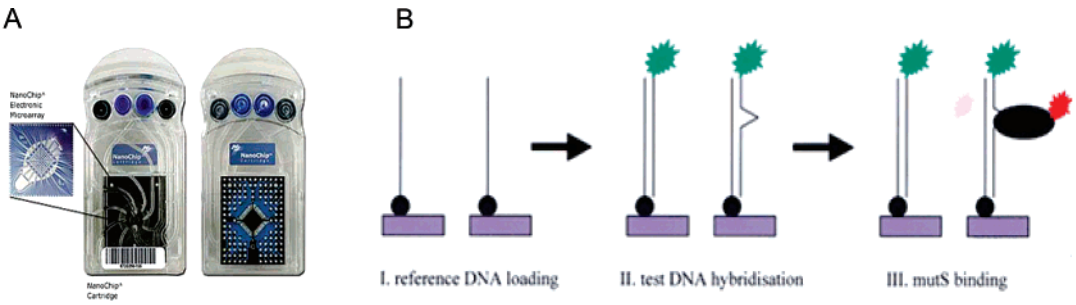

Figure 9. (A) NanoChip Microelectronic Array.[124] (B) Schematic representation of the mutS chip described by Behrensdorf et al.[163] (I) Biotinylated reference strands (e.g., PCR products) are first addressed to individual test sites of the array using electronic biasing. (II) Cy3-labeled complementary test strands are "electronically" hybridized to the reference strands, thereby generating heteroduplex DNA. (III) The Cy5/mutS protein binds preferentially to mismatched heteroduplex DNA. Hybridization and binding events are monitored by fluorescence scanning of the array. Reprinted with permission from ref 163. Copyright 2002 Oxford University Press.

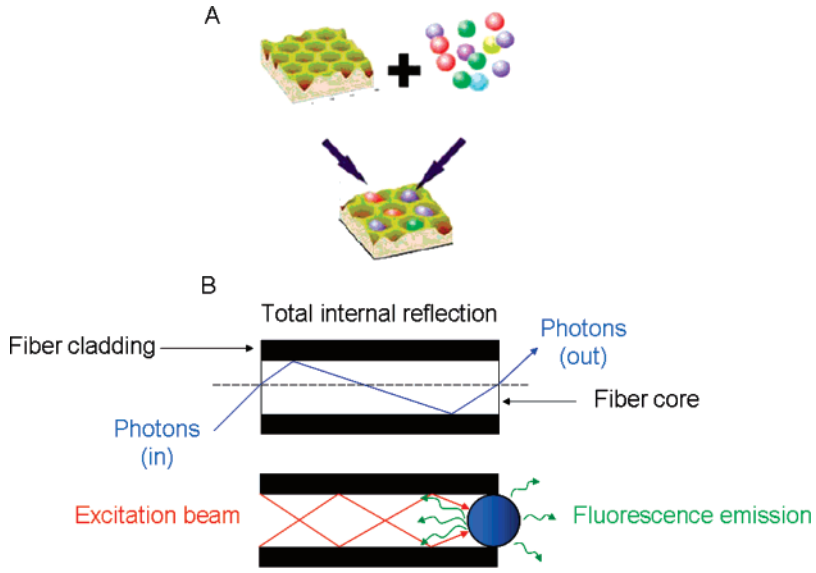

Figure 10. (A) BeadArray technology of Illumina. (B) An individual fiber conducts light to enable data acquisition and quantitation of a signal emitted by each bead.

porated by exploiting the chemical properties of amino-modified polystyrene microspheres swelled in tetrahydrofuran, enabling hydrophobic dyes to penetrate and become entrapped.[168] The different microspheres were then mixed and randomly distributed and fixed in micrometer-sized wells on the fiber-optic substrate. The arrays were dipped into a hybridization buffer, and DNA targets hybridized to the complementary probe located on each bead. In general, the targets were amplified and labeled with fluorescent dye using PCR. Upon hybridization, the microspheres fluoresced when the target bound to the probe beads.[171] This fiber-optic microsphere-based approach provided many advantages in comparison to other array-based methods.[47,168,170] This platform provided a high-density array with the smallest available individual feature sizes. The miniature array size enabled the analysis of extremely small sample volumes. Because many array interactions are diffusion-dependent, more rapid responses are possible with reduced volumes. In addition, microsphere-based arrays could be regenerated for more than 100 hybridizations using high temperatures or organic denaturants without significant signal degradation. This fiber-optic array was also flexible because it can incorporate different nucleic acid detection schemes such as fluorescence resonance energy transfer (FRET)-based molecular beacon assays.[170,175]

Molecular beacons can be used to detect DNA by fluorescence. MBs are single-stranded ODN probes that possess a stem-and-loop structure. The loop portion is complementary to the target (Figure 11). A fluorophore and a quencher are linked to the two ends of the stem. In its native state, the probe is a hairpin, and the two ends of the MB are in close proximity. Consequently, the fluorescence of the fluorophore is quenched by energy transfer. The hairpin stem is less stable than the binding between the loop and the target. In the presence of the target, the MB undergoes a conformational reorganization because the loop hybridizes with the target. The structure is opened, separating the fluorophore and the quencher. In this case, the MB emits an intensive fluorescent signal.[176−180] This class of DNA probes presents many advantages, such as their ease of synthesis, unique functionality, inherent signal transduction mechanism, molecular specificity, and structural tolerance

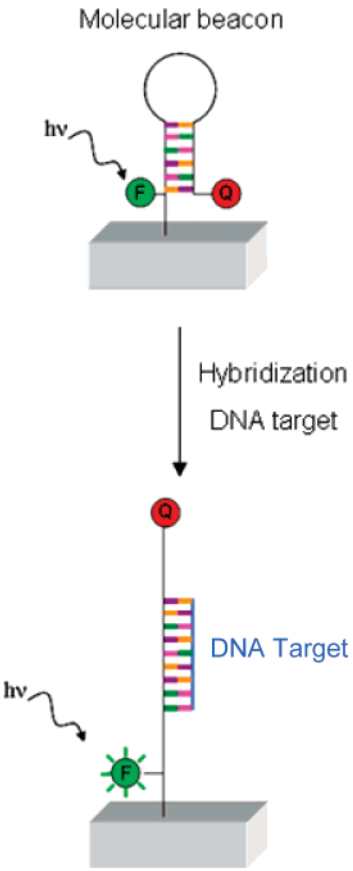

Figure 11. DNA biosensor based on the molecular beacon principle

to various modifications. Their unique structural and thermodynamic properties provide a high degree of molecular specificity, with the ability to differentiate between two target DNA sequences that differ by as little as a single nucleotide. Stem-loop DNA probes appear to be better alternatives to conventional linear probes for mismatch discrimination. By analysis of free energy phase diagrams of MBs in solution with matched and mismatched targets, structurally constrained MBs have been shown to distinguish mismatches over a wider range of temperatures than the one obtained with unstructured (linear) probes. Unfortunately, when MB

probes are immobilized onto solid supports, they display lower sensitivities than in solution, so various solid supports have been explored, and appropriate probes have been designed. In this case, the MBs have been used widely to develop biosensors for DNA detection.[181,182]

Some authors reported the use of a biotinylated ssDNA MB.[115,151] The 5′ end was linked to a fluorophore, tetramethylrhodamine (TMR), and the 3′ end was attached to the quencher, 4-(4-(dimethylamino)phenyl)azo)benzoic acid (DABCYL). The MB was immobilized on a solid surface via an avidin−biotin linkage. The MBs also were immobilized on silica microspheres through avidin−biotin interactions.[183] The MBs were labeled using carboxytetramethylrhodamine (TAMRA) and DABCYL. However, the ability of metals was noted to quench fluorescence.[184] It was demonstrated that fluorophore-tagged DNA hairpins attached to gold films can function as highly sensitive sensors for ODNs. The substrate material itself was used as the quenching agent. Rhodamine-labeled MBs are specific for genes known to confer methicillin resistance to *S. aureus* and were immobilized onto gold films. In the same way, ability of a gold-surface-immobilized MB to distinguish single-base mismatches was studied.[185] Apparently, MBs are very specific and can distinguish targets that differ by only single nucleotides. This specificity is due to their ability to form a stem-and-loop structure.[186] A MB fiber-optic DNA array also was developed.[170,175] In this work, MBs were immobilized on optically encoded microspheres that can be made by entrapping different dyes inside polystyrene microspheres. Fluorescein and DABCYL were used as the fluorophore and the quencher, respectively, for each MB. These immobilized MBs were incubated with the unlabeled target, and the fluorescence was monitored in real time. However, MBs were immobilized on an agarose film-coated slide, and the sensor was compared with a conventional system that directly immobilizes MBs on a glutaraldehyde-derived glass slide.[187] The MB array can identify a single-nucleotide difference in a 16-mer target DNA sequence. A streptavidin−biotin bridge also was used to immobilize MB on a glass slide.[149] The response of the MB biosensor to its 19-mer target DNA is linear from 5×10^{-9} to 10^{-7} M (Table 1).

Quantum dots also could be used to detect DNA sequences instead of fluorophores. A quantum dot is a semiconductor particle (e.g., ZnS, CdSe, and CdS) that can be used as a fluorophore.[188−190] The size and the shape of these structures and the number of electrons that they contain can be controlled precisely. Colloidal semiconductors are very attractive for the labeling of biomolecules. They are candidates for replacing conventional fluorescent markers such as rhodamine in biodetection assays.

QDs are more photostable than an organic fluorophore. Moreover, QDs exhibit higher fluorescence quantum yields than conventional organic fluorophores, allowing greater sensitivity. They are characterized by a band gap between the valence and the conduction bands.[191] In natural bulk semiconductor material, there is practically no electron in the conduction band, but instead they occupy the valence band (Figure 12). The only way for an electron in the valence band to jump to the conduction band is to acquire enough energy to cross the band gap. When a photon having an excitation energy exceeding the band gap energy is absorbed by a QD, electrons are promoted from the valence gap to the conduction gap, creating a positively charged hole in the valence gap. The excited electron may then relax to its ground state by the emission of another photon with energy equal to the band gap. Emission properties of QDs depend on their composition and size. For example, the fluorescence spectra of CdSe QDs with different particle diameter sizes were reported.[192]

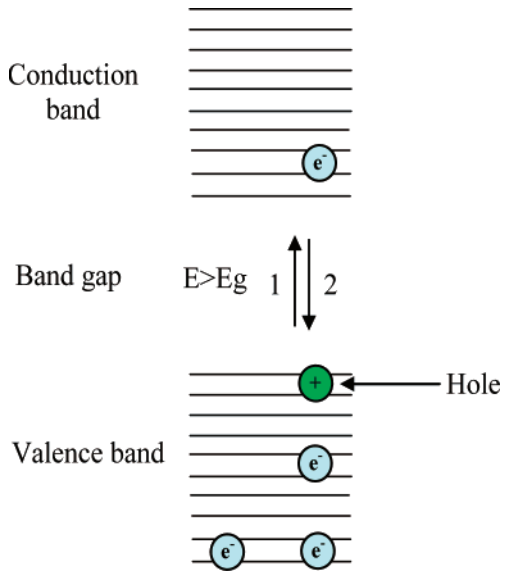

Figure 12. (1) Stimulus enables the electron to jump from the valence band to the conduction band, inducing the creation of a temporary hole in the valence band. For this, the electron must absorb radiation with energy greater or equal to the band gap energy ($E > E_g$). (2) A relaxing electron emits radiation at a fixed wavelength.

Instead of an organic fluorophore, DNA−nanocrystal conjugates are used to detect SNP mutations in the human p53 tumor suppressor gene, which has been found to be mutated in more than 50% of the known human cancers, and to target hepatitis B and C genotypes in the presence of background of human genes.[193] The p53 target sequence was a 25-mer whereas the hepatitis B and C virus were 75-mers. These three targets were conjugated to the nanocrystals, and the complementary sequences were bound on the DNA array. In this case, DNA probes with amino end modification were immobilized on glass slides on an aldehyde-activated surface.

Polychromatic microarrays were reported to analyze eight different *Bacillus anthracis* samples simultaneously. For that, beads coated with ssDNA probes were localized into etched wells of fiber-optic arrays. Biotinylated samples labeled with streptravidin−QD conjugates were then hybridized to the array.[194]

Energy-transfer mechanisms have been used widely to develop optical biosensors. Quantum dot photoemission properties allow efficient energy transfer with a conventional organic dye. For example, a streptavidin-coated QD with an emission peak at 585 nm was used as a donor and Cy5 was used as an organic fluorophore acceptor in a FRET measurement.[195]

3.1.2. Surface-Enhanced Raman Scattering Spectroscopy

This detection technique presents several advantages compared to fluorescence. A Raman dye can be either fluorescent or nonfluorescent, and a minor chemical modification of a dye molecule can lead to a new dye with a different Raman spectrum even if the two dyes exhibit virtually indistinguishable fluorescence spectra.[196,197] Moreover, the spectral specificity of a SERS gene probe is

excellent in comparison to that of the fluorescence method. For example, the spectral bandwidths of cresyl fast violet (CFV) in UV absorption and fluorescence are broad whereas the bandwidth of the SERS spectrum of the same CFV dye is narrower.[198] One of the major difficulties in the development of the SERS technique for genomics applications is the production of surfaces or media that can be readily adapted to the assay formats, e.g., DNA hybridization. The SERS substrates must have an easily controlled protrusion size and reproducible structures. Roughened metal electrodes and metal colloids were among the first SERS-active media used. A variety of solid-surface SERS substrates were developed on metal-covered nanoparticles.[199] However, DNA biosensors or microarrays that did not use a metallic SERS substrate also have been reported,[197,200] but in this case SERS detection is possible using ODN labeled with Au or Ag nanoparticles.

The SERS technique was reported as a tool for detecting specific nucleic acid sequences.[201] For that, the SERS-labeled probes were hybridized to fragments of DNA attached on nitrocellulose. After hybridization, probes and DNA fragments were transferred on a SERS-active substrate for analysis. A negative control that consisted of labeled DNA that was not complementary to the DNA probe exhibited no SERS signal. However, after hybridization between the CFV-labeled target and the probe, SERS peaks were observed. The SERS technique also was applied to detect DNA fragments of the human immunodeficiency virus (HIV).[198] In the same way, the development of SERS-active substrates for cancer gene detection was described.[202] The thiolated ssDNA probe was immobilized on a silver surface that was incubated in the presence of rhodamine-B-labeled DNA target. A signal was observed on the SERS spectrum only after the hybridization. The authors also demonstrated that the addition of silver colloids to the surface enhanced the Raman signal.

SERS was used to monitor DNA hybridization of a fragment of the BRCA1 breast cancer gene on modified silver surfaces, which were prepared by depositing a layer of silver onto glass slides, forming a microwell platform.[203] The ODN probe was covalently immobilized on the silver-coated glass slide covered with a SAM. In this work, the SERS-active dye, rhodamine B, labeled the DNA target.

Nanoparticles functionalized with ODNs and Raman labels, coupled with SERS spectroscopy, can be used to perform multiplexed detection of ODN targets.[197] A sandwich complex was formed between the immobilized DNA probe, the target, and a DNA signaling sequence attached to Cy3-labeled Au nanoparticles. Then, the chip was treated with Ag enhancement solution. Before Ag enhancement, no Raman scattering signal was detectable. After the treatment, the Ag particles could grow around the Cy3-labeled nanoparticle probes, leading to large Raman scattering enhancements. Other dyes than Cy3 also were used to create a large number of probes with distinct and measurable SERS signals for multiplexed detection. Six dissimilar DNA targets with six Raman labeled nanoparticle probes were distinguished. The current unoptimized detection limit of this method was 2×10^{-14} M.

3.1.3. Chemiluminescent Detection

Table 1 presents some performances of chemiluminescence- and electrochemiluminescence-based biosensors or microarrays.[84,204−210]

Luminescent reactions can be catalyzed by a biomolecule, such as hemin or horseradish peroxidase (HRP), or triggered by the application of a potential between the working electrode and a pseudo-reference. Luminol and derivatives are often used for chemiluminescent (CL) or electrochemiluminescent (ECL) reactions.

The chemiluminescent properties of luminol were first reported by Albrecht.[211] Luminol oxidation leads to the formation of an aminophthalate ion in an excited state, which then emits light on return to the ground state. The peroxidase-catalyzed chemiluminescent oxidation of luminol was largely used to detect analytes[212] or DNA concentration.

DNA-sensitive biochips that could be used to detect a biotin-labeled sequence were described.[207] The ODN-charged beads were entrapped in a poly(vinyl alcohol) bearing styrylpyridinium groups (PVA-SbQ) photopolymer, and the probes were hybridized with biotinylated $d(T)_{22}$ that could react with HRP-labeled streptavidin. The chemiluminescent reaction is catalyzed by this enzyme in the presence of luminol, H_2O_2, and *p*-iodophenol. This microarray allowed DNA detection with a detection limit of 25×10^{-11} M (Table 1). In the same way, beads bearing DNA were spotted onto a poly(vinyl chloride) (PVC) master and were transferred then to a PDMS interface.[205] This biochip enabled the quantitative detection of biotinylated ODN concentrations from 5.8×10^{-11} to 2.3×10^{-8} M (Table 1). However, a detection system for specific nucleic acid sequences was developed using a chemiluminescent signal based on alkaline phosphatase (PAL).[208] After immobilization of the DNA probe on a gold surface, the biotinylated target was hybridized with this sequence. Therefore, detection was possible due to interaction with avidin−PAL. 3-(2′-Spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane (AMP-PD) was used as a direct chemiluminescent substrate for this enzyme.[213] In this case, the detection limit was 15×10^{-12} M (Table 1).

A DNAzyme capable of forming a supramolecular G-quadruplex structure with hemin was used to detect the DNA target.[84] First, a thiolated probe complementary to the 5′ end of the target (34-mer) was immobilized on a gold surface. The free part of the target hybridized a nucleic acid that included the complementary sequence and a biocatalytic sequence that can form a supramolecular complex with hemin. This hemin G-quadruplex structure acted as a biocatalyst for the chemiluminescence in the presence of H_2O_2 and luminol. The detection limit was 10^{-9} M (Table 1). DNAzyme-functionalized gold nanoparticles also were reported as catalytic labels.[204] In this case, nanoparticles were modified with nucleic acids including the biocatalytic sequence and the complementary sequence. The detection limit for the DNA target (36-mer) is 0.1×10^{-9} M (Table 1).

Mallard et al. also developed an opto-electronic DNA chip at the level of 1 pixel.[206] The probes were grafted on the surface of a low-cost complementary metal oxide semiconductor (CMOS) photodetector. After hybridization of the HRP-labeled target, a chemiluminescent substrate of the enzyme is added. The photons produced are captured by a photodiode that converts them into electrons. The detection limit of this system was around 5×10^{-13} M.

Beads bearing glucose-oxidase-labeled DNA were immobilized at the surface of PDMS.[210] The biochip could detect DNA due to ECL reaction of luminol with H_2O_2 enzymatically produced by the oxidase. In this case, the detection limit was 20 fmol (10^{-8} M) (Table 1). *N*-(4-

Aminobutyl)-*N*-ethylisoluminol (ABEI) is a derivative of luminol that can be used as a chemiluminescence marker to label an ODN sequence. Electropolymerization on a GCE of a copolymer of pyrrole units and pyrrole monomers functionalized with an ODN also was described.[214] In this work, the ECL label was a biotinylated ABEI grafted to the target (16-mer) through an avidin−biotin link. An ABEI-labeled DNA probe also was used to recognize the target ssDNA immobilized on a PPy-modified electrode.[209] In this case, the intensity of the ECL was linearly related to the concentration of the complementary sequence (24-mer) in the range of 9.6×10^{-11}−9.6×10^{-8} M (Table 1). A novel detection method for DNA hybridization based on the ECL of $Ru(bpy)_3^{2+}$ (Tris-(2,2′-bipyridyl)dichloro-ruthenium(II) hexahydrate) using DNA-binding intercalators has been developed.[215] DNA probes were immobilized on the cylindrical gold surface of a through-hole array, which was designed to prevent interference from the electric field of the electrodes and to focus luminescence from each electrode by separated holes. After hybridization to the DNA target, the surface was incubated with the intercalator containing 1 mM $Ru(bpy)_3^{2+}$. When a voltage of +1.19 V vs Ag/AgCl was applied to the working electrode, $Ru(bpy)_3^{2+}$ was oxidized to $Ru(bpy)_3^{3+}$ and consequently converted into its excited form by a redox reaction with an intercalator near the electrode. Then, the compound generated an orange light when it returned to the ground state. The amount of light generated was proportional to the amount of intercalator and thus to the concentration of the target DNA after hybridization.

Applied Biosystems[216] developed Expression Array System Microarray assays based on a chemiluminescent detection of digoxigenin-labeled targets. An immobilized ODN was hybridized with a digoxigenin-labeled cDNA target. Then, the anti-digoxigenin antibody−PAL conjugate interacted with digoxigenin. Interaction of the enhancer, PAL, and the chemiluminescent substrate produces light with an emission maximum at 458 nm.

3.1.4. Colorimetric Detection

A bead sensor was described based on the two-dimensional aggregation of single-stranded ODN-modified gold nanoparticle probes upon hybridization with the complementary target.[217] Two types of gold nanoparticles carrying different DNA sequences complementary to the target were adsorbed on a lipid layer by electrostatic forces. This lipid layer formed on an organic or an inorganic substrate allowed the nanoparticles to move along the surface. The sensor was then incubated with the target. The color change described as a colorimetric signal in the three-dimensional (3D) system[218−220] cannot be used with the two-dimensional (2D) system, where detection is based on the desorption properties of the nanoparticles by adding chemical species to the solution. Dextran sulfate was added to the solution after hybridization. When hybridization was carried out with a noncomplementary target, dextran sulfate induced complete nanoparticle desorption. Consequently, a discoloration of the sample was observed. However, when the hybridization was carried out with complementary DNA, no desorption occurred. Thus, the sample remained red. This 2D colorimetric DNA sensor was highly specific and allowed the detection of DNA mismatches and damages.[221]

A two-color labeling of an ODN array was described to analyze two different DNA targets in a solution.[222] The immobilized DNA probe, the ODN-functionalized nanoparticle labels, and the targets to be detected were designed to cohybridize in a three-component sandwich assay (Figure 13). The 50 and 100 nm diameter gold nanoparticles functionalized with ODNs were used to identify two different target sequences. Green light was observed when 50 nm Au particles were attached to the surface, and orange light was observed only for attached 100 nm particles.

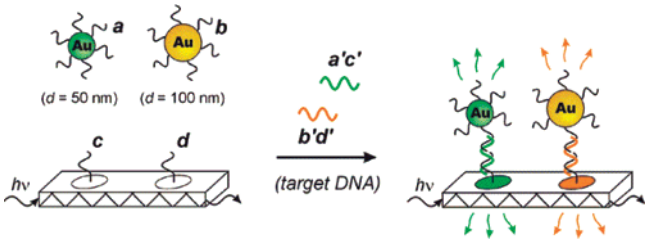

Figure 13. Two-color labeling of oligonucleotide arrays via size-selective scattering of nanoparticle probes. Reprinted from ref 222. Copyright 2001 American Chemical Society.

3.1.5. Dual Polarization Interferometry

Dual polarization interferometry (DPI) is a new approach for label-free, quantitative, and real-time measurement of interactions. DPI uses nondiffractive optics to interrogate and resolve the size and density of a biomolecule at a solid solution interface in real time.[223−225] This technique was employed previously to characterize the immobilization of a single-stranded DNA probe by simple adsorption on a silanized surface or via an avidin−biotin bridge and to examine the effect of probe concentration on hybridization efficiency.[225] More recently, the covalent immobilization of a ssDNA probe and the selective detection of target DNA hybridization were investigated using DPI. In this work, two immobilization protocols were employed to immobilize probe molecules on an amino-derivatized surface. In the first case, probes were attached directly via the formation of a Schiff's base whereas in the second case a 1,2-homo-bifunctional cross-linker molecule was employed to attach an amine-modified probe to the surface. DPI allowed the determination of probe orientation and the measurement of probe coverage at different stages of the immobilization process in real time and in a single experiment. The results showed that a probe molecule attached to the surface via a cross-linker group had greater mobility to hybridize to target DNA than one attached by the direct method.

3.1.6. Surface-Plasmon-Based Detection

Performances of SPR-based biosensors or microarrays are presented in Table 1.[226,227]

SPR is an optical technique that investigates what happens at the interface of a thin metal-coated prism in contact with a solution. SPR is used for determining refractive index changes at a surface. When light is incident on the prism side at a particular angle called the resonance angle, the intensity of the reflected light is at its minimum. In the presence of biomolecules on the metal (gold) surface, this angle variation is very sensitive. Changes in reflectivity give a signal that is proportional to the mass of the biomolecules bound to the surface. To detect one molecule, such as a DNA target, ligand (e.g., DNA probe) is immobilized onto the surface. As the analyte binds to the ligand, the mass and the refractive index increase. As SPR can detect the binding of the analyte on a surface without any label,[228−231] this technique has been used frequently to develop biosensors,

especially to detect DNA−DNA hybridization. SAMs constituted of either COOH-terminated thiol molecules[232] or branched probe DNA-containing single- and double-stranded portions[233] were reported. In the same way, a DNA SAM made of a probe containing dsDNA and ssDNA portions was constructed.[85] A DNA-based SPR biosensor also was developed by immobilizing thiolated ODNs onto suitable photolithographic patterned gold substrates.[234] Two different immobilization approaches were reported for the analysis of ODNs: DNA amplified by PCR and enzymatically digested genomic DNA.[226] In this work, two instruments based on SPR were used: Biacore X and Spreeta. When thiolated probes were immobilized on a gold surface, a detection limit of 2.5×10^{-9} M was observed for the P35S target (25-mer) using Biacore X, whereas the detection limit was 10×10^{-9} M with Spreeta. Jiang et al.[235] reported a method for detecting TP53 mutations using the commercially available Spreeta. In this work, the thiolated probe immobilized on a bare gold sensor surface was hybridized to its complementary (26-mer) or mismatched sequence. Recently, SPR spectroscopy was also used to study DNA assembly, DNA hybridization, and protein−DNA interactions on a planar (2D) streptavidin and a Biacore streptavidin chip (3D).[236] The first chip was a biotin-containing thiol-treated gold disk onto which streptavidin molecules were adsorbed through one or two biotin linkages. In the second case, the streptavidin was covalently immobilized on a 50 nm dextran matrix through amine coupling.

However, if low-molecular-weight molecules such as short-chain DNA molecules are involved in binding or if the packing density of the film is very small, then the resonance angle shifts are very slight and SPR is no longer sensitive enough to monitor these binding events or interaction accurately. Thus, there is a need to incorporate signal amplifications that can be used to monitor all of these interfacial interactions. Fluorescence tagging of molecules can be combined with the resonant excitation of surface plasmons.

Surface plasmon field-enhanced fluorescence spectroscopy (SPFS) has become a powerful tool for the detection and the quantitative evaluation of interfacial binding reactions.[237−240] For example, biotinylated probes were bound to free binding pockets of streptavidin molecules at the sensor surface. The hybridization of the labeled target to these surface-attached capture probe ODNs brought the fluorophore into the evanescent optical field of the surface plasmon mode excited at the gold/dielectric interface.

Surface plasmon microscopy (SPM)[241−243] allows the imaging of systems without any addition of fluorescent dyes. However, surface plasmon field-enhanced fluorescence microscopy (SPFM) also was used to detect hybridization reactions between targets, which are labeled with organic dyes or with semiconducting QDs, and electrochemically immobilized ODN probes.[244] This detection method allowed for the observation of ODN hybridization between labeled targets to a complementary probe immobilized via a monolayer of streptavidin-SH chemisorbed to a gold surface.[245]

3.2. Electrochemical DNA Biosensors and Microarrays

Electrochemical transducers have often been used for detecting DNA hybridization due to their high sensitivity, small dimensions, low cost, and compatibility with micromanufacturing technology.[53,246] There are numerous labeled electrochemical DNA biosensors where the tag can be an enzyme, ferrocene, an interactive electroactive substance (a groove binder, such as Hoechst 33258, or an intercalator), or nanoparticles. Other label-free electrochemical DNA biosensors also have been reported (Table 2).

3.2.1. Enzyme Label

Some performances of enzyme-labeled biosensors and microarrays are presented in Table 2A.[34,49,70,247−261]

The hybridization event is commonly detected by labeling the target DNA sequence or the reporter DNA probe with a redox-active enzyme (e.g., HRP or glucose oxidase, GOD).

A DNA biosensor was described using a peroxidase label that converts the *o*-phenylenediamine (OPD) into 2,2′-diaminobenzene (DAA), which is a chromophore and also an electroactive product.[70] Thus, this molecule can be detected by differential pulse voltammetry (DPV). The electrochemical system was 83-fold more sensitive than the colorimetric hybridization system (detection limit of 6×10^{-16} M, Table 2A).

An electrochemical detection of 26-mer DNA sequences was performed using Faradaic impedance spectroscopy.[249] A thiolated ODN containing the sequence complementary to a part of the target was immobilized on a gold electrode. The probe was hybridized to a duplex constituted of the target and a biotinylated signaling ODN forming a sandwich structure. This double-stranded assembly was then treated with avidin-labeled HRP. The association of the enzyme label with the assembly enabled the biocatalytic oxidation of 4-chloro-1-naphthol by H_2O_2 and formed a precipitate at the electrode surface, which acted as a barrier for interfacial electron transfer. The sensor can detect a DNA target at a concentration of 2.3×10^{-9} M (Table 2A). Liposomes labeled with HRP also were used in connection with Faradaic impedance spectroscopy.[248] The first steps were the same as those described by Patolsky et al.[249] However, in this case, the bifunctional double-stranded assembly on the electrode surface reacted with biotinylated HRP-labeled liposomes via an avidin−biotin bridge (Figure 14). The DNA target was detected at a concentration of 6.5×10^{-13} M (Table 2A).

Pividori and co-workers used a nylon membrane modified with a DNA target integrated onto a transducer based on graphite−epoxy composite (GEC) using different formats. In all cases, hybridization was detected electrochemically with a labeling system using an HRP−streptavidin conjugate in the presence of H_2O_2 and hydroquinone as a mediator.[262−265] Encapsulation of streptavidin in siloxane−poly(propylene oxide) (PPO) films also was reported.[266] Biotinylated probes were immobilized via streptavidin−biotin interactions. After hybridization, an avidin−peroxidase conjugate allowed the detection of the target DNA by amperometric measurement. The HRP-biocatalyzed oxidation of 4-chloro-1-naphtol also was used.[267] Electropolymerization involved a conducting polymer bearing an ODN unit. HRP was linked through a molecular assembly using a streptavidin−biotin bridge. The enzyme-catalyzed oxidation of 4-chloro-1-naphtol was followed by precipitation of the insoluble product, 4-chloro-1-naphton, on the support. This local deposition caused a change in conductivity, which was detected by scanning electrochemical microscopy (SECM).

Cationic redox polymers containing osmium−bipyridine complexes coated on the electrode to improve the electron-transfer efficiency between the enzyme reaction and the electrode were used for the first time by Heller and

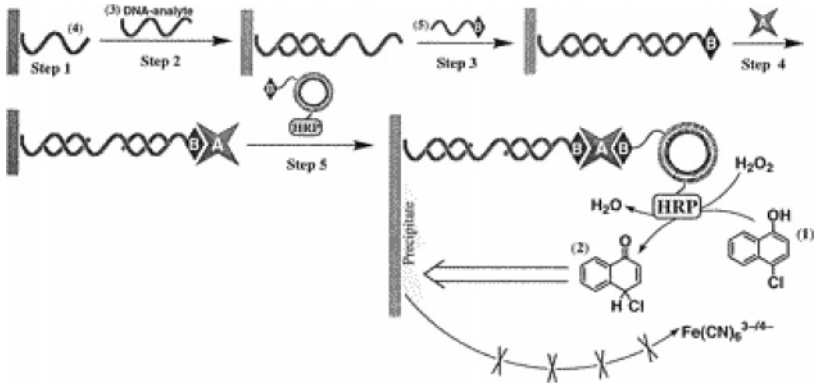

Figure 14. Amplified detection of a target DNA by biotin-tagged HRP-functionalized liposomes and the biocatalyzed precipitation of an insoluble product on the electrode. Reprinted from ref 248. Copyright 2001 American Chemical Society.

co-workers. A redox film of acrylamide and vinylimidazole modified with a hydrazine and osmium complex was formed on the electrode allowing an electrical contact between the peroxidase redox center and the electrode.[94] The probe was immobilized onto the hydrazides of the hydrogel by carbodiimide coupling. The complementary strand labeled by HRP was hybridized with the probe. Finally, the H_2O_2 electrocatalytic reduction current was measured. This approach also was employed using the thermostable soybean peroxidase (SBP) to detect a single-base mismatch in an 18-mer ODN.[268] Probes were covalently immobilized onto a redox film of PAA, acrylhydrazide, 1-vinylimidazole, and $[Os(4,4'\text{-dimethyl-}2,2'\text{-bipyridine})_2Cl]^{+/2+}$, which was complexed with the imidazole function. Hybridization with the SBP-labeled target was monitored by amperometry. Upon hybridization, electrical contact was realized between the enzyme heme centers and the electrode via the redox polymer. This enabled the electrocatalytic reduction of H_2O_2. This approach was used in the development of highly sensitive sandwich assays. An enzyme-amplified amperometric sandwich hybridization also was described to detect DNA using a detection sequence that was HRP-labeled.[269] In this work, avidin was electrodeposited on a carbon electrode with the polymer. The biotinylated capture probe was then bound on the electrode. The ODN-modified redox polymer film was the base of the sandwich. The 75-mer complementary target was cohybridized with the probe and with an HRP-labeled ODN. The presence of the target was evaluated electrochemically by measuring the H_2O_2 reduction current. Another osmium-containing redox polymer was used to develop an enzyme-amplified sandwich-type amperometric sensor.[270] A copolymer of 4-vinylpyridine and acrylamide with some of the pyridines complexed with $[Os(4,4'\text{-bipyridine})_2Cl]$ comprising a DNA capture sequence was electrodeposited on a carbon electrode. This surface was then exposed to the target DNA, followed by the detection sequence to which HRP was covalently attached. In this case, a 38-mer DNA strand was detected at a 0.5×10^{-15} M concentration.

CombiMatrix[271] developed an ODN microarray platform that contains 12 544 individual electrodes per square centimeter, and each working electrode is 44 μm in diameter.[272] First, a different ODN probe was synthesized at each microelectrode. After hybridization with the biotinylated target, the HRP−streptavidin conjugate allowed the electrochemical detection. The enzyme label catalyzed the oxidation of tetramethylbenzidine by H_2O_2. The detection limit was 7.5×10^{-13} M.

Glucose oxidase also can be used as an enzyme label. A sandwich-type assay was described, based on a cationic redox polymer containing osmium−bipyridine complexes that interact with GOD through layer-by-layer electrostatic self-assembly.[251] After the immobilization of ssDNA probes followed by hybridization with a 73-mer target and an enzyme-labeled ODN detection probe, the redox polymer interacted with anionic enzymes and displayed high electron mobility. In the presence of glucose, the current generated from enzymatic oxidation is detected amperometrically. The detection limit was 10^{-15} M, and the dynamic range extended up to 8×10^{-13} M (Table 2A).

Other enzymes also have been used for ODN labeling based on electrochemical transduction. An electrochemical genosensor was described, based on the coupling of a PAL conjugate and biotinylated target sequences.[256] This enzyme catalyzes the hydrolysis of the electroinactive α-naphthyl phosphate to the electroactive α-naphthol, which was detected by DPV. The system was characterized using synthetic ODNs. This genosensor could detect a 25-mer DNA target up to 2.5×10^{-8} M with a detection limit of 2.5×10^{-10} M (Table 2A). In the same way, an electrochemical array for the rapid and simultaneous detection of food-contaminating pathogenic bacteria was developed.[273] Mixtures of DNA samples from different bacteria were detected at the nanomolar level without any cross-interference. A voltammetric enzyme genosensor based on streptavidin-modified screen-printed carbon electrodes using PAL as an enzyme label also was developed.[259] In this case, electrochemical detection was achieved with an anti-FITC PAL-labeled antibody that recognized FITC bound to the DNA target. This sensor could detect 1.63×10^{-11} M (Table 2A) of a 20-mer ODN target and discriminated between a complementary target and a sequence with a three-base mismatch.

A DNA hybridization sandwich assay was reported using glucose-6-phosphate dehydrogenase (G6PDH) as an indicator.[260] However, an amperometric DNA sensor was constructed, employing pyrroquinoline quinone glucose dehydrogenase ((PQQ)GDH) for the labeling of the 18-mer DNA target.[261] The sensor response was found to be linearly related to the target concentration between 5×10^{-8} and 1×10^{-5} M (Table 2A). Recently, bilirubin oxidase (BOD) was reported as a novel enzyme label,[274] thus replacing HRP previously used[270] in the electrochemical sandwich assay. The sandwich was formed in an electron-conducting redox polymer where a DNA probe was immobilized. The target was cohybridized to a DNA probe and to a BOD-labeled signaling DNA sequence. The enzyme could catalyze the reduction of ambient O_2 to H_2O where no additional substrate (such as H_2O_2) was necessary.

Recently, a novel signal-amplification scheme for the ultrasensitive detection of nucleic acids involving enzymatically catalyzed conducting-polymer formation and template-guided deposition to amplify nucleic acid hybridization events was reported.[34] A sandwich structure was formed between the immobilized probe, the target, and an HRP-labeled ODN detection probe. The biosensor was incubated with a mixture of aniline and H_2O_2 in a 0.1 M phosphate buffer at pH 4. The hybridized anionic nucleic acid molecules served as templates, providing the requisite local environment to facilitate para-coupling of aniline molecules, HRP acting as a catalyst. Indeed, HRP is known to catalyze the oxidative polymerization of aniline in the presence of H_2O_2.[275,276] The deposition of PAn occurred exclusively at the hybridized nucleic acid molecules, and the electroactivity of this polymer allowed the ultrasensitive electrochemical quantification of targets. The linear range extended from 2×10^{-15} to 1×10^{-12} M with a detection limit of 10^{-15} M.

3.2.2. Ferrocene

Performances of ferrocene-labeled DNA biosensors and microarrays are presented in Table 2B.[277−283]

Ferrocene (Fc) can be used to label ODN sequences due to its good stability. DNA labeled with Fc can be synthesized using different strategies. Electrochemically active ODNs were prepared by covalent linkage of a ferrocenyl group to the 5′-aminohexyl-terminated synthetic ODNs.[284] However, Fc-containing phosphoramidites were incorporated at various positions along an ODN.[285] ODNs labeled with Fc were synthesized by solid-phase coupling of an Fc derivative to an ODN-containing 5′-iodouridine.[286] Another strategy proposed the replacement of a nucleotide by an Fc unit during automated solid-phase DNA synthesis.[287]

Farkas and co-workers[288,289] reported the use of Fc-labeled ODNs in a sandwich-type assay. A gold electrode was coated with a SAM containing DNA probes to capture the unlabeled targets at the electrode surface. Afterward, a signaling probe, containing adenosine residues modified with a ferrocene group, was hybridized in a region of the target adjacent to the capture probe binding site. The electron transfer from Fc to the gold electrode, through the SAM, can be detected as a Faradaic current. At the redox potential of the Fc, the peak of the Faradaic current was directly proportional to the number of Fc moieties fixed at the electrode surface. Consequently, the peak was proportional to the number of targets.[288] In another work, the probe was immobilized onto a gold electrode through the specific chemisorption of successive phosphorothioates that were introduced onto the 5′-end of the ODN.[290] The target DNA (19-mer) was hybridized to the immobilized ODN and an Fc-modified ODN. This DNA sensor allowed one point mutation to be distinguished from the fully complementary target.

Wang and co-workers reported the use of Fc-capped gold nanoparticle−streptavidin conjugates for the detection of polynucleotide targets. They used a sandwich complex between the thiolated capture probe, the target, and the biotinylated ODN detection probe that binds Fc-capped gold nanoparticle−streptavidin conjugates.[277] To simplify the method, Baca et al. carried out a direct hybridization between a thiolated probe that was mixed with hexanethiol and the biotinylated target and detected the surface reaction in the presence of the Fc-capped gold nanoparticle−streptavidin conjugates.[278] For the analysis of a biotinylated 30-mer target at a mixed SAM, a concentration level as low as 2.5×10^{-13} M could be detected (Table 2B). This remarkable detection limit was attributed to the amplification of the voltammetric signal by the large number of Fc moieties present on the conjugates.

A sensor for viral DNA detection based on the generation of a redox-active replica was developed.[291] A thiolated nucleic acid was immobilized on a gold electrode and hybridized with the cyclic viral DNA. The replication of the immobilized DNA was then triggered in the presence of polymerase and a mixture of dNTP that included the synthetic ferrocene-tethered dUTP that was incorporated in the replicated DNA. Ferrocene units act as electron-transfer mediators between the redox enzymes and the electrode.

It is also possible to use a molecular beacon labeled with Fc.[150,292] This MB possessing a terminal thiol and an Fc group was immobilized on a gold electrode. In the absence of target DNA, the Fc was in close proximity to the electrode surface, thus allowing an efficient electrochemical oxidation/reduction of Fc. In the presence of the target, the distance between the label and the electrode increased, thereby lowering the electrochemical signal. This system allowed the detection of the target DNA (27-mer) at concentrations as low as 10×10^{-12} M.[292]

A novel strategy was described for DNA detection and point mutation identification based on the combination of a hairpin structure and DNA ligase.[280] A gold electrode was modified with a mixed SAM of a DNA probe and mercaptoethanol. A sandwich structure was formed between the DNA probe, the target, and an Fc-labeled signaling DNA sequence. Then, a ligation reaction was carried out between the signaling sequence and the probe. The target was released from the electrode surface by thermal denaturation. The ligation product formed a hairpin structure after the removal of target DNA, bringing the ferrocene label in close proximity to the electrode surface. Target DNA was determined by alternating current voltammetry (ACV) in the range from 3.4×10^{-12} to 1.4×10^{-7} M with a detection limit of 10^{-12} M (Table 2B).

Electrochemical detection of DNA based on an Fc-functionalized cationic water-soluble polymer was described.[279] Neutral PNA capture probes complementary to the target were immobilized on a gold electrode. The cationic polymer can interact strongly with the negatively charged backbone of the target bound to the PNA probes but not with the PNA alone. In the presence of the 20-mer target, an oxidation peak, attributed to the oxidation of Fc, was observed by square-wave voltammetry (SWV). The detection limit was around 5×10^{-10} M (Table 2B).

Ferrocene derivatives are also commonly used as electroactive indicators. For example, electrochemical detection of sequence-specific DNA using a DNA probe labeled with aminoferrocene (AFc) was reported.[282] However, the signaling probes could be modified with Fc carboxylic acid (FcAc).[293] Takenaka and co-workers[283,294] used a naphthalene diimide derivative carrying ferrocenyl moieties that behaves as an intercalator. Cyclic voltammetry and DPV gave an electrochemical signal due to the redox oxidation of ferrocenylnaphthalene diimide that was bound to the dsDNA on the electrode. According to Takenaka et al., the target DNA (dT_{20}) can be linearly detected in the range from 10^{-14} to 10^{-12} M (Table 2B).[283]

Motorola's eSensor is based on a sandwich hybridization protocol.[295] This system employs two ssDNA probes: one for capturing the target to the electrode surface and the

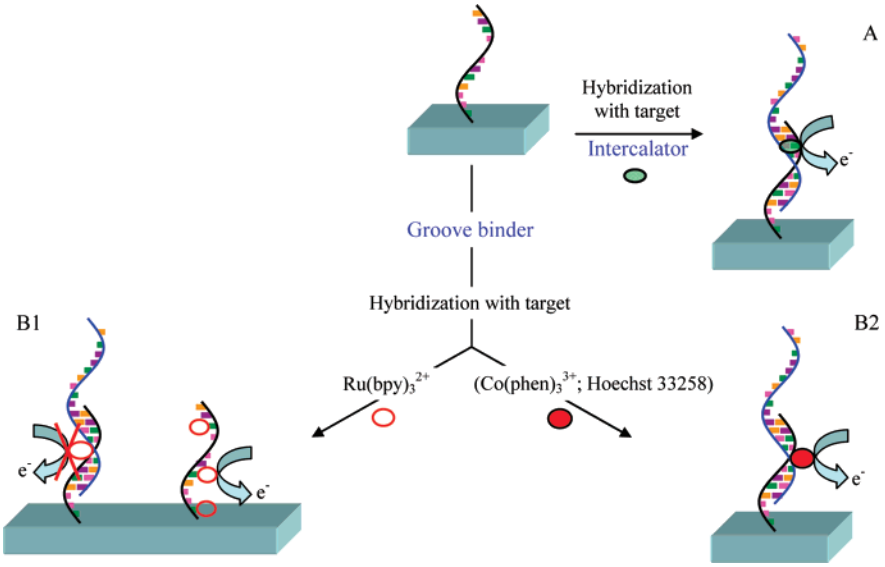

Figure 15. Different strategies to develop redox-indicator-based electrochemical DNA biosensors. (A) Intercalator interacts with the DNA duplex. (B1) $Ru(bpy)_3^{2+}$ interacts with guanines (green bases) of ssDNA whereas the formation of the double helix precludes the collision of $Ru(bpy)_3^{2+}$with guanine bases. (B2) $Co(phen)_3^{3+}$ or Hoechst 33258 interact with the DNA duplex, thus allowing DNA detection.

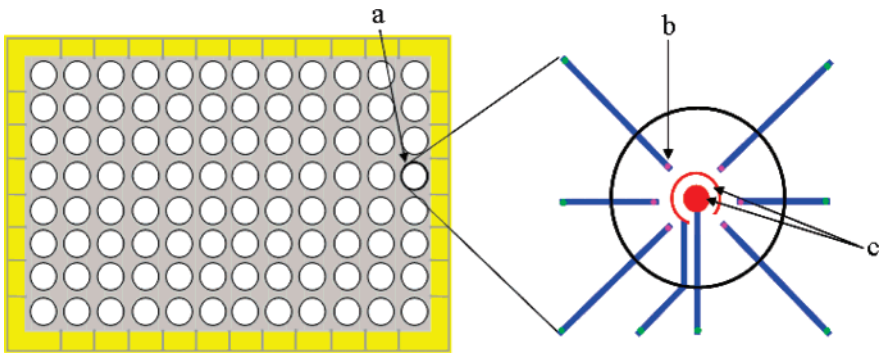

Figure 16. Schematic representation of the Xanthon Xpression Analysis Plate:[300] (a) one well of the plate; (b) working electrode; (c) reference and auxiliary electrodes

second for bioelectronic signaling. A gold electrode in a printed circuit board is coated with a SAM containing unlabeled capture probes. The signaling probe tagged with an Fc serves to label the target upon hybridization.[296]

3.2.3. Interacting Electroactive Substances

The most common electrochemical strategies for detecting the hybridization of DNA rely on interacting electroactive substances such as a groove binder (e.g., $Co(phen)_3^{3+}$, Hoechst 33258, or $Co(bpy)_3^{3+}$) or intercalating organic compounds (e.g., acridine orange) that interact in different ways with ssDNA or dsDNA. The electrochemical detection of DNA via an interacting electroactive substance is an attractive approach for ODN hybridization measurements because the target DNA does not need to be chemically modified.

3.2.3.1. Groove Binders. Table 2Ca presents performances of some groove-binder-based biosensors.[31,297,298]

Nucleic acids can be detected using $Ru(bpy)_3^{2+}$, which is a redox-active mediator used to oxidize guanine

$$Ru(bpy)_3^{2+} \xrightarrow{1.05\ V\ vs\ Ag/AgCl} Ru(bpy)_3^{3+} + e^-$$

$$Ru(bpy)_3^{3+} + guanine \rightarrow Ru(bpy)_3^{2+} + guanine^+$$

In this catalytic cycle, $Ru(bpy)_3^{2+}$ is oxidized to $Ru(bpy)_3^{3+}$, which in turn removes an electron from guanine in DNA and converts it to an oxidized guanine. Guanine oxidation causes the mediator reduction. In the absence of guanine, the mediator is oxidized only once, whereas a catalytic cycle occurs in the presence of guanine in DNA. Consequently, DNA is detected by a current increase due to the numerous oxidations of the mediator.[2] An electrochemical sensor for DNA hybridization using a probe strand containing only A, T, and C was developed[299] (Figure 15 B1). These inosine-substituted probes are hybridized to a target containing seven guanines. Because $Ru(bpy)_3^{2+}$ oxidizes guanine, a high catalytic current is measured after hybridization.

Xanthon Inc.[300] developed an electrochemical DNA chip called the Xanthon Xpression Analysis System. It is presented in a 96-well microplate format (Figure 16). Each well of the plate contains seven 200 μm tin-doped indium oxide (ITO) working electrodes. The approach is based on the electrochemical oxidation of the guanine base moieties of the target nucleic acids. The probes attached to a working electrode are exposed to a sample containing target DNA or RNA. The nucleic acid hybrid was then detected using the redox-active mediator, $Ru(bpy)_3^{2+}$. The interrogation period is short and allows 5 min of processing time per plate.

Cobalt complexes also can be used as electroactive hybridization indicators. $Co(bpy)_3^{3+}$ and $Co(phen)_3^{3+}$ (phenanthroline) preconcentrate at the electrode surface through association with dsDNA. They bind electrostatically in the minor groove of the DNA helix (Figure 15B2). For example,

Millan and co-workers[301,302] covalently immobilized probes onto GCEs. In these works, hybridization was detected voltammetrically using $Co(bpy)_3^{3+}$. $Co(phen)_3^{3+}$ also was used as an active indicator to develop DNA chips.[74] After probe immobilization onto an activated SPE, hybridization was monitored by changes in the guanine oxidation signal using chronopotentiometry. The response increased linearly with the 21-mer target concentration up to 0.6×10^{-6} M.

Hoechst 33258 is a DNA minor groove binder[303] that recognizes adenine/thymine-rich sequences of DNA within the helix (Figure 15B2). Hoechst 33258 was described as an acceptable electroactive hybridization indicator.[304,305] After DNA probe immobilization and hybridization, the indicator bound the DNA hybrids. Thus, the anodic current derived from linear sweep voltammetry (LSV) of Hoescht 33258 increased with the quantity of the hybridized target. Recently, Choi and co-workers[297,306,307] developed a 32 channel electrode array to detect target DNA (29-mer) using this electroactive marker. For that, they immobilized probe DNA on the gold surface by a DNA arrayer utilizing the affinity between gold and sulfur. Electrochemical gene detection was monitored using LSV or DPV in the presence of the DNA minor groove binder. The DNA target was linearly detected in the concentration range of $10^{-10}-10^{-7}$ M (Table 2Ca).

Toshiba Corp. developed an electrochemical DNA chip called Genelyzer that is able to analyze SNPs and common DNA sequence variations.[308] Capture probes are immobilized onto gold electrodes through a SAM. After the hybridization reaction with the target DNA, Hoechst 33258 is added. When an appropriate potential is applied, the oxidative current from the dye is proportional to the amount of bound target DNA.[296] A typical analysis of SNPs in a sample takes only about 1 h. This DNA chip has been tested and proved in its application to a system for providing personalized drug therapies for people suffering from hepatitis C. It was applied to a system for screening patients to ensure that they received appropriate medication.

3.2.3.2. Intercalators. Table 2Cb presents performances some intercalator-based biosensors or microarrays.[89,309−320]

Anthracycline antibiotics such as daunomycin and doxorubicin are currently used in cancer therapy. The interaction of daunomycin with DNA has been largely studied by Chaires and co-workers.[321−324] It appears that daunomycin intercalates into duplex DNA with preferential binding to G−C base pairs (Figure 15A). The voltammetric behavior of different intercalators such as acridine dyes, anthracycline antibiotics, ethidium dyes, tetracycline antibiotics, and bisbenzimide dyes was studied.[71] The selectivity of these molecules for dsDNA was evaluated. Daunomycin was the optimal intercalator for DNA sensors. In this work, probes were adsorbed on a polished BPPG electrode. After hybridization, the sensor was immersed in daunomycin. In the same way, the interaction of daunomycin with dsDNA in solution and at the electrode surface was studied by CV and by constant-current chronopotentiometric stripping analysis (CPSA).[325] Probes also were immobilized onto an aminoethanethiol SAM-modified gold electrode to detect cDNA (24-mer) by LSV using the intercalation of daunomycin.[310] Concentrations of the target were linearly detected between 1.26×10^{-11} and 1.26×10^{-8} M (Table 2Cb). Doxorubicin is also a specific intercalator[326,327] that is often used. For example, a thiolated probe immobilized on a gold electrode was hybridized to the complementary target (27 bases), and the dsDNA assembly on the electrode was treated by doxorubicin.[311] Electrochemical reduction of this agent led to the electrocatalyzed reduction of O_2 to H_2O_2, which caused, in the presence of HRP, the oxidation of 4-chloronaphthol to an insoluble product that precipitated on the electrode. The precipitate increased the interfacial electron-transfer resistance, which can be followed by Faradaic impedance spectroscopy. The detection limit of the DNA target was around 10^{-10} M (Table 2Cb). Technobiochip's electrochemical DNA biosensor enables DNA detection using PSA.[328] The ssDNA probe was immobilized on a carbon electrode using the avidin−biotin interaction. In this case, the detection involved the use of daunomycin, an electroactive indicator, which intercalated the dsDNA. This system was adapted to detect mutations related to neuroblastoma, the most common solid extracranial cancer in children.

Anthraquinone derivatives, such as 2,6-anthraquinone disulfonic acid (AQDS), are anionic intercalators that can be used to develop an electrochemical DNA hybridization biosensor based on long-range electron transfer through dsDNA to a redox intercalator. Wong and Gooding immobilized thiolated probes on a gold electrode.[329] After hybridization to the 20-mer complementary target, voltammetric peaks due to the oxidation and reduction of AQDS were observed. The selectivity of an AQDS biosensor has been studied, and anthraquinone-2-sulfonic acid (AQMS), which is less negatively charged than AQDS, also has been used. The performances of these two agents have been compared with those of two cationic intercalators.[87,330] It has been found that cations intercalated more rapidly than the anions, but significant nonspecific signals were observed in this case. However, anionic intercalators showed negligible nonspecific signals. Sensors based on AQMS or AQDS can detect a 20-mer target with a detection limit of 1.8×10^{-7} M.

Hybridized DNA can be detected by using electroactive ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide; EB), which is a specific intercalator for dsDNA. For example, after covalent immobilization of the probes onto a graphite electrode and hybridization to the 24-mer DNA target, EB intercalation into the dsDNA allowed the detection of cDNA by CV.[312] The currents were linear with concentrations of cDNA ranging from 1.26×10^{-10} to 1.26×10^{-8} M (Table 2Cb).

Methylene blue has been used widely as an electrochemical intercalator to monitor the DNA hybridization because ssDNA and dsDNA have different affinities for it. According to Ozsoz and co-workers,[331,332] MB had a higher affinity for ssDNA compared to that for dsDNA because it has a strong affinity for free guanine bases. A decrease in the peak current of methylene blue was observed upon hybridization of the probe to the 21-mer target.[89] The detection limit of this DNA biosensor was 7.2×10^{-8} M (Table 2Cb). In the same way, probes were immobilized to carboxyl groups on CNTs, and hybridization between the probe and the target (21-mer) was monitored by DPV of methylene blue.[88] Barton and co-workers reported a new strategy for the electrochemical detection of single-base mismatches in ODNs based upon the reduction of $Fe(CN)_6^{3-}$ mediated by methylene blue bound to a hybrid-modified surface.[333−337] Gold electrodes were modified with thiolated double-stranded ODNs to form densely packed DNA films that blocked the electrochemical reduction of ferricyanide in solution. The presence of methylene blue allowed this electrocatalytic reduction at the DNA-modified electrode. Methylene blue is reduced to

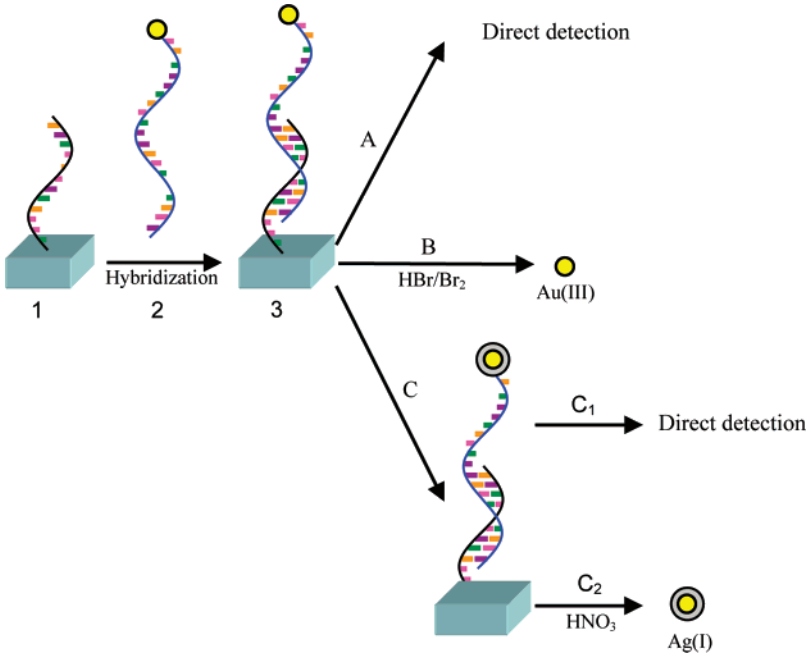

Figure 17. Detection strategies for gold nanoparticles. An immobilized DNA strand (1) is hybridized to a gold-tagged DNA target (2). The gold-labeled duplex (3) is then detected according to the following strategies: (A) direct detection of gold nanoparticles on the bare electrode; (B) dissolution of gold nanoparticles with HBr/Br_2 treatment and then detection by stripping techniques; (C) silver deposited on gold nanoparticles and detection by stripping techniques via a silver-enhanced signal; (C1) direct detection of nanoparticles covered with Ag; (C2) gold nanoparticles covered by Ag dissolved with HNO_3 treatment and then detected by stripping techniques. Detection is based on potentiometric and voltammetric stripping assays.

leucomethylene blue, which can easily reduce ferricyanide and regenerate the redox-active intercalative marker. This process can be repeated as long as the potential of the gold electrode is sufficiently negative to reduce methylene blue. Recently, methylene blue was used to develop an electrochemical DNA biosensor for the detection of chronic myelogeneous leukemia DNA.[319] GeneOhm Sciences Inc.[338] produced an 18-electrode array featuring microelectrode pads to make an easy detection test for SNPs using this principle.[296,339] A probe is immobilized onto a gold electrode surface. After hybridization with the target DNA, the electrode is exposed to an intercalator solution containing methylene blue. If the hybrid is perfectly matched, then the current flows through the well-stacked DNA to reduce methylene blue. If it contains mismatches, then the flow of current is blocked, and no signal can be measured.

Proflavine (3,6-diaminoacridine) also was used as an intercalator.[340] This molecule is positively charged but is not electrochemically active, contrary to the other previously described indicators. $[Fe(CN)_6]^{3-}$ was the only electrochemically active species present in solution. With a bare gold electrode, reduction and oxidation of $[Fe(CN)_6]^{3-}$ occurred. When thiolated probes were immobilized on the electrode surface, the negative charges of ssDNA repelled $[Fe(CN)_6]^{3-}$, and the voltammetric signal was very low. After hybridization with the complementary target (20-mer), proflavine bound the DNA duplex. In this case, the negative charges of dsDNA were compensated by the positive charges of proflavine, allowing the oxidation and reduction of $[Fe(CN)_6]^{3-}$. The high sensitivity of this system has been demonstrated by detecting 10^{-14} M of the DNA target.

3.2.4. Metal Nanoparticles for DNA Labeling

Table 2D presents the performances of some metal-nanoparticle-labeled DNA biosensors or microarrays.[64,73,138,341−349]

Nanoparticles are more and more used for signal hybridization in various DNA detection assays. Most of the work has been carried out using silver[348] or gold[350−352] in metal form.

Direct detection of nanoparticles on the electrode can be considered (Figure 17A). For example, an electrochemical genosensor was developed to detect the factor V Leiden mutation from PCR amplicons.[353] The DNA target was immobilized onto a pencil graphite electrode (PGE) before being hybridized to complementary probes conjugated to gold nanoparticles. The Au-tagged 23-mer probes were challenged with the synthetic 23-mer target, 131-base ssDNA, or denaturated 256-base PCR amplicon. The electroactivity of gold nanoparticles was used for the detection of hybridization without any external indicators. Thus, the specific sequences of DNA were directly detected by anodic analysis of the gold colloids. Recently, the electric detection of DNA hybridization by a nanoparticle nanoswitch was described.[354] The probe DNA was immobilized in a narrow gap between two electrodes. Then, a 24-mer target DNA was cohybridized to the probe on the electrode and to a signaling probe on gold nanoparticles. After the insertion of DNA-modified gold nanoparticles into the nanogap, the electric conductance across the electrodes increased, thus revealing the presence of the target.

The electrochemical signal of the gold nanoparticle can be measured after dissolving it with a particular treatment.[355] The gold nanoparticles can be dissolved by HBr/Br_2 treatment (Figure 17B). The Au(III) ions obtained are preconcentrated by electrochemical reduction onto an electrode and then detected by stripping techniques.[356] Nanoparticle-based electrical detection of DNA hybridization using this principle was described.[357] Hybridization involved a biotinylated target strand (19-mer) to ODN probe-coated magnetic beads. Then, the streptavidin-coated gold nanoparticles were bound to the

captured target before a dissolution process with HBr/Br_2. Finally, the dissolved gold tags were detected at a disposable thick-film carbon electrode by PSA.

After hybridization, silver deposition on the gold nanoparticles can be done to enhance the electrochemical signal (Figure 17C).[64,342,355,358−362] In this case, gold nanoparticles can be treated with a silver enhancer solution containing a reducing agent such as hydroquinone. In its presence, colloidal gold could catalyze the chemical reduction of silver ions (from silver lactate or silver acetate) to metallic silver on the nanoparticle surface.[64] Nevertheless, the reducing agent that triggers silver precipitation is difficult to control, and nonspecific silver deposition on the substrate cannot be avoided. To eliminate this drawback, the chemical silver reduction can be replaced by an electrochemical deposition process,[363] which has been reported previously for other applications related to trace metal quantitations.[364,365] After silver deposition using a reducing agent or an electrochemical potential, two techniques can be considered for DNA detection. In the first case, the silver deposition on the gold nanoparticles and the strategy based on the metal dissolution have been combined (Figure 17C2). For example, a biotinylated oligomer target was hybridized to the biotinylated DNA probe immobilized on a streptavidin-coated magnetic latex sphere.[362] Streptavidin-coated colloidal gold was then bound to the target. Silver metal was deposited on the gold nanoparticles before being dissolved in a solution containing HNO_3 and detected at a disposable thick-carbon electrode using PSA. The use of an ODN-functionalized electroconducting polymer coupled with a gold nanoparticle-based hybridization indicator was reported.[342] Poly(2-aminobenzoic acid) was formed on an ITO electrode and was used to covalently immobilize an amino-modified probe. Signal transduction was achieved by binding the gold nanoparticle label to the hybridized target. The amount of silver deposited on the gold nanoparticle label was determined by measuring the electrochemical oxidative silver dissolution response during a PSA scan. The sensor could linearly detect a 16-mer target from 10×10^{-12} to 10×10^{-9} M (Table 2D). In the second case, silver deposition on the gold nanoparticle was successful but not the acidic dissolution (Figure 17C1). An electrochemical detection method based on the catalytic precipitation of silver onto the gold nanoparticle label also was reported.[64] In this work, a 32-mer target DNA was adsorbed on a chitosan-modified GCE. After silver deposition on the gold nanoparticles, hybridization was monitored by DPV. The sensor response showed a linear relationship to the DNA target from 10^{-10} to 10^{-8} M (Table 2D). The silver-deposition technique also was exploited to construct a DNA sensor based on conductivity measurements. The target DNA was detected at concentrations as low as 5×10^{-13} M (Table 2D).[346]

Numerous protocols rely on the use of colloidal gold tags. Nevertheless, silver nanoparticles can be used instead of gold ones.[348] The 30-base target DNA was immobilized on gold colloid particles associated with a cysteamine monolayer on the gold electrode surface. Then, the target was hybridized with a silver nanoparticle−ODN DNA probe. The silver particles, which were bound to the hybrids, were dissolved by HNO_3 treatment. Anodic stripping voltammetry (ASV) allowed the detection of the DNA target in the range from 10^{-11} to 8×10^{-10} M (Table 2D).

It is also possible to use polymeric microbeads carrying gold nanoparticle tags. For instance, polystyrene beads carrying numerous gold nanoparticles were reported.[341] The DNA target (19-mer) labeled with the gold nanoparticle carrier beads were hybridized with probes captured on magnetic beads. The hybridization process was followed by a nanoparticle-promoted precipitation of gold. A gold dissolution process was achieved prior to electrochemical stripping detection. The detection limit was around 6×10^{-12} M (Table 2D).

Iron-containing particles were used for electrochemical detection of DNA hybridization.[349] Two strategies have been contemplated. The first possibility involved probes labeled with iron−gold core−shell nanoparticles. The biotinylated target, immobilized onto streptavidin-coated polystyrene beads, was hybridized to the iron−gold-particle-labeled probe. The second protocol relied on the use of commercial iron-containing magnetic beads. The probes were immobilized onto the chitosan-modified microwell and were then hybridized with the magnetic-bead-labeled target. In both cases, the captured iron-containing particles were dissolved, and the released iron was quantified by cathodic stripping voltammetry (CSV) in the presence of the 1-nitroso-2-naphthol ligand and a bromate catalyst. The 32-mer DNA target detection, based on gold-coated iron nanoparticle tags, enabled a detection limit of around 4.7×10^{-9} M. The 37-base DNA detection based on magnetic-sphere redox tags presented a detection limit of 1.6×10^{-9} M (Table 2D).

Quantum dots also can be used to electrochemically detect the DNA target. After the ODN sample entrapment into a PPy film, the surface was exposed to complementary probes or CdS-tagged ODN probes.[137,138] According to them, the use of CdS nanoparticle-labeled ODNs resulted in a higher sensitivity and a significantly improved detection limit of the sensor. In this case, the impedance signal was linear to the logarithm of ODN concentration between 3.7×10^{-9} and 3.7×10^{-7} M (Table 2D).

3.2.5. Label-Free Electrochemical Detection

Table 2E presents performances of some label-free DNA biosensors or microarrays.[75,101,102,134,136,366−377]

DNA detection can be based on the natural electroactivity of the nucleotide residues present in DNA. This has been exploited in particular by Palecek group who showed that DNA and RNA are electroactive compounds producing reduction and oxidation signals after hybridization.[378] Signals of adenine, cytosine, and guanine can be observed on oscillopolarograms of ssDNA, whereas these signals are absent with dsDNA. Guanine was described as the most redox-active nitrogeneous base in DNA.

Immobilized guanine-free probes can be used to develop a label-free electrochemical DNA hybridization biosensor. Inosine can be substituted with guanine.[75,379] While the inosine moiety preferentially forms a base pair with the target cytosine residue, its oxidation signal is well-separated from the guanine response. After hybridization, the appearance of the guanine oxidation signal of the 29-mer target allowed the detection of the DNA hybrid by PSA. The detection limit was estimated to be around 1.25×10^{-8} M (Table 2E).

Pividori and co-workers also exploited the guanine oxidation signal for detecting duplex formation.[79] For example, an inosine-substituted probe was immobilized by wet adsorption on the GEC.[376] In this case, the 23-mer target was detected by DPV using the oxidation signal of guanine. The linear range for target detection was observed from 3.95×10^{-6} to 1.98×10^{-5} M (Table 2E). Recently, a genomagnetic assay was developed using a GEC electrode containing a

small magnet as the transducer.[377] A biotinylated inosine-substituted capture probe was immobilized onto streptavidin-coated magnetic beads. After hybridization with the 56-mer complementary target, the oxidation signal of guanine also was measured by DPV. The detection limit was estimated at 9.68×10^{-12} M (Table 2E).

Kerman and co-workers developed label-free electrochemical DNA sensors based on the oxidation of guanine. They covalently immobilized an adenine probe on a SAM-modified gold electrode.[372] Then, this sequence was hybridized with a thymine tag of an inosine-substituted capture probe, which recognized the 21-mer target DNA. The guanine oxidation signal was measured at +0.73 V vs Ag/AgCl to confirm duplex formation on the gold electrode. The lowest detected target concentration was 1.25×10^{-9} M (Table 2E). However, the synthesis of guanine-free probes that includes inosine bases is expensive. To overcome this problem, electrochemical transduction of the hybridization between a probe without inosine bases and the 17-mer complementary target was reported.[374] The oxidation signals of these bases were studied by DPV. The detection limit was 1.2×10^{-8} M (Table 2E). It appeared that the signal obtained from the ssDNA-modified carbon paste electrode (CPE) was higher than that from the dsDNA-modified CPE due to the accessible unbound adenine and guanine bases. PNA was used to develop a label-free DNA sensor based on guanine oxidation.[375] This sensor detected 14-mer DNA target with a detection limit of 2.7×10^{-10} M (Table 2E).

Generally, hybridization and electrochemical detection of this hybridization occur at the same electrode. However, a new technology was proposed in which an oligo$(T)_{25}$ was immobilized on magnetic beads and the hybridization with an oligo$(A)_{25}$ was then detected at a mercury electrode.[380,381] The detection was based on the CSV of adenine that is released from DNA after acid treatment.

Changes in some intrinsic properties of the double helix (such as impedance, capacitance, or conductivity) also can be used to monitor the hybridization.

It has been demonstrated that impedance can be used for direct in situ detection of hybridization between complementary homo-oligomer DNA strands.[382] Since this discovery, impedance is a technique often used to study interfacial properties.[383]

A DNA probe was also reported to be grafted to a conducting polythiophene film.[384] Hybridization to a 37-mer single-stranded complementary target was measured by electrochemical impedance spectroscopy (EIS). The conducting properties of the film changed upon hybridization.

Probe ODNs also were attached to a SAM on a gold electrode via a streptavidin−biotin bridge.[114] Impedance measurements were made using a $[Fe(CN)_6]^{4-/3-}$ redox couple as a signal reporter to enhance the sensitivity.

DNA probes were also reported to be grafted onto a copolymer of poly[pyrrole-*co*-4-(3-pyrrolyl)butanoic acid]. In the presence of the complementary target (18-mer), the CV of the ODN probe-modified copolymer showed significant changes.[385] AC impedance showed an increased charge-transfer resistance and double-layer capacitance of the sensor film after hybridization.

Piro et al. immobilized, covalently, a DNA probe on a quinone-containing polymer.[386] The quinone group acts as an immobilized redox indicator of hybridization. In this work, hybridization was monitored by CV and EIS. The shape of the cyclic voltammogram was modified. Upon hybridization, the electrochemical impedance spectra obtained in the presence of the 20-mer complementary target were attributed to changes in the conformation on the polymeric film. The electrode surface was partially blocked after probe grafting and liberated after hybridization.

An electrochemical DNA biosensor was developed using EIS to detect HIV.[371] First, a polypyrrole film was deposited on a platinum electrode surface by CV. Then, negatively charged gold and silver nanoparticles were directly bound onto a PPy film that is positively charged. Finally, thiolated ODNs were self-assembled onto the nanoparticles. When hybridization occurs, a decrease of impedance values was observed, allowing 21-mer target DNA detection with good selectivity. This sensor detected cDNA ranging from 10^{-9} to 10^{-6} M with a detection limit of 5×10^{-10} M (Table 2E).

An anionic oligo$(dG)_{20}$ probe was reported to be incorporated within a growing PPy film.[133] ODN probes served as sole counteranions and maintained their hybridization activity upon entrapment. When a potential of +0.15 V vs Ag/AgCl was applied to the electrode, an anionic peak was obtained after addition of the complementary oligo$(dC)_{20}$ target whereas the opposite signal was observed in the presence of noncomplementary sequences.

Korri-Youssoufi and co-workers developed an electrochemical biosensor based on a precursor polymer bearing an ester group on which amino-ODNs were directly immobilized by substitution. The hybridization event was studied by CV.[101,103] In the presence of the complementary sequence, a significant modification in the voltammogram was observed. The results showed a decrease of the intensity of the oxidation wave and a shift of the oxidation wave to a higher potential. The same immobilization method also was coupled to non-Faradaic EIS.[102,103] A significant modification in the Nyquist plot was observed upon hybridization. The results showed an increase in the charge-transfer resistance as a function of the concentration of the 25-mer target. The detection limit was estimated to be 2×10^{-10} M (Table 2E).

Standard "top-down" semiconductor processes also can be used to immobilize a probe on silicon nanowires.[387] Hybridization to the label-free target produced a change in conductance. The authors easily detected 2.5×10^{-11} M of target DNA that hybridized with a 12-mer ODN probe.

Polymerization of a terthiophene monomer containing a carboxyl group on a GCE was described.[93] The 19-mer ODN was covalently bound to the polymer. In this work, impedance and admittance changes according to frequency variation were observed before and after hybridization. The difference revealed a reduction of the resistance and an enhancement of the conductance after hybridization.

Arrays of highly ordered n-type silicon nanowires (SiNWs) were fabricated for direct rapid, ultrasensitive, and label-free electrical DNA detection.[33] A monolayer of PNA probes was self-assembled onto the individual SiNWs via silane chemistry. The interaction of PNA with a sample DNA formed a heteroduplex, bringing a high density of negative charges on the SiNW surface. The formation of an electrical field at the SiNW surface provided the sensitivity for the detection of DNA. The resistance of the SiNW increased linearly with the DNA concentration, and the dynamic range was found to be from 2.5×10^{-14} to 5×10^{-12} M with a detection limit of 10^{-14} M.

3.3. Gravimetric DNA Biosensors

3.3.1. Quartz Crystal Microbalance Sensors

A QCM sensor is a mass-sensitive sensor capable of measuring very small mass changes.[388] It consists of a thin quartz disc sandwiched between a pair of electrodes. Quartz is a piezoelectric material that deforms when an electric field is applied across the electrode. The quartz crystal has a resonant frequency dependent on the total oscillating mass. This frequency increases with an increase in material on the QCM surface. In addition to electrochemical and optical detection of DNA, mass sensors are also capable of detecting label-free ODNs.[389,390] Table 3 presents performances of some QCM-based biosensors or microarrays.[39,66,391−396]

Willner and co-workers described a method to amplify DNA detection involving the use of functional liposomes.[391,392,397] They described two strategies to amplify the ODN−DNA processes.[392] In the first case, a thiolated primer was assembled on a gold-quartz crystal, followed by hybridization with the 27-mer analyte DNA. Then, this double-stranded assembly interacted with a liposome functionalized with another ODN complementary to one part of the target. The detection limit was estimated to be 5×10^{-12} M. In the second configuration, the double-stranded assembly interacted with a biotinylated ODN. The resulting assembly interacted with avidin and then with a biotin-functionalized liposome. The use of liposomes containing ODNs allowed the formation of dendritic structures. The lower limit of detection for the target by this amplification method was 10^{-13} M (Table 3). Willner et al. described three methods for the amplified detection of a single-base mismatch in DNA where a guanine in the mutant gene substituted for an adenine in the normal gene.[391] In the three cases, a thiolated primer complementary to the analyte was assembled on a gold-quartz crystal. After hybridization, a biotinylated dCTP was incorporated to the mutation site in the presence of DNA polymerase I. Thus, the biotin label indicated the presence of the single-base mutation in the sequence. As previously described, the first strategy involved the association of avidin- and biotin-labeled liposomes. The second method used an Au nanoparticle−avidin conjugate whereas the third protocol required an avidin−PAL bioconjugate that bound the biotin label introduced in the polymerase-induced process. This enzyme catalyzed the oxidative hydrolysis of 5-bromo-4-chloro-3-indolyl phosphate to an insoluble product that precipitated on the gold-quartz crystal. The protocol using an Au nanoparticle−avidin conjugate is the most sensitive method (detection limit of 3×10^{-16} M, Table 3).

A system was developed for the detection of a point mutation in DNA.[395] For that, the authors immobilized a ssDNA probe on a QCM surface via streptavidin−biotin interaction. After hybridization, the MutS protein was used to recognize mutation. The MutS application caused a frequency change that can be attributed to the MutS binding. DNA containing a single T:G mismatch and one to four unpaired mutation(s) has been discriminated from perfectly matched DNA down to target concentrations of 1×10^{-9} M (Table 3).

A QCM DNA sensor was developed for the detection of *Escherichia coli* O157:H7 using a nanoparticle amplification method.[393] A thiolated ssDNA probe specific to the *E. coli* O157:H7 eaeA gene was immobilized on the QCM surface. After bovine serum albumin blocking, the probe was exposed to the biotinylated target DNA; however, the frequency change was negligible. Therefore, to amplify this frequency change, streptavidin conjugated Fe_3O_4 nanoparticles were attached to the target. This sensor could then detect DNA concentrations as low as 10^{-12} M (Table 3).

Okahata and co-workers largely have used QCM to detect DNA. They immobilized a DNA probe that was complementary to the EcoRI binding site of single-stranded M13 phage DNA.[394] In the presence of the target, the frequency decreased with time. The minimum concentration that could be detected was 10^{-12} M (Table 3). A biotinylated DNA probe also could be immobilized either via avidin, which was covalently attached to the QCM surface, or via electrostatic interaction with poly(allylamine hydrochloride).[398] The authors also formed multilayer films of DNA by successive deposition of avidin and poly(styrenesulfonate) to improve nucleic acid detection. A commercially available 27 MHz QCM also was used to study the in situ binding of avidin to a modified electrode, the immobilization of the single-stranded biotin DNA, and the hybridization with the complementary target.[399] It appeared that the 27 MHz QCM was 10 times more sensitive than the commonly used 9 MHz QCM. The same 27 MHz QCM also was used by immobilizing a 10−30-mer ODN on the surface.[400] The effect of 1, 2, or 3 mismatching bases in the target sequence on hybridization was studied. When the number of mismatching bases increased, the frequency increased, showing that the binding amount clearly decreased.

DNA dendrimers also were used with numerous peripheral single-stranded arms as a probe to detect *Cryptosporidium* DNA.[39] Adsorption and electropolymeric entrapment were used to immobilize DNA dendrimers on the crystal. For both immobilization techniques, the response increased linearly with the 38-mer target concentration up to 8×10^{-6} M (100 μg/mL) with a detection limit around 8×10^{-8} M (1 μg/mL) (Table 3).

A DNA genetic sensor was developed for the diagnosis of β-thalassaemia using a QCM technique.[66] An 18-mer DNA probe complementary to the site of genetic β-thalassaemia mutations was immobilized on the QCM electrodes. Different methods for immobilizing the DNA probe were tested. Mono- or multilayered DNA probes were formed by avidin−biotin interaction, chemisorption, or electrostatic adsorption on a polyelectrolyte film. The DNA sensor based on the immobilization of biotinylated DNA probe to avidin provides fast sensor response and high hybridization efficiency. The optimum concentration range of the target DNA sequence is 8.4×10^{-6} M (50 μg/mL)−1.2×10^{-5} M (70 μg/mL) (Table 3).

A biotinylated single strand ODN was immobilized on streptavidin, which is covalently attached to a dextran-modified thiol layer on the piezoelectric crystal.[401] This system could detect DNA that was complementary to the immobilized probe with good selectivity and high reproducibility.

Technobiochip[328] described a QCM DNA sensor for the detection of genetically modified organisms (GMOs). As previously described, the gold-quartz surface is functionalized by a biotinylated DNA probe layer through its high affinity with streptavidin deposited on the surface.[402] This DNA sensor called μLibra is a reliable, fast, and cost-effective method for GMO screening in food samples.

Although not very common, piezoelectric devices can be found in a multiarray format. A piezoelectric microarray was developed to detect the hepatitis B virus quantitatively in

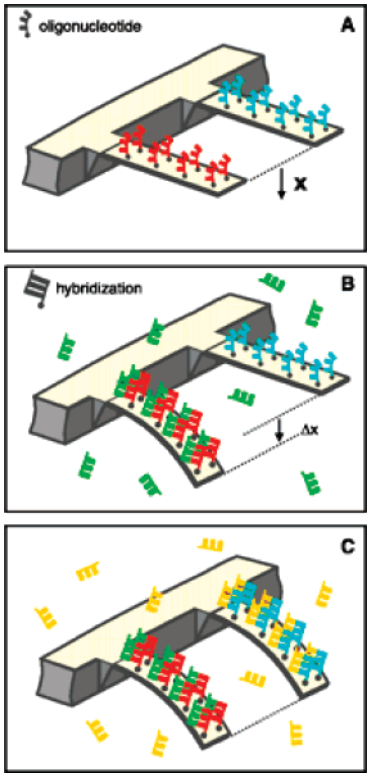

Figure 18. Cantilever sensor principle. (A) Each cantilever is functionalized on one side with a different oligonucleotide base sequence (red or blue). (B) After injection of the first complementary oligonucleotide (green), hybridization occurs on the cantilever that provides the matching sequence (red), increasing the differential signal, Δx. (C) Injection of the second complementary oligonucleotide (yellow) causes the cantilever functionalized with the second oligonucleotide (blue) to bend. Reprinted with permission from *Science* (http://www.aaas.org), ref 408. Copyright 2000 American Association for the Advancement of Science.

clinical samples.[403] A bis-PNA probe was designed and immobilized on the surface of the microarray. Every crystal could oscillate independently without interfering with the others.

3.3.2. Microcantilever Sensors

Microcantilever sensors recently have emerged as a promising tool to detect biomolecular interactions.[404−407] These systems offer many advantages compared to conventional sensors: label-free detection, high precision, reliability, reduced size, easy manufacture of multielement sensor arrays, and small thermal mass. Microcantilever sensors are based on a response due to either surface stress variation or mass loading. Interaction between an immobilized ligand (e.g., a DNA probe) and an analyte (e.g., a DNA target) causes a change of the surface stress of the cantilever and can be detected as changes in the cantilever deflection (Δx).

Thiolated ODNs were covalently immobilized on the gold-covered side of the cantilevers[408] (Figure 18). One cantilever was functionalized with a 12-mer ODN, whereas the other was functionalized with a 16-mer ODN (Figure 18A). The arrays were brought to equilibrium in a hybridization buffer until the differential signal became stable. The complementary 16-mer sequence (Figure 18B) and the complementary 12-mer ODN (Figure 18C) were then injected into the liquid cell. Hybridization of the target to the matching ODNs immobilized on the cantilever surfaces occurred, causing the cantilever deflection.

Hansen's group developed microcantilever-based sensors for DNA detection. They immobilized a 5′-thiol-modified probe on the Au-coated cantilever surface. The functionalized cantilever was then exposed to a solution containing complementary target ssDNA. They demonstrated that cantilever deflection varied with the complementary oligomer length.[409] A change in deflection was caused by hybridization of a ssDNA probe (20-mer) complementary to the distal end of target ssDNA of four different lengths (9-, 10-, 15-, and 20-mers). They also could discriminate full and partial complementary sequences.[410] For example, hybridization of 10-mer complementary target resulted in net positive deflection whereas hybridization with sequences containing one or two internal mismatches resulted in net negative deflection.

Covalently immobilization of ssDNA onto AFM tips also was reported.[104] Interaction between the cantilever and the target was quantified by obtaining the percent separation distance (PSD)

$$\mathrm{PSD} = (L_2 - L_1) \times 100$$

where L_1 is the separation distance (nm) for the buffer solution and L_2 is the separation distance (nm) for the same buffer solution containing the target ssDNA. They found a correlation between the concentration of the target in the medium and the PSD value. After hybridization between the immobilized probe and its complementary sequence, the PSD values significantly increased.

4. Conclusion

Different strategies used to develop DNA biosensors and DNA microarrays have been reported in this review. A critical step is the DNA probe immobilization on a surface. To develop microarrays, the probes can be made base-by-base on the support or presynthesized and then spotted on the surface. Adsorption, covalent immobilization, and avidin−biotin interactions can be contemplated for the development of DNA microarrays but also for the manufacture of DNA biosensors. Regeneration of a surface-immobilized probe also has been described, allowing the reuse of DNA biosensors and microarrays without the loss of hybridization activity. Three requirements have to be taken into account with probe immobilization: the immobilization chemistry needs to be stable, the probes have to remain functional after attachment, and biomolecules have to be immobilized with an appropriate orientation and configuration.[178] None of the immobilization techniques is ideal, and each one obviously presents some drawbacks. For example, covalent attachment is often used because immobilized probe stability is high, even if a time-consuming surface treatment is needed and immobilization yields are rather low. Associated with the DNA probe immobilization, most of the detection methods, which are used to develop DNA biosensors and microarrays, are open to criticism.[411] Optical, electrochemical, or gravimetric techniques can be used for DNA detection. Frequently, the detection methods are based on a tagging approach. Among them, the fluorescence-based technique is often used due to its high sensitivity. But the entire optical system including the laser diode, photodiode, and filter is not very suited for miniaturization and appears very costly. Electrochemistry is a promising tool often employed to develop DNA biosensors and DNA microarrays. Labels, such as enzymes, redox-active indicators, or nanoparticles, can be used, but label-free electrochemical detection of hybridization

is also a very attractive approach. Moreover, the electrochemistry-based approach should be more appropriate for on-site testing with portable analyzers.

Sensitivity and specificity are important features to consider. Molecular beacon probes are known to offer high specificity and appear as better alternatives to linear probes for mismatch discrimination. However, PNA also appears to be a good candidate for DNA detection, presenting remarkable sequence specificity, including the detection of single-base mismatches. DNA dendrimers also can be used to obtain a higher sensitivity related to an increase in hybridization capacity. On the basis of these different kinds of DNA probes or on more conventional linear DNA sequences, two types of biosensors and microarrays have been developed: systems for DNA hybridization detection and systems for detection of DNA mutations. Moreover, electrochemical DNA biosensors able to detect low-molecular-weight compounds, such as toxins, pollutants, or drugs with affinity for DNA, have been described. The determination of such compounds is measured by their effect on the oxidation signal of the guanine peak of DNA immobilized on an electrode surface and investigated by chronopotentiometric or voltammetric analysis. For example, Mascini and co-workers developed a DNA biosensor allowing the detection of aflatoxin, which is among the most potent environmental mutagens and is known as a liver carcinogen, and polychlorinated biphenyls (PCBs), which are environmental pollutants. Single-stranded calf thymus DNA was immobilized on the electrode surface by applying a fixed potential.[309,412] The guanine base in ssDNA was reported to be more available for oxidation than that in dsDNA, and the peak area of guanine decreased in the presence of aflatoxine or PCB concentrations. Detection limits of 0.2 and 10 mg/mL were obtained for PCB and aflatoxin, respectively. An electrochemical DNA biosensor also was reported as a screening device for the rapid detection of toxic compounds in water and wastewater samples.[413] Double-stranded calf thymus DNA was immobilized onto a screen-printed electrode surface. Potentially, toxic compounds, such as aromatic amines or 4-nitroquinoline-*N*-oxide present in samples, were evaluated by changes in the electrochemical signals of guanines. In the same way, the effects of four aromatic compounds (1,2-diaminoanthraquinone, 2-anthramine, 2-naphthylamine, and acridine orange) on dsDNA-coated electrodes were compared.[414] A decrease of the guanine signal was observed probably due to the surface accessibility of guanine upon intercalative binding of the aromatic amines to dsDNA. Submicromolar detection limits have been obtained for molecules after a 2 min accumulation. An SPR-based DNA biosensor also was described for the analysis of bioactive compounds with applications in drug and herbal drug screening.[415] Wang and co-workers also detected environmental and toxic compounds using a DNA biosensor.[416] They reported a dsDNA-coated CPE as a sensitive biosensor for the detection of hydrazine compounds.[417] The detection limit for methyl- and dimethylhydrazines was 0.5 μg/L.

Biosensors and microarrays both appear as highly efficient devices with enormous potential. Both are rapid and sensitive and give results in real time. Biosensors can be used for single-shot measurements whereas microarrays allow multiple simultaneous detections. Some issues still remain to be considered. Biosensors are difficult to commercialize due to their inherent instability. Although a diversity of microarrays for diagnostic and therapeutic applications has been described in research laboratories worldwide, only some of these chips have entered the clinical market, and more chips are awaiting commercialization. DNA microarray technology (section 2.2) and sample preparation have limitations that influence sensitivity, accuracy, specificity, and reproducibility of the results.[418−420] Gene-expression profiling relies on the preservation of small amounts of mRNA species from the tissue of interest. In situ synthesis of DNA probes (section 2.2.1) is also a critical parameter to consider. In this case, ODN probes are built up base-by-base on the surface through repeated cycles of deprotection and coupling that must be well controlled to prevent any mismatch that would lead to an incorrect hybridization. Splice variants also can introduce difficulties in microarray analysis. It is estimated that at least half of the human genes are alternatively spliced. Thus, measurements can reflect the concentration of either all splice variants present in the sample or a specific splice variant of a given gene. A cross-hybridization signal, produced by targets that are not fully complementary to the probe but have an important sequence similarity with it, also can explain the discrepancies in microarray measurements. When working with DNA microarrays, users have to consider the signal-to-noise ratio instead of the absolute signal. DNA microarrays can allow the comprehensive measurement of the expression levels of hundreds of genes simultaneously, giving a large amount of data that makes analysis complex. Thus, statistic and data processing technologies are necessary. There are many techniques in bioinformatics for the analysis of DNA microarray data.[421−423] For example, Affymetrix developed the GeneChip Scanner 3000, which is a 16-bit sophisticated opto-mechanical confocal scanner measuring fluorescence intensity emitted by the labeled cRNA bound to the probe arrays. It can detect as few as 400 phycoerythrin molecules in a 20 $\times$ 20 μm probe site. A computer workstation running the GeneChip Operating Software converts the hybridization intensity data into a presence/absence call for each gene using appropriate algorithms. Statistical techniques (e.g., algorithms) are also used to model the hybridization and cross-hybridization processes.[424−426]

The commercial array platforms differ in probe preparation methods and array surface chemistry. Thus, the availability of such multiple platforms raises the question of cross-platform agreement in gene-expression measurements.[427−435]

Several comparative studies have been carried out showing poor correlation.[429−431,436] More especially, a widely cited study[429] achieved by Cam and co-workers reported the results of gene-expression measurements generated from identical RNA preparations using three commercially available microarray platforms. They compared Affymetrix, Agilent, and Amersham (Codelink) microarrays. They showed a Venn diagram of overlapping circles representing the number of genes that were the most or least active on each device. From a set of 185 common genes selected, only four behaved consistently on all three platforms.

Although results raise doubts about the repeatability, reproducibility, and comparability of microarray technology, several studies also have been recently published showing increased reproducibility of microarray data generated at different test sites and/or using a different platform.[432,437−439] For example, two different arrays (Affymetrix GeneChips and spotted long ODN arrays) were used to analyze gene expression in two human RNA samples.[432] Expression measurements for 7344 genes that were represented in both types of arrays were compared. Strong correlations between

relative gene-expression measurements were noted between spotted long ODN probes and Affymetrix GeneChips.

As with every emerging technology, standards need to be established to avoid the doubts about the lack of reproducibility, repeatability, and compatibility across platforms and laboratories. For that, the microarray community and regulatory agencies have developed a consortium to establish a set of quality assurance and quality control criteria to ensure data quality, to identify critical factors affecting data quality, and to optimize and standardize microarray procedures.[440,441] The MicroArray Quality Control (MAQC)[442] Consortium involves 137 participants from 51 government, commercial, and academic organizations that include the U. S. Food Drug Administration (FDA), the U. S. Environmental Protection Agency, the National Institutes of Health, all the major whole-genome gene-expression platform providers, and several alternative gene-expression platform providers. The MAQC Consortium's main conclusion confirms that, with careful experimental design and appropriate data transformation and analysis, microarray data indeed can be reproducible and comparable among different formats and laboratories, irrespective of sample labeling format. Moreover, they demonstrated that fold-change results from microarray experiments correlate closely with results from assays such as quantitative reverse transcription PCR. The MAQC Consortium project tested six commercially available microarray platforms:[443] Applied Biosystems, Affymetrix, Agilent Technologies, GE Healthcare, Illumina, and Eppendorf.[444] In addition, scientists at the National Cancer Institute generated spotted microarrays using ODNs obtained from Operon. The RNA sample types also were tested on three alternative gene-expression platforms: TaqMan Gene Expression Assays from Applied Biosystems, StaRT-PCR from Gene Express, and QuantiGene assays from Panomics. These studies showed that all of the platforms actually correlate quite well with one another. "Normalization plays a critical role for successful microarray experiments. And now we know that all of the microarray platforms are viable" (from R. Shippy, a R&D scientist and biostatistician, GE Healthcare). Two other groups, The Center for Drug and Evaluation Research and the External RNA Control Consortium also work on this subject. The FDA plans to publish its recommendations in December 2007.

5. List of Abbreviations

ABEI	*N*-(4-aminobutyl)-*N*-ethylisoluminol
ACV	alternating current voltammetry
AFc	aminoferrocene
AFM	atomic force microscopy
AMPPD	3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane
APEX	arrayed primer extension
AQDS	2,6-anthraquinone disulfonic acid
AQMS	anthraquinone-2-sulfonic acid
ASV	anodic stripping voltammetry
BOD	bilirubin oxidase
BPPG	basal plane pyrolytic graphite
bpy	bipypridine
CCD	charge-coupled device
CFV	cresyl fast violet
CL	chemiluminescence
CMOS	complementary metal oxide semiconductor
CNT	carbon nanotube
CPE	carbon paste electrode
CPSA	chronopotentiometric stripping analysis
CSV	cathodic stripping voltammetry
CV	cyclic voltammetry
Cy	cyanine
DAA	2,2′-diaminoazobenzene
DABCYL	4-(4-(dimethylamino)phenyl)azo)benzoic acid
DNA	deoxyribonucleic acid
cDNA	complementary DNA
dsDNA	double-stranded DNA
ssDNA	single-stranded DNA
DPI	differential polarization interferometry
DPV	differential pulse voltammetry
EB	ethidium bromide (2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide)
ECL	electrochemiluminescence
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EIS	electrochemical impedance spectroscopy
Fc	ferrocene
FCA	ferrocenecarboxyaldehyde
FcAc	ferrocene carboxylic acid
FDA	Food Drug Administration
FITC	fluorescein isothiocyanate
FRET	fluorescence resonance energy transfer
G6PDH	glucose-6-phosphate dehydrogenase
GCE	glassy carbon electrode
GEC	graphite−epoxy composite
GMO	genetically modified organism
GOD	glucose oxidase
GOPS	3-glycidoxypropyltrimethoxysilane
HIV	human immunodeficiency virus
HRP	horseradish peroxidase
ITO	indium tin oxide
LSV	linear sweep voltammetry
MAQC	MicroArray Quality Control
MB	molecular beacon
ND	not determined
NHS	*N*-hydroxysuccinimide
ODN	oligonucleotide
OPD	*o*-phenylenediamine
PAA	polyacrylate
PAAH	poly(allylamine)hydrochloride
PAL	alkaline phosphatase
PAn	polyaniline
PBA	4-(3-pyrrolyl)butanoic acid
PCR	polymerase chain reaction
PDMS	poly(dimethylsiloxane)
PGE	pencil graphite electrode
Phen	phenanthroline
pI	isoelectric point
PNA	peptide nucleic acid
PPO	poly(propylene oxide)
PPy	polypyrrole
(PQQ)GDH	pyrroquinoline quinone glucose dehydrogenase
PSA	potentiometric stripping analysis
PSD	percent separation distance
PSS	poly(styrenesulfonate)
PVA-SbQ	poly(vinyl alcohol) bearing styrylpyridinium groups
PVC	poly(vinyl chloride)
Py	pyrrole
QCM	quartz crystal microbalance
QD	quantum dot
RNA	ribonucleic acid
$Ru(bpy)_3^{2+}$	Tris-(2,2′-bipyridyl)dichloro-ruthenium(II) hexahydrate
SAM	self-assembled monolayer
SBP	soybean peroxidase
SDS	sodium dodecyl sulfate
SECM	scanning electrochemical microscopy
SERS	surface-enhanced Raman scattering
SiNW	silicon nanowire
SNP	single-nucleotide polymorphism

SPE	screen-printed electrode
SPFM	surface plasmon field-enhanced fluorescence microscopy
SPFS	surface plasmon field-enhanced fluorescence spectroscopy
SPM	surface plasmon microscopy
SPR	surface plasmon resonance
SWNT	single-walled carbon nanotube
SWV	square-wave voltammetry
TAMRA	carboxytetramethylrhodamine
TMR	tetramethylrhodamine
UV	ultraviolet

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CR0684467