Review

Electrochemical DNA Hybridization Sensors Based on Conducting Polymers

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Abstract: Conducting polymers (CPs) are a group of polymeric materials that have attracted considerable attention because of their unique electronic, chemical, and biochemical properties. This is reflected in their use in a wide range of potential applications, including light-emitting diodes, anti-static coating, electrochromic materials, solar cells, chemical sensors, biosensors, and drug-release systems. Electrochemical DNA sensors based on CPs can be used in numerous areas related to human health. This review summarizes the recent progress made in the development and use of CP-based electrochemical DNA hybridization sensors. We discuss the distinct properties of CPs with respect to their use in the immobilization of probe DNA on electrode surfaces, and we describe the immobilization techniques used for developing DNA hybridization sensors together with the various transduction methods employed. In the concluding part of this review, we present some of the challenges faced in the use of CP-based DNA hybridization sensors, as well as a future perspective.

Keywords: electrochemical DNA sensors; conducting polymers; immobilization techniques; transduction mechanism
1. Introduction

Deoxyribonucleic acid (DNA) detection plays a prominent role in myriad disciplines related to human health, including the diagnosis of infectious diseases, identification of genetic mutations, drug discovery, forensics, and food technology [1–3]. For analyzing specific DNA sequences, reliable techniques based on either direct sequencing or DNA hybridization have been developed [2,3]. DNA sequencing is the process of determining the precise order of four nucleotides bases (adenine, guanine, cytosine, and thiamine) in a strand of DNA. DNA sequencing technology based on 2-D thin-layer chromatography was invented by Maxam and Gilbert and Sanger et al. in the 1970s [4,5]. The Maxam–Gilbert sequencing method rapidly became popular owing to the short sequence-reading times involved and because purified DNA could be directly used in this method. However, the requirement of using large amounts of purified DNA and complicated purification steps, combined with a shortage of available sequencers, limited the use of this method. Other major challenges associated with the Maxam–Gilbert method have been the extensive use of hazardous chemicals and difficulties with sample scale-up. By contrast, the chain-termination method developed by Sanger and coworkers made DNA sequencing comparatively more practical, because it required lesser amounts of purified DNA than the Maxam–Gilbert method did and it also provided multiple options for labeling the sequencing template. Of the two methods, the Sanger method is more efficient, uses fewer toxic chemicals, and requires the use of lower amounts of radioactivity. Furthermore, radioactive phosphorus labeling or the use of a primer labeled at the 5’ end with a fluorescent dye allows an optical set-up to be employed in the sequencing performed using the Sanger method; this facilitates easy analysis and the use of inexpensive automation. In order to enhance the sensitivity of this method, dye-terminator sequencing chemistry has been introduced [6–8]. However, dye-terminator sequencing has limited practical utility owing to the “dye effect” that arises from the difference in the incorporation of the dye-labeled chain terminators into the DNA fragment, which generates unequal peak heights and shapes in the DNA sequencing chromatogram. DNA sequencing by hybridization onto a solid support (e.g., nitrocellulose, nylon membrane, or lysine-coated glass slide) performed using fluorescently or radioactively tagged DNA became a common method for DNA analysis in the early 1990s [9,10]. This detection method appeared to be a promising tool for the real-time analysis of multiple DNA sequences, and it depended on the anchoring of multiple DNA-specific probes onto solid surfaces [11–13]. Such an array system might be useful in genome-wide genetic mapping, physical mapping, proteomics, and gene expression studies. However, the main challenges involved in using solid supports are the lack of commonly used DNA probes in “user-friendly” assays and an immobilization method that is fully compatible with the hybridization process, and low sensitivity and reproducibility [14]. To enable rapid, sensitive, and label-free DNA detection, numerous approaches have been suggested based on optical [15–17], acoustic [18], and electrochemical techniques [19–21].

Electrochemical methods are typically inexpensive and rapid methods that allow distinct analytes to be detected in a highly sensitive and selective manner [22–25]. Although electrochemical DNA sensors exploit a range of distinct chemistries, they all take advantage of the nanoscale interactions among the target present in solution, the recognition layer, and the solid electrode surface. This has led to the development of simple signal transducers for the electrochemical detection of DNA hybridization by using an inexpensive analyzer. DNA hybridization can be detected electrochemically by using various
strategies that exploit the electrochemistry of the redox reaction of reporters [26] and enzymes immobilized onto an electrode surface [27], direct or catalytic oxidation of DNA bases [28–31], electrochemistry of nanoparticles [32–35], conducting polymers (CPs) [35–37], and quantum dots [38].

CPs are organic conjugated polymers that feature an extended π-orbital system through which electrons can move from one end of the polymer to the other. In 2000, H. Shirakawa, A. MacDiarmid, and A. Heeger were awarded the Nobel Prize in chemistry for their revolutionary research on the conductive behavior of polymers and provocative research based on CPs. Unlike saturated polymers, CPs exhibit several distinctive properties such as excellent electrical conductivity, low ionization potentials, and high electron affinity. The electrical conductivity of CPs is responsible for the excitation of polarons, bipolarons, and solitons during the doping processes. The ground state p-bonds ($p - p^*$) of CPs are partially localized as a result of Peierls distortion [39]. However, depending on the doping concentration, the formation of polarons, bipolarons, and solitons creates distinct band gaps between the self-localized excitations and the localized electronic states. CPs also exhibit very high flexibility, which can be modulated together with their electrical properties by using appropriate chemical modeling and synthesis [40–42]. These distinctive properties of CPs have broadened their application in various technological fields, such as in the design of light-emitting diodes [43], anti-static coating [44], electrochromic devices [45], solar cells [46], anti-corrosion coatings [47], chemical sensors and biosensors [48], and drug-release systems [49,50]. To date, diverse CPs have been developed and used in sensing applications, such as poly(acetylene), polypyrrole (PPy), polythiophene (PTh), poly(terthiophene), polyaniline (PANI), poly(fluorine), poly(3-alkylthiophene), poly(tetrathiafulvalene), poly(naphthalene), poly(p-phenylene sulfide), poly(para-phenylene vinylene), and poly(thionine) (PTH); these CPs are reviewed elsewhere [51]. Previous studies have also examined the growth and stabilities of PANI [52,53], PPy [54,55], poly(azulene) [56], and PTh [57,58]. Apart from these commonly used CPs, various reversibly doped and undoped CPs, which exhibit considerable changes in conductivity, have been studied using electrochemical methods [59]. The electrical conductivity of CPs depends substantially on the pH and the applied potentials, which can vary over several orders of magnitude [60]. Moreover, the grafting of organometallic compounds can aid in the tuning of the physical properties of CPs [61–64]. The electronic structure, chain conformation, and orientation of CPs can cause extremely sensitive changes in the polymeric chain environment of CPs. For example, a change in the delocalized electronic structure of CPs during DNA hybridization alters their optical and electrical properties [65]. These advantages offered by CPs make them suitable for developing sensitive electrochemical DNA hybridization sensors. In this article, we comprehensively review recent works on various CPs, as well as their application and implementation for electrochemical DNA sensing. Furthermore, we discuss the most commonly used methods of immobilizing DNA probes for developing DNA biosensors along with the transduction mechanism employed.

2. Immobilization Techniques Used for Developing DNA Biosensors

To enhance the sensitivity of DNA biosensors, the DNA probe used must be sufficiently immobilized [66]. Nonspecific adsorption and stabilization of the immobilized DNA probe are crucial for achieving high sensitivity and specificity. Moreover, minimizing nonspecific adsorption is essential for
ensuring the high reactivity, accessibility, orientation, and stability of surface-confined DNA probes [66]. DNA probes immobilized on sensor surfaces can be used in a manner similar to enzyme-based biosensors; these probes are immobilized by means of adsorption, covalent immobilization, or avidin (or streptavidin)-biotin interaction [67,68]. Figure 1 shows the unique design of a CP-based DNA sensor. In this sensor, single-stranded DNA (ss-DNA) probes are immobilized on or within a CP layer, and target DNA base-pairing to the probe generates a recognition signal that can be recorded using an electrode (e.g., an electrode made of gold, platinum, or glassy carbon). Identification occurs at the CP/electrolyte edge, and the generated recognition signal reaches the transducer through the CP layer. In this section, we discuss the various methods used for immobilizing a DNA probe in order to develop a DNA hybridization sensor.

![Figure 1. Schematic representation of a general electrochemical DNA sensor based on conducting polymers.](image)

2.1. Adsorption

Adsorption is the simplest immobilization method in which a DNA probe can be immobilized without any modification of the probe [69]. Hirayama et al. developed an enhanced and simplified dry-adsorption protocol for DNA probe immobilization that increased hybridization sensitivity [70]. The efficiencies of DNA adsorption and retention were increased 1.4–6.5 and 4.2–19.6 times, respectively, compared with the efficiencies achieved using conventional methods such as incubation and decantation. Moreover, the use of this simple protocol reduces the consumption of DNA and increases the hybridization efficiency substantially. Another method involves the use of the amino group of the natural cationic chitosan polymer that can readily form a strong complex with the negatively charged phosphate backbone of DNA [71]. Xu et al. successfully immobilized a DNA probe labeled
with aminoferrocene (AFC) on a chitosan-modified glassy carbon electrode (GCE) by means of adsorption [72]. The AFC-labeled DNA probe formed a duplex only with the complementary target DNA, and the detection limit was 2.0 nM. DNA that is either physically or chemically adsorbed onto a solid electrode surface can also be used for studying the electrochemical behavior of DNA and its interaction with other molecules [73–76]. For example, Azek et al. developed a disposable DNA sensor by physically adsorbing amplified human cytomegalovirus DNA onto a screen-printed electrode (SPE) [77]. The extent of hybridization of the target DNA was determined using horseradish peroxidase-conjugated streptavidin, and the detection limit was measured to be $6 \times 10^{-7}$ nM.

DNA probes and DNA composites can also be immobilized on electrodes by applying an electric potential [78,79]. Wang et al. electrochemically adsorbed a DNA probe on an electrochemically pretreated carbon-paste electrode (CPE) at an applied potential of $-0.5$ V (vs. Ag/AgCl) [80]. This DNA sensor requires only nanogram quantities of DNA owing to the low background response of the potentiometric-stripping mode. Recently, Wu et al. electrodeposited silver nanoparticle-DNA composites at a controlled dimension on a GCE by reducing silver in the presence of DNA [81]. The inclusion of DNA with the silver nanoparticles prevents nanoparticle aggregation and enhances the catalytic activity. Lahiji et al. electrochemically deposited a uniform DNA-carbon nanotube (CNT) composite on an Au substrate by maintaining a $+0.5$ V potential [82]. This immobilization technique does not require prior DNA or substrate functionalization, and this is combined with a new method of generating a modified DNA electrode that offers the advantage of the high electron-transporting capacity of CNTs for sensing.

2.2. Covalent Immobilization

DNA probes have frequently been immobilized through covalent binding to various solids [83–88]. Ligaj et al. developed a stearic acid-modified conventional CPE in order to covalently immobilize DNA probes through an ethylenediamine linker [86]. This process does not alter the structural flexibility of the DNA probe, and it enhances DNA hybridization. Raymond et al. developed a simple and specific method that does not require the labeling of the target before hybridization [87]. The amino-linker of the probe DNA allows it to be covalently attached to a functionalized glass surface. Functionalized CP films, such as poly(3-pyrrololacrylic acid) (Py-co-PAA), poly(5-(3-pyrrolyl) 2,4-pentadienoic acid) (Py-co-PPDA), and poly(3-pyrrolylpentanoic acid) (PPA), were previously used for covalently immobilizing a DNA probe [88]. Based on the principle of chemisorption, thiol-metal interactions have also been frequently employed to covalently bind thiol-functionalized DNA probes onto gold surfaces [89–92].

The distinctive electrical, thermal, chemical, mechanical, and 3-D spatial characteristics of CNTs suggest that it is possible to construct DNA biosensors with high sensitivity, selectivity, and multiplexing by exploiting Watson–Crick base-pairing [93]. Rodríguez et al. covalently attached single-walled CNTs (SWNTs) to a gold surface modified with 11-amino-1-undecanethiol (AUT) and subsequently immobilized a DNA probe on the Au/AUT/SWNTs through covalent linkage [94]. The interaction and covalent immobilization of DNA probes on functionalized CNTs and CNT/CP-composite-modified electrodes have been widely studied [95,96]. Yang et al. developed a sensitive electrochemical DNA sensor based on the synergistic effects of a PANI nanofiber and a
multiwall CNT (MWNT) composite on a chitosan film [97]. The covalent immobilization of the DNA probe on the PANI-MWNT-composite film enhanced DNA hybridization, which was highly reproducible and stable.

2.3. Avidin/Streptavidin-Biotin Interaction

Avidin and streptavidin are large tetrameric proteins containing four identical biotin-binding sites that can be used for forming tetravalent avidin/streptavidin-biotin bonds in order to develop DNA-coated electrodes; in these electrodes, surface-confined avidin/streptavidin reacts with biotinylated DNA [98]. Because of the innate aqueous immobility of the avidin-biotin complex, this system is easy to use. Pan et al. generated a mixture of self-assembled monolayers (SAMs) by using 2-mercaptoethanol (ME) and 11-mercaptoundecanoic acid (MUA) on an Au electrode and attached a DNA probe to the activated MUA through streptavidin-biotin chemistry, as shown in Figure 2 [99]. Caruso et al. employed the quartz crystal microbalance (QCM) technique in order to immobilize biotin-DNA on an avidin-modified QCM electrode [100]. Several strategies have been developed for attaching biotin to modified CP electrodes, including the biotin-sandwich technique for immobilizing DNA [101–106]. Guillerez et al. designed a DNA sensor by using an electropolymerized biotinylated-PPy film [101]. Biotinylated DNA probes were immobilized onto PPy-biotin films through an intercalated layer of avidin (PPy-biotin/avidin/DNA probe). Furthermore, to detect DNA hybridization, fluorescently labeled or non-labeled avidin and biotinylated DNA probes have also been immobilized on a biotin derivative (photobiotin)-modified poly(dimethylsiloxane) (PDMS) chip by using biotin/avidin/biotin chemistry [106].

Figure 2. Schematic illustration of DNA probe immobilization through avidin-biotin chemistry and the hybridization of target DNA on a self-assembled monolayer (SAM)-modified Au electrode. (Reproduced with permission from [99]. Copyright 2014, American Chemical Society.)
3. Transduction Methods

Sensing and/or transduction must occur in order to convert a recognition event into a readable signal. Depending on the type of measurement, transduction can occur by electrochemical, optical, mass-based, or thermal means [107–109]. Among these, electrochemical transduction has been shown to be appropriate for DNA sensing, wherein a biorecognition event directly gives rise to an electrical signal, and this allows the sensing system to be miniaturized [110]. Recently, various convenient systems for analysis and on-site monitoring have been developed using distinct solid contact materials such as gold, ferrocene-labeled PNAs (peptide nucleic acids), CNTs, and CPs [111–113]. CPs have been widely used because they can create a high redox capacitance that makes the recorded signal highly stable [114–116]. The transduction mechanism of poly(3,4-ethylenedioxythiophene) (PEDOT) was comprehensively studied using various electrochemical techniques, including electrochemical impedance spectroscopy (EIS) [117,118]. Although CPs clearly offer advantages over other transducing materials, a few drawbacks are also associated with CPs depending on their type. For example, PPy exhibits slight chemical instability in the presence of certain components of ambient media, such as oxygen, acids, bases, redox reactants, nonreactive ions, and surfactants [119,120]. The possible formation of a water layer at the boundary between CPs and polymeric ion-selective membranes is also a hurdle that must be addressed [121]. In the case of CP-based electrochemical DNA sensors, the polymers not only serve as an immobilization template, but also actively participate in signal transduction. When using electrochemical methodologies, reversible doping and dedoping of CPs markedly alter the electrochemical responses. Doping and dedoping modulate the interaction of the probe-target complex and can regulate the sensitivity and stability of DNA detection [122,123]. The change in the signal after probe-DNA immobilization and target-DNA hybridization can also be quantified using diverse approaches, such as by measuring the change in current as a function of the applied potential (voltammetry), the change in current at a fixed applied potential (amperometry), or the change in conductivity (conductometry), impedance (impedimetry), or potential (potentiometry). Among these transduction methods, voltammetric methods (e.g., cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV)) are most widely used for the detection of DNA hybridization. Voltammetric measurement requires the use of two- or three-electrode electrochemical cell systems together with a potentiostat, which allows the application of the potential and the measurement of the resultant current. Voltammetry such as CV, DPV, and SWV depend on the pattern of the applied potential, which also potently controls the sensitivity of the current response.

4. Development of DNA Sensors Based on Distinct CPs

4.1. DNA Sensors Based on Polypyrrole and Its Derivatives

PPy is formed from a number of connected pyrrole ring systems and is highly biocompatible. PPy synthesized at neutral pH is extensively used as a versatile immobilization matrix in the design of biosensors, such as catalytic biosensors, immunosensors, and DNA sensors and in molecular-imprinting technologies [124]. When deposited on the electrode surface, PPy provides an effective DNA-sensing platform, in which PPy itself acts as an interface for the attachment of the DNA probe. Notably, PPy-modified electrodes have also facilitated the development of indicator- and label-free detection of
Livache et al. developed a novel method for electro-synthesizing a PPy-DNA composite through co-polymerization [126]. To synthesize a PPy film harboring covalently linked DNA, a mixture of pyrrole and a pyrrole bearing a specific DNA probe was electrooxidized. This was the first study to detect DNA hybridization on the surface of modified macroelectrodes. Soon after this study, the same group also generated a DNA chip constructed of three components: silicon chips bearing a matrix of 50-μm or 4-μm microelectrodes for genotyping hepatitis C virus (HCV), a QCM, and a non-patterned gold/glass slide featuring 500-μm spots [127]. Wang et al. described the incorporation of DNA dopants into a PPy network by using an electrochemical QCM (EQCM) and showed that it exhibited a strong affinity for target DNA [128,129]. Youssoufi et al. developed a new type of electrochemical DNA hybridization sensor based on DNA-functionalized PPy [130]. The prepared PPy precursor contained a loosely bound ester group that was directly substituted with an amino-labeled DNA probe of various sequence lengths. The electrochemical response of this sensor was analyzed in aqueous media containing distinct target DNA sequences. The voltammetric signals obtained for DNA-PPy remained unchanged in the presence of a noncomplementary target DNA sequence; however, the signal changed considerably when a complementary target DNA was added. This was quantified using amperometry, and the detection limit of the biosensor was determined to be approximately $1 \times 10^{-2}$ nmol in the absence of any signal processing. Other researchers also developed a similar type of DNA hybridization sensor by functionalizing PPy; these researchers introduced PPy nanotubes in which PPy was functionalized with, for example, poly[pyrrole-co-4-(3-pyrrolyl) butanoic acid] or carboxylic acid [131–133]. The acid-functionalized PPy is a favorable alternative for fabricating label-free sensors because it enables versatile immobilization of DNA, proteins, and enzymes by using various pendant groups: –SH, –NH$_2$, and –COOH. Peng et al. prepared a poly[pyrrole-co-4-(3-pyrrolyl) butanoic acid]-modified platinum electrode for DNA hybridization, which exhibited high electroactivity in aqueous medium [131]. An NH$_2$-substituted DNA probe was covalently grafted onto the surfaces of this polymer in a one-step procedure. Komarova et al. developed a prototype amperometric sensor for the detection of a biowarfare pathogen, the virus *Variola major*, based on DNA-doped ultrathin PPy films [134]; the investigators determined that thinner films harboring smaller or more highly concentrated dopant ions produced stronger amperometric signals than did thicker films bearing larger or less concentrated dopant ions. After the film surface was blocked with fragmented calf-thymus DNA, the nonspecific signal disappeared completely when ultrathin (Langmuir–Blodgett) films were tested; however, the specific signal from the complementary DNA remained unaffected. Under optimal conditions, the detection limit for the target DNA was $16 \times 10^{-3}$ nM. Ease of use and rapid detection are the primary advantages of this sensor; however, steric hindrance and poor accessibility of the probe to the analyte in the film can reduce hybridization efficiency and substantially limit sensitivity and selectivity [68].

Several groups have developed DNA sensors based on PPy and PPy derivatives by using a modified fluorine-doped tin oxide (FTO) electrode [135–137]. Eguiluz et al. developed a PPy/FTO electrode and used Ag/Au-nanoparticle labels to detect *Alicyclobacillus acidoterrestris* in pure cultures by means of reverse-transcription polymerase chain reaction (RT-PCR) [136]. The sensor sensitivity could also be enhanced by performing asymmetric nested RT-PCR of the amplicon and using Ag/Au-based electrochemical detection, which was able to detect 2 colony-forming units/mL of spores. In this electrochemical bioassay, the detection and quantification limits for the target *A. acidoterrestris*
were 7.07 and 23.6 nM, respectively. Riccardi et al. developed a new type of label-free PPy-based DNA sensor for identifying HCV [137]; in this approach, HCV is detected through the electrostatic modulation of the ion-exchange kinetics of PPy films. Here, the PPy layer was electropolymerized in order to immobilize a synthetic, single-stranded, 18-mer HCV genotype-1-specific probe DNA on a 2,5-bis (2-thienyl)-N-(3-phosphoryl-n-alkyl)pyrrole film. HCV DNA sequences (244-mer) obtained through RT-PCR amplification of the original viral RNA were examined based on the disruption of the ion-exchange properties of the PPy film. However, with this sensor, the selectivity, sensitivity, and reproducibility of DNA detection were poor.

In order to overcome the poor selectivity, sensitivity, and detection limit of DNA sensors, researchers have introduced PPy composites, such as PPy-CNTs, PPy-nanoparticles, PPy-nanoengineered materials, and pyrrole-derivative bilayers [138–140]. Xu et al. developed an impedimetric DNA biosensor based on a GCE modified with a PPy-MWNT composite [138]. COOH-MWNTs and PPy were electrodeposited on the GCE to facilitate the immobilization of the NH$_2$-DNA probe. The hybridization reaction of this DNA/PPy/MWNT-COOH/GCE results in a decrease in impedance, which is attributed to the electron-transfer resistance through double-stranded DNA (ds-DNA) being lower than that through ss-DNA. The PPy/MWNT-COOH-modified electrode exhibited high electron-transport capacity and also featured an increased specific surface area. Consequently, the sensitivity and selectivity of DNA hybridization were increased and the detection limit was 5.0 × 10$^{-3}$ nM. Table 1 summarizes the characteristics of some of the reported electrochemical DNA sensors based on PPy and PPy derivatives, together with their immobilization methods, detection method, detection limit, and sensitivity.
| Matrix | Immobilization Method | Detection Method | Detection Limit | Sensitivity | Ref. |
|--------|-----------------------|------------------|----------------|-------------|------|
| Copolymerizations of 5’ pyrrole-labeled DNA and pyrrole | DNA entrapment | Fluorescence microscopy | - | $>10^{-11}$ M | [127] |
| Copolymerizations of DNA probe within PPy | DNA entrapment | CV/amperometry | $>6$ µg | 1.08 nA/µg | [129] |
| Poly[3-acetic acid pyrrole, 3-N hydroxyphthalimide pyrrole]] | Covalent | CV | $1 \times 10^{-2}$ nmol | - | [130] |
| Poly[pyrrole-co-4-(3-pyrrolyl) butanoic acid] | Covalent | CV/EIS | - | 10.5, 3.0 and 1.7 µA/cm²/nM of complementary DNA for 23, 57, and 114 nm film thicknesses, respectively. | [131] |
| Carboxylic acid-functionalized PPy nanotubes (CPPy NTs) | Covalent | Photoluminescence | - | High sensitivity ($\Delta R/R_0 = 1.7$) even at low concentration (1 nmol) of target DNA | [132] |
| Poly [3-acetic acid pyrrole, 3-N-hydroxyphthalimide pyrrole] | Covalent | EIS | $1 \times 10^{-3}$ nmol | 21.6 Ω cm$^{-2}$/µM | [133] |
| PPy doped with an DNA | DNA entrapment | Chronoamperometry | $16 \times 10^{-3}$ nM | - | [134] |
| Poly(Py-co-PAA) | Covalent | QCM/EIS | 0.98 nM | - | [135] |
| PPy-DNA | DNA entrapment | CV/Linear Sweep Voltammetry (LSV) | - | - | [136] |
| 2,5-bis(2-thienyl)-N-(3-phosphorylpropyl)pyrrole | DNA entrapment, Mg$^{2+}$ ion serve as a linker | CV/LSV | $1.82 \times 10^{-12}$ nM | - | [137] |
| PPy/MWNTs | Carbodiimide cross linking between amine and carboxyl group | CV/EIS | $5.0 \times 10^{-3}$ nM | - | [138] |
| PPy–polyaniline–Au | HS-DNA bind on Au via Au-thiol chemistry | EIS | $1.0 \times 10^{-4}$ nM | - | [141] |
| PPy–poly(3,4-ethylenedioxythiophene)–Ag | HS-DNA bind on Ag via Ag-thiol chemistry | EIS | $5.4 \times 10^{-4}$ nM | - | [142] |
| Copolymer of PPy and 3-pyrrolacrylic acid (PAA) | Covalent | EIS | - | - | [143] |
4.2. DNA Sensors Based on Polythiophene and Its Derivatives

PTh and functionalized PTh demonstrate a variety of remarkable solid-state properties and hold tremendous potential for use in molecular electronic devices, solid-state batteries, and sensors [144,145]. Carboxylic acid- and ester group-functionalized PTh polymers (e.g., 3'-carboxyl-5,2',5',2''-terthiophene, poly(thiophen-3-yl-acetic acid 1,3-dioxo-1,3-dihydro-isoindol-2-yl ester) (PTAE), and 3-((2':2'',5''':2''''-terthiophene)-3'''-yl) acrylic acid (TAA)) have been widely used for developing electrochemical DNA sensors [146–149]. Lee et al. used a poly(3'-carboxyl-5,2',5',2''-terthiophene)-modified GCE and reported that only a short hybridization time (1 h) was required [146]. The amine group linked to the 5' end of the DNA probe (a 19-mer) was covalently attached with the carboxyl (-COOH) group-terminated polymer, which corroborated the hybridization of the target DNA (Figure 3I). The hybridization of fully complementary target DNA induced a significant decrease of the impedance values (Figure 3II). The difference in impedance values before and after hybridization of target DNA can be ascribed by the change in conductivity and capacitive current. This method is more advantageous than other methods because of its selectivity, short response time, and minimal use of intercalators and fluorescent tags. Cha et al. described a synthetic route for thiophen-3-yl-acetic acid 1,3-dioxo-1,3-dihydro-isoindol-2-yl ester (TAE), which can be readily electropolymerized on a Pt chip electrode and allow for the direct substitution of its exiting group with a prosthetic group that contains a terminal amino group on the DNA probe [149]. The sensitivity of this sensor was 0.62 μA/nmol and the detection limit was 1 nmol.

Figure 3. Schematic representation of the immobilization of probe DNA and the hybridization of a target sequence (I), and plots of (A) impedance and (B) admittance before and after hybridization in a phosphate buffer solution (II). (Redrawn and reproduced with permission from [146]. Copyright 2014, American Chemical Society.)

Peng et al. fabricated a poly(TAA)-modified DNA sensor that exhibited a qualitatively unique response when compared to functionalized PPy sensors [148,149]. The applicability of these two polymers as active substrates for DNA sensors was confirmed by covalently attaching NH₂-DNA probes to the –COOH group of both polymers. Here, the hybridization of complementary DNA can be detected by an increase in the admittance without the requirement for an indicator or any sample modification.
Experimental results suggested that PPy functionalized with long unsaturated carbon side chains exhibited more favorable DNA-sensing properties and a larger difference in the impedance signal. This difference was due to the disparities in the movement of the dominant ions (CF₃SO₃⁻ and ClO₄⁻) at the interface of the polymer film and the electrolyte, which was confirmed using an EQCM.

Considerable emphasis has been placed on producing portable and inexpensive devices for DNA detection because of their importance in forensics, medical diagnostics, and evolutionary studies [150–153]. Shiddiky et al. developed an ultrasensitive technique for detecting DNA and proteins based on poly-5,2':5',2''-terthiophene-3'-carboxylic acid (pTTCA) (Figure 4) [154]. Dendrimer (DEN) and hydrazine were covalently linked to the pTTCA film and the signal was amplified by the pTTCA/DEN assembly loaded with Au nanoparticles (AuNPs). The target DNA- or protein-linked hydrazine labels (avidin-hydrazine) adsorbed onto the pTTCA/DEN film, and DPV measurements revealed a linear dynamic range for the electrocatalytic detection of DNA and protein. The simplicity, low detection limit, and reproducibility (RSD < 4.3% for n = 10) of the sensor make this a promising tool that can be developed in the future for practical applications.

Figure 4. Schematic illustration of the poly-5,2':5',2''-terthiophene-3'-carboxylic acid (pTTCA)/dendrimer (DEN)/Au nanoparticles (AuNP)/biomolecule-linked avidin-hydrazine assembly developed for (A) DNA and (B) protein sensors, based on the electrocatalytic activity of hydrazine. (Reproduced with permission from [154]. Copyright 2014, American Chemical Society.)

Functionalized PTh was also used for developing a DNA sensor. Fang et al. developed a novel methodology for detecting DNA by using ferrocene-functionalized PTh deposited on a nanogold-modified electrode [155]. Nanogold-modified electrodes substantially increase the quantity of immobilized PNA probes and thus cause an increase in the electrical signal. Positively charged ferrocene-functionalized PTh does not bind electrostatically with the PNA probes because of the absence...
of anionic phosphate groups. This limitation can be resolved by performing an initial DNA–PNA hybridization. Adsorption of cationic PTh onto the DNA backbone results in the generation of a detectable hybridization-recognition signal in DPV. Thus, PNA could be used as a highly sensitive, selective, and reversible coupling substrate for DNA immobilization. Table 2 summarizes the characteristics of electrochemical DNA sensors based on PTh and PTh derivatives, together with their immobilization techniques, detection method, detection limit, and sensitivity.

### Table 2. Polythiophene (PTh)- and PTh-derivative-based DNA sensors and their performance in the detection of DNA hybridization.

| Matrix | Immobilization Method | Detection Method | Detection Limit | Sensitivity | Ref. |
|--------|-----------------------|------------------|-----------------|-------------|-----|
| PTh    | Covalent              | Fluorescence     | -               | -           | [144]|
| Poly(3'-carboxyl-5,2',5',2''-terthiophene) | Covalent | EIS             | -              | 5.608 (ng/cm²)/Hz | [146] |
| Poly(thiophen-3-yl-acetic acid 1,3-dioxo-1,3-dihydro-isoindol-2-yl ester) | Covalent | CV              | 1 nmol         | 0.62 μA/nmol | [149] |
| Poly (3-[(S)-5-amino-5-carboxyl-3-oxapentyl]-2,5-thiophenylene hydrochloride) | Hydrogen bond | Fluorometric | 1 × 10⁻² nmol | - | [151] |
| Cationic PTh | Covalent | Fluorometric | 3.6 × 10⁻¹² nM | - | [152] |
| Poly(5,2':5,2''-terthiophene-3'-carboxylic Acid) | Covalent | Electrophoresis | 1.14 × 10⁻⁴ nM | 0.20 nA (fg/μL)⁻¹ | [153] |
| PTh functionalized- methylene blue | HS-DNA bind on Au via Au-thiol chemistry | DPV | - | - | [156] |

### 4.3. DNA Sensors Based on Polyaniline and Its Derivatives

The environmental stability of PANI and its easy synthesis (either chemically or electrochemically) have broadened the application of PANI as a chemical sensor [157,158] and biosensor [159–161]. PANI is the best-recognized semi-flexible rod-like CP with chemically and structurally flexible –NH₂ linkage on its surrounding molecules, thus making it suitable for binding biomolecules [159]. PANI undergoes two redox reactions and can be functionalized, and this makes it a favorable material for DNA sensing. The electrical conductivity of PANI has been established to strongly depend on pH, and most previous studies on PANI were performed at a pH below 4.0. However, using a neutral pH solution is critical for developing biosensors because most biocatalytic and immunological reactions occur at neutral pH [162]. Therefore, a challenge is to incorporate biological molecules in the conventional pH-dependent PANI. Research has shown that N-substituted aniline does not exhibit pH sensitivity because an alkyl chain is covalently bound to the nitrogen atom in order to prevent the formation of an emeraldine base (the deprotonated form). Moreover, self-doped PANI, commonly referred to as sulfonated PANI, exhibits redox activity even at neutral pH [163].

Wu *et al.* synthesized PANI-intercalated graphite-oxide nanocomposites (PANI/GO) enveloped in CPE [164]. This PANI/GO-modified CPE displayed electrochemical activity and two sharp peaks at 668 and 207 mV in SWV measurements. These results indicated that ss- and ds-DNA transformed the redox characteristics of PANI/GO, and this could be used to monitor probe immobilization and the hybridization of complementary DNA, with the hybridization peak occurring at ~270 mV. DNA detection performed using the PANI/GO-modified CPE was highly stability and reproducible. Gu *et al.* developed an impedimetric DNA hybridization sensor based on a PANI/polyacrylate (PANI/PAA)-modified
boron-doped diamond (BDD) electrode [165]. An ultrathin film of the PANI-PAA copolymer was electropolymerized onto BDD surfaces in order to enhance the availability of the –COOH for binding the DNA probe. The hybridization event was sensed based on the direct oxidation of guanine and adenine in the DNA double helix.

Immobilization of probe DNA on a polymer matrix revealed the limitations in the selectivity and specificity of hybridization [166]. In this context, PNA is recognized to provide enhanced stability and specificity in the detection of targets containing a single mismatch [167]. Gao et al. developed a novel signal-amplification method for ultrasensitive detection of DNA, which involved enzymatically catalyzed PANI formation and template-guided deposition for enhancing DNA hybridization [168]. The hybridization was quantified by examining the electroactivity of the deposited PANI by using SWV. This DNA sensor was extremely sensitive—it had a femtomolar detection limit—and it was highly selective for sequences mismatched by one, two, and three bases. The biosensor was used for detecting His4, RCA1, and GAPDH, and the results obtained were similar to those obtained from northern-blotting analysis of the same samples.

An ultrasensitive technique for detecting DNA hybridization has also been developed by using PANI nanowires or nanotubes or methylene blue (MB) as an indicator [169,170]. MB was used to distinguish between ss- and ds-DNA by using various electrochemical techniques [171,172]. MB specifically binds to unpaired bases of DNA/PNA. The redox reaction of MB was used for monitoring the native and denatured states of DNA [173]. Prabhakar et al. covalently immobilized 20-base-long NH2-DNA and PNA probes on a PANI/Au electrode in order to detect DNA hybridization and used MB as an indicator (Figure 5) [174]. PNA-PANI/Au and DNA-PANI/Au electrodes were used for detecting the presence of complementary target *Mycobacterium tuberculosis* by using SWV, and the response time was short (30 s). The study revealed that the PNA electrode exhibits a higher affinity for complementary DNA sequence and an improved detection limit and higher specificity than do electrodes used in other methods. Table 3 lists the characteristics of electrochemical DNA sensors based on PANI and PANI derivatives, together with their immobilization techniques, detection method, detection limit, and sensitivity.

**Table 3.** Polyaniline (PANI)- and PANI-derivative-based DNA sensors and their performance in the detection of DNA hybridization.

| Matrix                          | Immobilization Method | Detection Method | Detection Limit   | Sensitivity   | Ref.     |
|--------------------------------|-----------------------|------------------|-------------------|--------------|---------|
| PANI-intercalated graphite oxide nanocomposite | Covalent | SWV | - | 0.77 µA/µg/mL | [164] |
| PANI/PAA-modified boron-doped diamond (BDD) | Covalent | EIS | 20 nM | - | [165] |
| Avidin modified-PANI | Avidin interaction | DPV | $5 \times 10^{-10}$ n mole | - | [166] |
| Polyaniline nanowire | Covalent immobilization | DPV | $1 \times 10^{-3}$ nM | - | [169] |
| PANI nanotube | Covalent | CV/DPV | $1 \times 10^{-6}$ nM | 1 pM | [170] |
| Graphene/PANI | Non-covalent binding | DPV | $3.2 \times 10^{-5}$ nM | - | [175] |
| PANI-Au | HS-DNA bind on Au via Au-thiol chemistry | DPV | 0.1 nM | - | [176] |
4.4. DNA Sensors Based on Quinone and Its Derivatives

Quinone is an electronically conductive redox polymer that has attracted substantial interest as a chemical sensor [177] and biosensor [178,179] because of its capacity to transport charges inside films and the nature of the ionic flux at the interface of the polymer and the solution (anionic and cationic, respectively). The characteristics of quinone-based polymers differ from those of conventional p-doped electronically conducting polymers (ECPs). The sensitivity of ECP-based biosensors depends on the amplification of the interaction between the electrochemical transducer and biomolecules [180].

Piro et al. constructed a new electroactive film, poly(JUG-co-JUGA), by co-electrooxidizing 5-hydroxy-1,4-naphthoquinone and 5-hydroxy-3-thioacetic acid-1,4-naphthoquinone [180]. This poly(JUG-co-JUGA) copolymer presents both electroactive and chemically reactive groups for sensing DNA and L-lactate [181]. An NH2-DNA probe was covalently immobilized on poly(JUG-co-JUGA) and the electroactivity of the quinone group was used for detecting hybridization. The main feature of this DNA hybridization sensor is the transformation between ss- and ds-DNA at the solution/polymer interface. Therefore, the rate of charge (ion) diffusion from the solution to the polymer/solution interface is primarily affected by hybridization. After hybridization, the current increases in SWV measurements because of electrostatic and/or steric effects. Piro and coworkers also examined PNA probe-based DNA hybridization sensors (Figure 6) [182]. The PNA probe was covalently attached to poly(JUG-co-JUGA), and upon hybridization with the target complementary DNA, the flexibility of the PNA probe was altered, which caused changes in the electrochemical signal at the polymer/solution interface. This simple and reagent-free DNA hybridization sensor can discriminate single mismatches and can be regenerated after a simple dehybridization step. The main drawback of this sensor is its
detection limit, 10 nM, which does not constitute the theoretical limit. In order to improve the detection limit, Acevedo et al. fabricated a sequential multilayer CNT that featured an increased area for the oxidation of soluble redox couples [183]. The electropolymerization of quinine and quinone derivatives onto the MWNT-modified electrode produced an interpenetrated CP/CNT network that is electroactive in both aqueous and nonaqueous media. The effective current response was enhanced up to 19 times, which increased the sensitivity and lowered the detection limit.

Figure 6. (1) Immobilization of the peptide nucleic acid (PNA) probe; and (2) hybridization of the target DNA onto poly(JUG-co-JUGA)-modified glassy carbon electrode (GCE) together with the corresponding square wave voltammetry (SWV) signals for (1) PNA probe immobilization, and (2) target DNA hybridization. (Reproduced with permission from [182]. Copyright 2014, Elsevier.)

4.5. DNA Sensors Based on Miscellaneous Conducting Polymers and Their Derivatives

Poly(triamine) (PTyr) contains one primary aliphatic amine per triamine moiety, which represents an extremely high concentration of surface reactive sites for biomolecule immobilization [184]. Tran et al. first prepared a PTyr film by electrooxidizing 4-hydroxiphenylethylamine in perchloric acid, which left one reactive amine group per moiety [185]. DNA was immobilized on the polymer film through a phosphoramidate covalent bond [186], and this yielded a high surface concentration of DNA (~500 pmol/cm²). DNA can also readily bind to PTyr through nonspecific adsorption, and thus differentiating between adsorbed and covalently bonded DNA could be challenging. However, DNA probes that were weakly adsorbed were removed by washing with SL salmon-testis DNA. The remaining covalently bonded DNA probes were used for the hybridization of GEM DNA, a complementary DNA sequence derived from HIV gag protein. For detecting DNA hybridization, Li et al. prepared a new type of conjugated CP, poly(indole-5-carboxylic acid) (PICA), on GCE by means of anodic oxidation (Figure 7) [187]. PICA exhibited optimal electrochemical behavior and thermal stability, with a conductivity of $10^{-2} \text{ S/cm}$ and high redox activity compatible with the concept of molecular-wire transduction. The PICA-modified sensor showed comparable sensitivity and its detection limit was 1.0 nM, which can be further improved by increasing the side chain length because longer side chains permit greater freedom of movement and more enhanced hybridization [35].
Certain other CPs have been synthesized and employed in DNA sensing applications in which fluorescence techniques are used. These CPs include poly({2,5-bis[3-(N,N-diethylamino)-1-oxapropyl]-para-phenylenevinylene}-alt-para-phenylenevinylene)dibromide [188], poly(9,9-bis(6'-N,N,N-trimethylammonium)-hexyl)-fluorene phenylene) [189], and poly(fluorene-co-phenylene) [190]. The potential application of these CPs for the development of an electrochemical DNA sensor is in high demand.

**Figure 7.** Schematic representation of the preparation of an electrochemical DNA sensor based on a poly(indole-5-carboxylic acid) (PICA) conducting polymer. (Redrawn from [187]. Copyright 2014, Elsevier.)

### 5. Conclusions and Outlook

This review has summarized the diverse strategies used for developing DNA biosensors by using electroactive CPs. CPs have been extensively used for developing electrochemical label-free methods of DNA detection. This is because CPs exhibit highly favorable electrical conductivity or charge-transport properties. Over the last decade, researchers have developed numerous types of CPs and their derivatives for highly sensitive and selective electrochemical detection of DNA. Adsorption, covalent immobilization, and avidin-biotin interactions have been used for developing DNA biosensors by modifying the electrode surface with CPs and their derivatives. To develop CP-based DNA sensors, regeneration of a surface-immobilized probe and the reuse of DNA biosensors must be addressed. The key factors that must be considered for probe immobilization are the following: the immobilization chemistry must be stable, the probes must retain their functionality after attachment, and immobilized biomolecules must maintain proper orientation and configuration [191]. CP films must be deposited on inert substrates because hydrophobic interactions and the consequential electrochemical oxidation and reduction accompanied by the movement of ions in and out of the CP film can result in the delamination of CP films. With regard to the immobilization of DNA probes, most detection methods that are used for developing DNA biosensors and microarrays are open to criticism [192]. CP-based DNA sensors are expected to be highly sensitive, selective, and reproducible and to enable multi-analyte determination.

Although we have focused on CPs and various CP-CNT composites, other materials could also be used for developing electrochemical DNA hybridization sensors. For example, graphene, a new
allotrope of carbon composed of $sp^2$-hybridized carbon atoms arranged in a honeycomb lattice, is an ideal 2-D material for developing DNA sensors. Graphene can be readily functionalized and doped with various functional groups (e.g., –COOH, –OH) and atoms, which might facilitate the immobilization of DNA probes in a biocompatible manner.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Barhoumi, A.; Halas, N.J. Label-Free detection of DNA hybridization using surface enhanced raman spectroscopy. *J. Am. Chem. Soc.* 2010, 132, 12792–12793.
2. Cai, B.; Wang, S.; Huang, L.; Ning, Y.; Zhang, Z.; Zhang, G.-J. Ultrasensitive label-free detection of PNA-DNA hybridization by reduced graphene oxide field-effect transistor biosensor. *ACS Nano* 2014, 8, 2632–2638.
3. Ahmed, M.U.; Nahar, S.; Safavieh, M.; Zourob, M. Real-time electrochemical detection of pathogen DNA using electrostatic interaction of a redox probe. *Analyst* 2013, 138, 907–915.
4. Maxam, A.M.; Gilbert, W. A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA* 1997, 74, 560–564.
5. Sanger, F.; Nicklen, S.; Coulson, A.R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 1977, 74, 5463–5467.
6. Marciaq, F.; Sauvaigo, S.; Issartelte, J.P.; Mouret, J.F.; Molko, D. Synthesis and enzymatic incorporation of Morpholino thymidine-S-triphosphate in DNA fragments. *Tetrahedron Lett.* 1999, 40, 4673–4676.
7. Reeve, M.A.; Fuller, C.W. A novel thermostable polymerase for DNA sequencing. *Nature* 1995, 376, 796–797.
8. Lee, L.G.; Spurgeon, S.L.; Heiner, C.R.; Benson, S.C.; Rosenblum, B.B.; Menchen, S.M.; Graham, R.I.; Constantinescu, A.; Upadhya, K.G.; Cassel, J.M. New energy transfer dyes for DNA sequencing. *Nucleic Acids. Res.* 1977, 25, 2816–2822.
9. Wallace, R.B.; Shaffer, J.; Murphy, R.F.; Bonner, J.; Hirose, T.; Itakura, K. Hybridization of synthetic oligodeoxyribonucleotides to phi chi 174 DNA: The effect of single base pair mismatch. *Nucleic Acids Res.* 1979, 6, 3543–3557.
10. Wallace, R.B.; Johnson, M.J.; Hirose, T.; Miyake, T.; Kawashima, E.H.; Itakura, K. The use of synthetic oligonucleotides as hybridization probes. II. Hybridization of oligonucleotides of mixed sequence to rabbit beta-globin DNA. *Nucleic Acids Res.* 1981, 9, 879–894.
11. Pease, A.C.; Solas, D.; Sullivan, E.J.; Cronin, M.T.; Holmes, C.P.; Fodor, S.P. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc. Natl. Acad. Sci. USA* 1994, 91, 5022–5026.
12. Fodor, S.P.; Rava, R.P.; Huang, X.C.; Pease, A.C.; Holmes, C.P.; Adams, C.L. Multiplexed biochemical assays with biological chips. *Nature* **1993**, *364*, 555–556.

13. Shalon, D.; Smith, S.J.; Brown, P.O. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res.* **1996**, *6*, 639–645.

14. Adessi, C.; Matton, G.; Ayala, G.; Turcatti, G.; Mermod, J.J.; Mayer, P.; Kawashima, E. Solid Phase DNA amplification: Characterisation of primer attachment and amplification mechanism. *Nucleic Acids Res.* **2000**, *28*, doi:10.1093/nar/28.20.e87.

15. Liu, J.; Tian, S.; Tiefenauer, L.; Nielsen, P.E.; Knoll, W. Simultaneously amplified electrochemical and surface plasmon optical detection of DNA hybridization based on ferrocene-streptavidin conjugates. *Anal. Chem.* **2005**, *77*, 2756–2761.

16. Peng, H.; Zhang, L.; Kjaellman, T.H.M.; Soeller, C.; Travas-Sejdic, J. DNA hybridization detection with blue luminescent quantum dots and dye-labeled single stranded DNA. *J. Am. Chem. Soc.* **2007**, *129*, 3048–3049.

17. Yin, M.-J.; Wu, C.; Shao, L.-Y.; Chan, W.K.E.; Zhang, A.P.; Lu, C.; Tam, H.-Y. Label-free, disposable fiber-optic biosensors for DNA hybridization detection. *Analyst* **2013**, *138*, 1988–1994.

18. Papadakis, G.; Tsortos, A.; Bender, F.; Ferapontova, E.E.; Gizeli, E. Direct detection of DNA conformation in hybridization processes. *Anal. Chem.* **2012**, *84*, 1854–1861.

19. Drummond, T.G.; Hill, M.G.; Barton, J.K. Electrochemical DNA sensor. *Nat. Biotechnol.* **2003**, *21*, 1192–1199.

20. Alfonta, L.; Singh, A.K.; Willner, I. Liposomes labeled with biotin and horseradish peroxidase: A probe for the enhanced amplification of antigen-antibody or oligonucleotide-DNA sensing processes by the precipitation of an insoluble product on electrodes. *Anal. Chem.* **2011**, *73*, 91–102.

21. Ji, H.; Yan, F.; Lei, J.; Ju, H. Ultrasensitive electrochemical detection of nucleic acids by template enhanced hybridization followed with rolling circle amplification. *Anal. Chem.* **2012**, *84*, 7166–7171.

22. Rahman, M.M.; Ahammad, A.J.S.; Jin, J.H.; Ahn, S.J.; Lee, J.J. A comprehensive review of glucose biosensors based on nanostructured metal-oxides. *Sensors* **2010**, *10*, 4855–4886.

23. Kim, Y.J.; Rahman, M.M.; Lee, J.J. Ultrasensitive and label-free detection of annexin A3 based on quartz crystal microbalance. *Sens. Actuators B Chem.* **2013**, *177*, 172–177.

24. Rahman, M.M.; Li, X.B.; Kim, J.C.; Lim, B.O.; Ahammad, A.J.S.; Lee, J.J. A cholesterol biosensor based on a bi-enzyme immobilized on conducting poly(thionine) film. *Sens. Actuators B Chem.* **2014**, *202*, 536–542.

25. Rahman, M.M.; Li, X.B.; Lopa, N.S.; Lee, J.J. Electrodeposition of Gold on Fluorine-doped tin oxide: Characterization and application for catalytic oxidation of nitrite. *Bull. Korean Chem. Soc.* **2014**, *35*, 2072–2076.

26. Bonanni, A.; Chua, C.K.; Zhao, G.; Sofer, Z.; Pumera, M. Inherently electroactive graphene oxide nanoplatelets as labels for single nucleotide polymorphism detection. *ACS Nano* **2012**, *6*, 8546–8551.

27. Walter, A.; Surkus. A.E.; Flechsig, G.U. Hybridization detection of enzyme-labeled DNA at electrically heated electrodes. *Anal. Bioanal. Chem.* **2013**, *405*, 3907–3911.

28. Hsieh, K.; Xiao, Y.; Soh, H.T. Electrochemical DNA detection via exonuclease and target-catalyzed transformation of surface-bound probes. *Langmuir* **2010**, *26*, 10392–10396.
29. Kerman, K.; Morita, Y.; Takamura, Y.; Tamiya, E. Label-free electrochemical detection of DNA hybridization on gold electrode. *Electrochem. Commun.* **2003**, *5*, 887–891.
30. Kang, B.; Yeo, U.; Yoo, K.H. Anodized aluminum oxide-based capacitance sensors for the direct detection of DNA hybridization. *Biosens. Bioelectron.* **2010**, *25*, 1592–1596.
31. Mousavi-Sani, S.Z.; Raoof, J.B.; Ojani, R.; Hamadi-Asl, E. Nano-Gold modified geneosensor for direct detection of DNA hybridization. *J. Chin. Chem. Soc.* **2013**, *60*, 650–656.
32. Cai, H.; Wang, Y.; He, P.; Fang, Y. Electrochemical detection of DNA hybridization based on silver-enhanced gold nanoparticle label. *Anal. Chim. Acta* **2002**, *469*, 165–172.
33. Castañeda, M.T.; Alegret, S.; Merkoçi, A. Electrochemical detection of DNA hybridization using micro and nanoparticles. *Methods Mol. Biol.* **2009**, *504*, 127–143.
34. Wang, J.; Liu, G.; Polsky, R.; Merkoci, A. Electrochemical stripping detection of DNA hybridization based on cadmium sulfide nanoparticle tags. *Electrochem. Commun.* **2002**, *4*, 722–726.
35. Bangar, M.A.; Shirale, D.J.; Purohit, H.J.; Chen, W.; Myung, N.V.; Mulchandani, A. Single conducting polymer nanowire based sequence-specific, sase-pair-length dependant label-free DNA sensor. *Electroanalysis* **2011**, *23*, 371–379.
36. Garnier, F.; Korri-Youssoufi, H.; Srivastava, P.; Mandrand, B.; Delair, T. Toward intelligent polymers: DNA sensors based on oligonucleotide-functionalized polypyrroles. *Synth. Met.* **1999**, *100*, 89–94.
37. Ma, H. Effect of electron-electron interactions on the charge carrier transitions in trans-polyacetylene. *J. Phys. Chem. A* **2010**, *144*, 5439–5444.
38. Patel, M.K.; Singh, J.; Singh, M.K.; Agrawal, V.V.; Ansari, S.G.; Malhotra, B.D. Tin oxide quantum dot based DNA sensor for pathogen detection. *J. Nanosci. Nanotechnol.* **2013**, *13*, 1671–1678.
39. Heeger, A.J. Nobel lecture: Semiconducting and metallic polymers: The fourth generation of polymeric materials. *Rev. Mod. Phys.* **2001**, *73*, 681–700.
40. Pernaut, J.M.; Reynolds, J.R. Use of conducting electroactive polymers for drug delivery and sensing of bioactive molecules. A redox chemistry approach. *J. Phys. Chem. B* **2000**, *104*, 4080–4090.
41. Park, S.M. Electrochemistry of π-conjugated polymers. In *Handbook of Organic Conductive Molecules and Polymers*; Nalwa, H.S., Ed.; Wiley: Chichester, UK, 1997; Volume 3, pp. 429–469.
42. Guiseppi-Elie, A.; Wallace, G.G.; Matsue, T. *Handbook of Conducting Polymers*, 2nd ed.; Skotheim, T.A., Elsenbaumer, R., Reynolds, J.R., Eds.; Dekker, M.: New York, NY, USA, 1998; Volume 963.
43. Kim, Y.H.; Lee, J.; Hofmann, S.; Gather, M.C.; Müller-Meskamp, L.; Leo, K. Achieving high efficiency and improved stability in ITO free transparent organic light-emitting diodes with conductive polymer electrodes. *Adv. Funct. Mater.* **2013**, *23*, 3763–3769.
44. Defieuw, G.; Samijn, R.; Hoogmartens, I.; Vanderzande, D.; Gelan, J. Antistatic polymer layers based on poly(isothianaphthene) applied from aqueous compositions. *Synth. Met.* **1993**, *57*, 3702–3706.
45. Shen, K.Y.; Hu, C.W.; Chang, L.C.; Ho, K.C. A complementary electrochromic device based on carbon nanotubes/conducting polymers. *Sol. Energy Mater. Sol. Cells* **2012**, *98*, 294–299.
46. Mengistie, D.A.; Ibrahim, M.A.; Wang, P.C.; Chu, C.W. Highly conductive PEDOT: PSS treated with formic acid for ITO-free polymer solar cells. *ACS Appl. Mater. Interfaces* **2014**, *6*, 2292–2299.

47. Baldissera, A.F.; Freitas, D.B.; Ferreira, C.A. Electrochemical impedance spectroscopy investigation of chlorinated rubber-based coatings containing polyaniline as anticorrosion agent. *Mater. Corros.* **2010**, *61*, 790–801.

48. Rahman, M.M.; Li, X.B.; Jeon, Y.D.; Lee, H.J.; Lee, S.J.; Lee, J.J. Simultaneous determination of ranitidine and metronidazole at poly(thionine) modified anodized glassy carbon electrode. *J. Electrochem. Sci. Technol.* **2012**, *2*, 90–94.

49. Svirskis, D.; Travas-Sejdic, J.; Rodgers, A.; Garg, S. Electrochemically controlled drug delivery based on intrinsically conducting polymers. *J. Control. Release* **2010**, *146*, 6–15.

50. Leprince, L.; Dogimont, A.; Magnin, D.; Champagne, S.D. Dexamethasone electrically controlled release from polypyrrole-coated nanostructured electrodes. *J. Mater. Sci. Mater. Med.* **2010**, *21*, 925–930.

51. Rahman, M.A.; Kumar, P.; Park, D.S.; Shim, Y.B. Electrochemical sensors based on organic conjugated polymers. *Sensors* **2008**, *8*, 118–141.

52. Ali, S.R.; Parajuli, R.R.; Balogun, Y.; Ma, Y.; He, H. A nonoxidative electrochemical sensor based on a self-doped polyaniline/carbon nanotube composite for sensitive and selective detection of the neurotransmitter dopamine: A review. *Sensors* **2008**, *8*, 8423–8452.

53. Dhaoui, W.; Bouzitoun, M.; Zarrouk, H.; Ouada, H.B.; Pron, A. Electrochemical sensor for nitrite determination based on thin films of sulfamic acid doped polyaniline deposited on Si/SiO₂ structures in electrolyte/insulator/semiconductor (E.I.S.) configuration. *Synth. Met.* **2008**, *158*, 722–726.

54. Dhaoui, W.; Bouzitoun, M.; Zarrouk, H.; Ouada, H.B.; Pron, A. Electrochemical sensor for nitrite determination based on thin films of sulfamic acid doped polyaniline deposited on Si/SiO₂ structures in electrolyte/insulator/semiconductor (E.I.S.) configuration. *Synth. Met.* **2008**, *158*, 722–726.

55. Shim, Y.B.; Park, S.M. Electrochemistry of conductive polymers XXII, Electrochemical and spectroelectrochemical studies of polyazulene growth and its characterization. *J. Electrochem. Soc.* **1997**, *144*, 3027–3033.

56. Uygun, A. DNA hybridization electrochemical biosensor using a functionalized polythiophene. *Talanta* **2009**, *79*, 194–198.

57. Kim, H.J.; Piao, M.H.; Choi, S.H.; Shin, C.H.; Lee, Y.T. Development of amperometric hydrogen peroxide sensor based on horseradish peroxidase-immobilized poly(thiophene-co-epoxythiophene). *Sensors* **2008**, *8*, 4110–4118.

58. Lee, J.W.; Park, D.S.; Shim, Y.B.; Park, S.M. Electrochemical characterization of poly (1,8-diaminonaphthalene): A functionalized polymer. *J. Electrochem. Soc.* **1992**, *139*, 3507–3514.

59. Paul, E.W.; Ricco, A.J.; Wrighton, M.S. Resistance of polyaniline films as a function of electrochemical potential and the fabrication of polyaniline-based microelectronic devices. *J. Phys. Chem.* **1985**, *89*, 1441–1447.
61. Mishra, A.; Ma, C.Q.; Bäuerle, P. Functional oligothiophenes: Molecular design for multidimensional nanoarchitectures and their applications. *Chem. Rev.* **2009**, *109*, 1141–1276.

62. Bhat, D.K.; Kumar, M.S. N and P doped poly(3,4-ethylenedioxythiophene) electrode materials for symmetric redox supercapacitors. *J. Mater. Sci.* **2007**, *42*, 8158–8162.

63. Turner, M.L. Inorganic and organometallic polymers. *Annu. Rep. Prog. Chem. Sect. A Inorg. Chem.* **1999**, *95*, 453–465.

64. Teles, F.R.R.; Fonseca, L.P. Applications of polymers for biomolecule immobilization in electrochemical biosensors. *Mater. Sci. Eng. C* **2008**, *28*, 1530–1543.

65. McQuade, D.T.; Pullen, A.E.; Swager, T.M. Conjugated polymer-based chemical sensors. *Chem. Rev.* **2000**, *100*, 2537–2574.

66. Sassolas, A.; Leca-Bouvier, B.D.; Blum, L.J. DNA biosensors and microarrays. *Chem. Rev.* **2008**, *108*, 109–139.

67. Fuentes, M.; Mateo, C.; García, L.; Tercero, J.C.; Guisán, J.M.; Fernández-Lafuente, R. Directed covalent immobilization of aminated DNA probes on aminated plates. *Biomacromolecules* **2004**, *5*, 883–888.

68. Peng, H.; Zhang, L.; Soeller, C.; Travas-Sejdic, J. Conducting polymers for electrochemical DNA sensing. *Biomaterials* **2009**, *30*, 2132–2148.

69. Pividori, M.I.; Merkoci, A.; Alegret, S. Electrochemical genosensor design: Immobilisation of oligonucleotides onto transducer surfaces and detection methods. *Biosens. Bioelectron.* **2000**, *15*, 291–303.

70. Hirayama, H.; Tamaoka, J.; Horikoshi, K. Improved immobilization of DNA to microwell plates for DNA–DNA hybridization. *Nucleic Acids Res.* **1996**, *24*, 4098–4099.

71. Kara, P.; Kerman, K.; Ozkan, D.; Meric, B.; Erdem, A.; Nielsen, P.E.; Ozsoz, M. Label-Free and label based electrochemical detection of hybridization by using methylene blue and peptide nucleic acid probes at chitosan modified carbon paste electrodes. *Electroanalysis* **2002**, *14*, 1685–1690.

72. Xu, C.; Cai, H.; He, P.; Fang, Y. Electrochemical detection of sequence-specific DNA using a DNA probe labeled with aminoferrrocene and chitosan modified electrode immobilized with ssDNA. *Analyst* **2001**, *126*, 62–65.

73. Wong, E.L.S.; Erohkin, O.; Gooding, J.J. A comparison of cationic and anionic intercalators for the electrochemical transduction of DNA hybridization via long range electron transfer. *Electrochem. Commun.* **2004**, *6*, 648–654.

74. Zhao, Y.D.; Pang, D.W.; Zhang, M.; Cheng, J.K. DNA-modified electrodes. Part 6. A new method for the determination of binding constants and binding site sizes. *Fresenius J. Anal. Chem.* **1999**, *363*, 708–709.

75. Zhao, Y.D.; Pang, D.W.; Wang, Z.L.; Cheng, J.K.; Qi, Y.P. DNA modified electrodes. Part 2. Electrochemical characterization of gold electrode modified with DNA. *J. Electroanal. Chem.* **1997**, *431*, 203–209.

76. Pang, D.W.; Abreuña, H.D. Micromethod for the investigation of the interactions between DNA and redox-active molecules. *Anal. Chem.* **1998**, *70*, 3162–3169.

77. Azek, F.; Grossiord, C.; Joannes, M.; Limoges, B.; Brossier, P. Hybridization Assay at a Disposable Electrochemical Biosensor for the attomole Detection of Amplified Human Cytomegalovirus DNA. *Anal. Biochem.* **2000**, *284*, 107–113.
78. Wang, J.; Cai, X.; Rivas, G.; Shiraiishi, H. Stripping potentiometric transduction of DNA hybridization processes. *Anal. Chim. Acta* **1996**, *326*, 141–147.

79. Marrazza, G.; Chianella, L.; Mascini, M. Disposable DNA electrochemical sensor for hybridization detection. *Biosens. Bioelectron.* **1999**, *14*, 43–51.

80. Wang, J.; Cai, X.; Jonsson, C.; Balakrishnan, M. Adsorptive stripping potentiometry of DNA at electrochemically pretreated carbon paste electrodes. *Electroanalysis* **1996**, *8*, 20–24.

81. Wu, S.; Zhao, H.; Ju, H.; Shi, C.; Zhao, J. Electrodeposition of silver-DNA hybrid nanoparticles for electrochemical sensing of hydrogen peroxide and glucose. *Electrochem. Commun.* **2006**, *8*, 1197–1203.

82. Lahiji, R.R.; Dolash, B.D.; McDonald, J.; Zemlyanov, D.; Bergstrom, D.E.; Reifenberger, R. Electrodeposition study of ODN:SWCNT hybrids on gold substrates. *Phys. Stat. Sol. A* **2008**, *205*, 1408–1411.

83. Tam, P.D.; Tuan, M.A.; Chien, N.D. DNA covalent attachment on conductometric biosensor for modified genetic soybean detection. *Commun. Phys.* **2007**, *17*, 234–240.

84. Peng, H.; Soeller, C.; Travas-Sejdic, J. Novel conducting polymers for DNA sensing. *Macromolecules* **2007**, *40*, 909–914.

85. Rasmussen, S.R.; Larsen, M.R.; Rasmussen, S.E. Covalent immobilization of DNA onto polystyrene microwells: The molecules are only bound at the 5' end. *Anal. Biochem.* **1991**, *198*, 138–142.

86. Ligaj, M.; Jasnowska, J.; Musial, W.G.; Filipiak, M. Covalent attachment of single-stranded DNA to carbon paste electrode modified by activated carboxyl groups. *Electrochim. Acta* **2006**, *51*, 5193–5198.

87. Raymond, F.R.; Ho, H.A.; Peytavi, R.; Bissonnette, L.; Boissinot, M.; Picard, F.J.; Leclerc, M.; Bergeron, M.G. Detection of target DNA using fluorescent cationic polymer and peptide nucleic acid probes on solid support. *BMC Biotechnol.* **2005**, *5*, doi:10.1186/1472-6750-5-10.

88. Niveleau, A.; Sage, D.; Reynaud, C.; Bruno, C.; Legastelois, S.; Thomas, V.; Dante, R. Covalent linking of haptens, proteins and nucleic acids to a modified polystyrene support. *J. Immunol. Methods* **1993**, *159*, 177–187.

89. Smith, E.A.; Wanat, M.J.; Cheng, Y.; Barreira, S.V.P.; Frutos, A.G.; Corn, R.M. Formation, Spectroscopic characterization, and application of sulphhydryl-terminated alkanethiol monolayers for the chemical attachment of DNA onto gold surfaces. *Langmuir* **2001**, *17*, 2502–2507.

90. Ajore, R.; Kumar, R.; Kaur, I.; Sobti, R.C.; Bharadwaj, L.M. DNA immobilization chemical interference due to aggregates study by dip and drop approach. *J. Biochem. Biophys. Methods* **2007**, *70*, 779–785.

91. Li, F.; Chen, W.; Zhang, S. Development of DNA electrochemical biosensor based on covalent immobilization of probe DNA by direct coupling of sol–gel and self-assembly technologies. *Biosens. Bioelectron.* **2008**, *24*, 781–786.

92. Liu, T.; Tang, J.; Jiang, L. The enhancement effect of gold nanoparticles as a surface modifier on DNA sensor sensitivity. *Biochem. Biophys. Res. Commun.* **2004**, *313*, 3–7.

93. Wang, J.; Lin, Y. Functionalized carbon nanotubes and nanofibers for biosensing applications. *Trends Anal. Chem.* **2008**, *27*, 619–626.

94. Santiago-Rodriguez, L.; Sánchez-Pomales, G.; Cabrera, C.R. Single-walled carbon nanotubes modified gold electrodes as an impedimetric DNA sensor. *Electroanalysis* **2010**, *22*, 399–405.
95. Gangopadhyay, R.; De, A. Conducting polymer nanocomposites: A brief overview. *Chem. Mater.* **2000**, *12*, 608–622.
96. Wahab, R.; Ansari, S.G.; Kim, Y.S.; Mohanty, T.R.; Hwang, I.H.; Shin, H.S. Immobilization of DNA on nano-hydroxyapatite and their interaction with carbon nanotubes. *Synth. Met.* **2009**, *159*, 238–245.
97. Yang, T.; Zhou, N.; Zhang, Y.; Zhang, W.; Jiao, K.; Li, G. Synergistically improved sensitivity for the detection of specific DNA sequences using polyaniline nanofibers and multi-walled carbon nanotubes composites. *Biosens. Bioelectron.* **2009**, *24*, 2165–2170.
98. Tichoniuk, M.; Ligaj, M.; Filipiak, M. Application of DNA hybridization biosensor as a screening method for the detection of genetically modified food components. *Sensors* **2008**, *8*, 2118–2135.
99. Pan, S.L.; Rothberg, L. Chemical control of electrode functionalization for detection of DNA hybridization by electrochemical impedance spectroscopy. *Langmuir* **2005**, *21*, 1022–1027.
100. Caruso, F.; Rodda, E.; Furlong, D.N.; Niikura, K.; Ohkohata, Y. Quartz crystal microbalance study of DNA immobilization and hybridization for nucleic acid sensor development. *Anal. Chem.* **1997**, *69*, 2043–2049.
101. Dupont-Filliard, A.; Billon, M.; Livache, T.; Guillerez, S. Biotin/avidin system for the generation of fully renewable DNA sensor based on biotinylated polypyrrole film. *Anal. Chim. Acta* **2004**, *515*, 271–277.
102. Calvo-Muñoz, M.L.; Dupont-Filliard, A.; Billon, M.; Guillerez, S.; Bidan, G.; Marquette, C.; Blum, L. Detection of DNA hybridization by ABEl electrochemiluminescence in DNA-chip compatible assembly. *Bioelectrochemistry* **2005**, *66*, 139–143.
103. Baur, J.; Gondran, C.; Holzinger, M.; Defrancq, E.; Perrot, H.; Cosnier, S. Label-Free Femtomolar Detection of Target DNA by Impedimetric DNA sensor based on poly(pyrrole-nitrilotriacetic acid) film. *Anal. Chem.* **2010**, *52*, 1066–1072.
104. Bonanni, A.; Pividori, M.I.; Valle, M.D. Application of the avidin-biotin interaction to immobilize DNA in the development of electrochemical impedance genosensors. *Anal. Bioanal. Chem.* **2007**, *389*, 851–861.
105. Cosnier, S.; Galland, B.; Gondran, C.; le Pellec, A. Electrogeneration of Biotinylated functionalized polypyrroles for the simple immobilization of enzymes. *Electroanalysis* **1998**, *10*, 808–813.
106. Shamansky, L.M.; Davis, C.B.; Stuart, J.K.; Kuhr, W.G. Immobilization and detection of DNA on microfluidic chips. *Talanta* **2001**, *55*, 909–918.
107. Piunno, P.A.E.; Krull, U.J.; Hudson, R.H.E.; Damha, M.J.; Cohen, H. Fiberloptic DNA sensor for fluorometric nucleic acid determination. *Anal. Chem.* **1995**, *67*, 2635–2643.
108. Liu, G.; Wan, Y.; Gau, V.; Zhang, J.; Wang, L.; Song, S.; Fan, C. An enzyme-based E-DNA sensor for sequence-specific detection of femtomolar DNA targets. *J. Am. Chem. Soc.* **2008**, *130*, 6820–6825.
109. Ferguson, B.S.; Buchsbaum, S.F.; Swensen, J.S.; Hsieh, K.; Lou, X.; Soh, H.T. Integrated micro fluidic electrochemical DNA sensor. *Anal. Chem.* **2009**, *81*, 6503–6508.
110. Wang, J. Portable electrochemical systems. *Trends Anal. Chem.* **2002**, *21*, 226–232.
111. Jin, Y. Label-free monitoring of site-specific DNA cleavage by EcoRI endonuclease using cyclic voltammetry and electrochemical impedance. *Anal. Chim. Acta* **2009**, *634*, 44–48.
112. Luo, X.; Hsing, I.M. Electrochemistry and adsorptive stripping voltammetric determination of amoxicillin on a multiwalled carbon nanotubes modified glassy carbon electrode. *Electroanalysis* 2009, 21, 1577–1586.

113. Karadeniz, H.; Erdem, A.; Caliskan, A. Electrochemical monitoring of DNA hybridization by multiwalled carbon nanotube based screen printed electrodes. *Electroanalysis* 2008, 20, 1932–1938.

114. Cadogan, A.; Gao, Z.; Lewenstam, A.; Ivaska, A.; Diamond, D. All-solid-state sodium-selective electrode based on a calixarene ionophore in a poly(vinyl chloride) membrane with a polypyrrole solid contact. *Anal. Chem.* 1992, 64, 2496–2501.

115. Michalska, A. Optimizing the analytical performance and construction of ion-selective electrodes with conducting polymer-based ion-to-electron transducers. *Anal. Bioanal. Chem.* 2006, 384, 391–406.

116. Bobacka, J. Conducting polymer-based solid-state ion-selective electrodes. *Electroanalysis* 2006, 18, 7–18.

117. Bobacka, J. Potential stability of all-solid-state ion-selective electrodes using conducting polymers as ion-to-electron transducers. *Anal. Chem.* 1999, 71, 4932–4937.

118. Bobacka, J.; Lewenstam, A.; Ivaska, A. Electrochemical impedance spectroscopy of oxidized poly(3,4-ethylenedioxythiophene) film electrodes in aqueous solutions. *J. Electroanal. Chem.* 2000, 489, 17–27.

119. Hatchett, D.W.; Josowicz, M. Composites of intrinsically conducting polymers as sensing nanomaterials. *Chem. Rev.* 2008, 108, 746–769.

120. Maksymiuk, K. Chemical reactivity of polypyrrole and its relevance to polypyrrole based electrochemical sensors. *Electroanalysis* 2006, 18, 1537–1551.

121. Sutter, J.; Pretsch, E. Response behavior of poly(vinyl chloride)- and polyurethane-based Ca$^{2+}$-selective membrane electrodes with polypyrrole- and poly(3-octylthiophene)-mediated internal solid contact. *Electroanalysis* 2006, 18, 19–25.

122. Shen, Z.; Burrows, P.E.; Bulovic, V.; Forrest, S.R.; Thompson, M.E. Three-color, tunable, organic light-emitting devices. *Science* 1997, 276, 2009–2011.

123. Lidzey, D.G.; Bradley, D.C.; Alvarado, S.F.; Seidler, P.F. Electroluminescence in polymer films. *Nature* 1997, 386, 135–135.

124. Ramanavičius, A.; Ramanavičienė, A.; Malinauskas, A. Electrochemical sensors based on conducting polymer-polypyrrole. *Electrochim. Acta* 2006, 51, 6025–6037.

125. Bidan, G.; Billon, M.; Galasso, K.; Livache, T.; Mathis, G.; Roget, A.; Torres-Rodriguez, L.M.; Vieil, E. Electropolymerization as a versatile route for immobilizing biological species onto surfaces: Application to DNA biochips. *Appl. Biochem. Biotechnol.* 2000, 89, 183–193.

126. Livache, T.; Roget, A.; Dejean, E.; Barthet, C.; Bidan, G.; Teoule, R. Preparation of a DNA matrix via an electrochemically directed copolymerization of pyrrole and oligonucleotides bearing a pyrrole group. *Nucleic Acids Res.* 1994, 22, 2915–2921.

127. Livache, T.; Fouque, B.; Roget, A.; Marchand, J.; Bidan, G.; Téoule, R.; Mathis, G. Polypyrrole DNA chip on a silicon device: Example of hepatitis C virus genotyping. *Anal. Biochem.* 1998, 255, 188–194.
128. Wang, J.; Jiang, M. Toward genolelectronics: Nucleic acid doped conducting polymers. *Langmuir* 2000, 16, 2269–2274.

129. Wang, J.; Jiang, M.; Fortes, A.; Mukherjee, B. New label-free DNA recognition based on doping nucleic-acid probes within conducting polymer films. *Anal. Chim. Acta* 1999, 402, 7–12.

130. Korri-Youssoufi, H.; Yassar, A. Electrochemical probing of DNA based on oligonucleotide functionalized polyppyrrole. *Biomacromolecules* 2001, 2, 58–64.

131. Peng, H.; Soeller, C.; Vigar, N.; Kilmartin, P.A.; Cannell, M.B.; Bownaker, G.A; Cooney, R.P.; Travas-Sejdic, J. Label-free electrochemical DNA sensor based on functionalized conducting copolymer. *Biosens. Bioelectron.* 2005, 20, 1821–1828.

132. Ko, S.; Jang, J. Label-free target DNA recognition using oligonucleotide-functionalized polypyrrole nanotubes. *Ultramicroscopy* 2008, 108, 1328–1333.

133. Tlili, C.; Jaffrezic-Renault, N.J.; Martelet, C.; Korri-Youssoufì, H. Direct electrochemical probing of DNA hybridization on oligonucleotide-functionalized polypyrrole. *Mater. Sci. Eng. C* 2008, 28, 848–854.

134. Komarova, E.; Aldissi, M.; Bogomolova, A. Direct electrochemical sensor for fast reagent-free DNA detection. *Biosens. Bioelectron.* 2005, 21, 182–189.

135. Peng, H.; Soeller, C.; Vigar, N.A.; Caprio, V.; Travas-Sejdic, J. Label-free detection of DNA hybridization based on a novel functionalized conducting polymer. *Biosens. Bioelectron.* 2007, 22, 1868–1873.

136. Eguiluz, K.I.V.; Salazar-Banda, G.R.; Elizabeth, M.; Huacca, F.; Alberice, J.V.; Carrilho, E.; Machado, S.A.S.; Avaca, L.A. Sequence-specific electrochemical detection of Alicyclobacillus acidoterrestris DNA using electroconductive polymer-modified fluorine tin oxide electrodes. *Analyst* 2009, 134, 314–319.

137. Riccardi, C.S.; Kranz, C.; Kowalik, J.; Yamanaka, H.; Mizaikoff, B.; Josowicz, M. Label-Free DNA detection of hepatitis C virus based on modified conducting polypyrrrole films at microelectrodes and atomic force microscopy tip-integrated electrodes. *Anal. Chem.* 2008, 80, 237–245.

138. Xu, Y.; Ye, X.; Yang, L.; He, P.; Fang, Y. Impedance DNA biosensor using electropolymerized polypyrrole/multiwalled carbon nanotubes modified electrode. *Electroanalysis* 2006, 18, 1471–1478.

139. Xu, Y.; Jiang, Y.; Cai, H.; He, P.G.; Fang, Y.Z. Electrochemical impedance detection of DNA hybridization based on the formation of M-DNA on polypyrrole/carbon nanotube modified electrode. *Anal. Chim. Acta* 2004, 516, 19–27.

140. Qi, H.; Li, X.; Chen, P.; Zhang, C. Electrochemical detection of DNA hybridization based on polypyrrole/ss-DNA/multi-wall carbon nanotubes paste electrode. *Talanta* 2007, 72, 1030–1035.

141. Wilson, J.; Radhakrishnan, S.; Sumathi, C.; Dharuman, V. Polypyrrole-polyaniline-Au (PPy-PANi-Au) nano composite films for label-free electrochemical DNA sensing. *Sens. Actuators B Chem.* 2012, 171, 216–222.

142. Radhakrishnan, S.; Sumathi, C.; Umar, A.; Kim, S.J.; Wilson, J.; Dharuman, V. Polypyrrole-poly(3,4-ethylenedioxythiophene)-Ag (PPy-PEDOT-Ag) nanocomposite films for label-free electrochemical DNA sensing. *Biosens. Bioelectron.* 2013, 47, 133–140.
143. Booth, M.A.; Harbison, S.A.; Travas-Sejdic, J. Effects of redox couple on the response of polypyrrole-based electrochemical DNA sensors. *Electroanalysis* **2012**, *24*, 1311–1317.

144. Ho, H.A.; Doré, K.; Boissinot, M.; Bergeron, M.G.; Tanguay, R.M.; Boudreau, D.; Leclerc, M. Direct molecular detection of nucleic acids by fluorescence signal amplification. *J. Am. Chem. Soc.* **2005**, *127*, 12673–12676.

145. Floch, F.L.; Ho, H.A.; Leclerc, M. Label-Free electrochemical detection of protein based on a ferrocene-bearing cationic polythiophene and aptamer. *Anal. Chem.* **2006**, *78*, 4727–4731.

146. Lee, T.Y.; Shim, Y.B. Direct DNA Hybridization Detection based on the oligonucleotide-functionalized conductive polymer. *Anal. Chem.* **2001**, *73*, 5629–5632.

147. Cheng, G.; Zhao, J.; Tu, Y.; He, P.; Fang, Y. A sensitive DNA electrochemical biosensor based on magnetite with a glassy carbon electrode modified by multi-walled carbon nanotubes in polypyrrole. *Anal. Chim. Acta* **2005**, *533*, 11–16.

148. Zhang, Z.; Liang, P.; Zheng, X.; Peng, D.; Yan, F.; Zhao, R.; Feng, C.L. DNA Immobilization/hybridization on plasma-polymerized pyrrole. *Biomacromolecules* **2008**, *9*, 1613–1617.

149. Cha, J.; Han, J.I.; Choi, Y.; Yoon, D.S.; Oh, K.W.; Lim, G. DNA hybridization electrochemical sensor using conducting polymer. *Biosens. Bioelectron.* **2003**, *18*, 1241–1247.

150. Peng, H.; Soeller, C.; Travas-Sejdic, J. DNA sensor based on conducting polymers functionalized with conjugated side chain. In Proceedings of the IEEE International Nanoelectronics Conference on Emerging Technologies, 28–31 October 2007, Atlanta, GA, USA; pp. 1124–1127.

151. Nilsson, K.P.R.; Inganäs, O. Chip and solution detection of DNA hybridization using a luminescent zwitterionic polythiophene derivative. *Nat. Mater.* **2003**, *2*, 419–424.

152. Ho, H.A.; Najari, A.; Leclerc, M. Optical detection of DNA and proteins with cationic polyporphenes. *Accounts Chem. Res.* **2008**, *2*, 168–178.

153. Shiddiky, A.J.M.; Shim, Y.B. Trace Analysis of DNA: Preconcentration, separation, and electrochemical detection in microchip electrophoresis using Au nanoparticles. *Anal. Chem.* **2007**, *79*, 3724–3733.

154. Shiddiky, A.J.M.; Rahman, M.A.; Shim, Y.B. Hydrazine-catalyzed ultrasensitive detection of DNA and proteins. *Anal. Chem.* **2007**, *79*, 6886–6890.

155. Fang, B.; Jiao, S.; Li, M.; Qu, Y.; Jiang, X. Label-free electrochemical detection of DNA using ferrocene-containing cationic polythiophene and PNA probes on nanogold modified electrodes. *Biosens. Bioelectron.* **2008**, *23*, 1175–1179.

156. Liu, M.; Luo, C.; Peng, H. Electrochemical DNA sensor based on methylene blue functionalized polythiophene as a hybridization indicator. *Talanta* **2012**, *88*, 216–221.

157. Huang, J.; Virji, S.; Weiller, B.H.; Kaner, R.B. Polyaniline nanofibers: Facile synthesis and chemical Sensors. *J. Am. Chem. Soc.* **2003**, *125*, 314–315.

158. Janata, J.; Josowicz, M. Conducting polymers in electronic chemical sensors. *Nat. Mater.* **2003**, *2*, 19–24.

159. Tahir, Z.M.; Alocilja, E.C.; Grooms, D.L. Indium tin oxide-polyaniline biosensor: Fabrication and characterization. *Sensors* **2007**, *7*, 1123–1140.
160. Lukachova, L.V.; Karyakin, A.A.; Karyakina, E.E.; Gorton, L. The improvement of polyaniline glucose biosensor stability using enzyme immobilization from water–organic mixtures with a high content of organic solvent. *Sens. Actuators B Chem.* **1997**, *44*, 356–360.

161. Sangodkar, H.; Sukeerthi, S.; Srinivasa, R.S.; Lal, R.; Contractor, A.Q. A biosensor array based on polyaniline. *Anal. Chem.* **1996**, *68*, 779–783.

162. Wei, D.; Ivaska, A. Electrochemical biosensors based on polyaniline. *Chem. Anal. (Wars.)* **2006**, *51*, 839–852.

163. Lindfors, T.; Ivaska, A. Potentiometric and UV-vis characterization of N-substituted polyanilines. *J. Electroanal. Chem.* **2002**, *535*, 65–74.

164. Wu, J.; Zou, Y.; Li, X.; Liu, H.; Shen, G.; Yu, R. A biosensor monitoring DNA hybridization based on polyaniline intercalated graphite oxide nanocomposite. *Sens. Actuators B Chem.* **2005**, *104*, 43–49.

165. Gu, H.; Su, X.D.; Loh, K.P. Electrochemical impedance sensing of DNA hybridization on conducting polymer film-modified diamond. *J. Phys. Chem. B* **2005**, *109*, 13611–13618.

166. Arora, K.; Prabhakar, N.; Chand, S.; Malhotra, B.D. Ultrasensitive DNA hybridization biosensor based on polyaniline. *Biosens. Bioelectron.* **2007**, *23*, 613–620.

167. Arora, K.; Prabhakar, N.; Chand, S.; Malhotra, B.D. *Escherichia coli* genosensor based on polyaniline. *Anal. Chem.* **2007**, *79*, 6152–6158.

168. Gao, Z.; Rafea, S.; Lim, L.H. Detection of nucleic acids using enzyme-catalyzed template-guided deposition of polyaniline. *Adv. Mater.* **2007**, *19*, 602–606.

169. Zhu, N.; Chang, Z.; He, P.; Fang, Y. Electrochemically fabricated polyaniline nanowire-modified electrode for voltammetric detection of DNA hybridization. *Electrochim. Acta* **2006**, *51*, 3758–3762.

170. Chang, H.; Yuan, Y.; Shi, N.; Guan, Y. Electrochemical DNA biosensor based on conducting polyaniline nanotube Array. *Anal. Chem.* **2007**, *79*, 5111–5115.

171. Erdem, A.; Kerman, K.; Meric, B.; Akarca, U.S.; Osos, M. Novel hybridization indicator methylene blue for the electrochemical detection of short DNA sequences related to the hepatitis B virus. *Anal. Chim. Acta* **2000**, *422*, 139–149.

172. Kerman, K.; Ozkan, D.; Kara, P.; Meric, B.; Gooding, J.J.; Ozsoz, M. Voltammetric determination of DNA hybridization using methylene blue and self-assembled alkanethiol monolayer on gold electrodes. *Anal. Chim. Acta* **2002**, *462*, 39–47.

173. Pänke, O.; Kirbs, A.; Lisdat, F. Voltammetric detection of single base-pair mismatches and quantification of label-free target ssDNA using a competitive binding assay. *Biosens. Bioelectron.* **2007**, *22*, 2656–2662.

174. Prabhakar, N.; Arora, K.; Singh, H.; Malhotra, B.D. Polyaniline based nucleic acid Sensor. *J. Phys. Chem. B* **2008**, *112*, 4808–4816.

175. Du, M.; Yang, T.; Li, X.; Jiao, K. Fabrication of DNA/graphene/polyaniline nanocomplex for label-free voltammetric detection of DNA hybridization. *Talanta* **2012**, *88*, 439–444.

176. Saberi, R.S.; Shahrokhian, S.; Marrazza, G. Amplified electrochemical DNA sensor based on polyaniline film and gold nanoparticles. *Electroanalysis* **2013**, *25*, 1373–1380.

177. Ge, Y.; Smith, D.K. Development of chemical sensors based on redox-dependent receptors. Preparation and characterization of phenanthrenequinone-modified electrodes. *Anal. Chem.* **2000**, *72*, 1860–1865.
178. Geng, L.; Boguslavsky, L.I.; Kovalev, I.P.; Sahni, S.K.; Kalash, H.; Skotheim, T.A. Amperometric biosensors based on dehydrogenase/NAD and heterocyclic quinones. *Biosens. Bioelectron.* 1996, 11, 1267–1275.

179. Bucur, B.; Mallat, E.; Gurba, A.M.; Goecheva, Y.; Velasco, C.; Marty, J.L.; Noguer, T. Strategies to develop malic acid biosensors based on malate quinone oxidoreductase (MQO). *Biosens. Bioelectron.* 2006, 21, 2290–2297.

180. Piro, B.; Reisberg, S.; Noel, V.; Pham, M.C. Investigations of the steric effect on electrochemical transduction in a quinone-based DNA sensor. *Biosens. Bioelectron.* 2007, 22, 3126–3131.

181. Haccoun, J.; Piro, B.; Tran, L.D.; Dang, L.A.; Pham, M.C. Reagentless amperometric detection of l-lactate on an enzyme-modified conducting copolymer poly(5-hydroxy-1,4-naphthoquinone-co-5-hydroxy-3-thioacetic acid-1,4-naphthoquinone). *Biosens. Bioelectron.* 2004, 19, 1325–1329.

182. Reisberg, S.; Dang, L.A.; Nguyen, Q.A.; Piro, B.; Noel, V.; Nielsen, P.E.; Le, L.A.; Pham, M.C. Label-free DNA electrochemical sensor based on a PNA-functionalized conductive polymer. *Talanta* 2008, 76, 206–210.

183. Acevedo, D.F.; Reisberg, S.; Piro, B.; Peralta, D.O.; Miras, M.C.; Pham, M.C.; Barbero, C.A. Fabrication of an interpenetrated network of carbon nanotubes and electroactive polymers to be used in oligonucleotide biosensing. *Electrochim. Acta* 2008, 53, 4001–4006.

184. Pham, M.C.; Lacaze, P.C.; Dubois, J.E. Voltammetric and XPS Analysis of Metal-Complexed Polytetramine Films: Geometry-Dependent Electron Transfer Therein. *J. Electrochem. Soc.* 1984, 131, 777–784.

185. Tran, L.D.; Prio, B.; Pham, M.C.; Doan, T.L.; Dao, L.H. Electropolymerized polytriamine films: Covalent binding of oligonucleotide and hybridization. *Synth. Met.* 2003, 137, 1439–1440.

186. Robinson, V.L.; Stock, A.M. High energy exchange: Proteins that make or break phosphoramide bonds. *Structure* 1999, 7, R47–R53.

187. Li, X.; Xia, J.; Zhang, S. Label-free detection of DNA hybridization based on poly(indole-5-carboxylic acid) conducting polymer. *Anal. Chim. Acta* 2008, 622, 104–110.

188. Peng, H.; Soeller, C.; Travas-Sejdic, J. A novel cationic conjugated polymer for homogeneous fluorescence based DNA detection. *Chem. Commun.* 2006, 35, 3735–3737.

189. Gaylord, B.S.; Heeger, A.J.; Bazan, G.C. DNA Hybridization Detection with Water-Soluble Conjugated Polymers and Chromophore-Labeled Single-Stranded DNA. *J. Am. Chem. Soc.* 2003, 125, 896–900.

190. Liu, B.; Bazan, G.C. Homogeneous Fluorescence-Based DNA Detection with Water-Soluble Conjugated Polymers. *Chem. Mater.* 2004, 16, 4467–4476.

191. Campas, M.; Katakis, I. DNA biochip arraying, detection and amplification strategies. *Trends Anal. Chem.* 2004, 23, 49–62.

192. Khanna, V.K. Existing and emerging detection technologies for DNA (deoxyribonucleic acid) finger printing, sequencing, bio- and analytical chips: A multidisciplinary development unifying molecular biology, chemical and electronics engineering. *Biotechnol. Adv.* 2007, 25, 85–98.

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