Chapter 27

DNA Detection Using Functionalized Conducting Polymers

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Abstract

A well-defined DNA bioconjugated surface is a key component in the development of efficient biosensor platforms for diseases, ranging from point-of-care detection of pathogens and viruses to personalized diagnostics and medication, as well as for drug discovery, forensics, and food technology. We herein describe a universal and rapid methodology to construct such surfaces based on functionalized conducting polymer thin films. The conducting polymers combine sensing properties with the ability to act as signal transducers for the biorecognition event. We have shown that biosensor designs based on conducting polymers display a number of advantageous features, such as a long-term stability, label-free sensing, fast analysis, and the capability to apply both electrochemical and fluorescent protocols for DNA detection.

Key words: Conducting polymer thin films, Biosensing, Polypyrroles, Polythiophenes, DNA, Electrochemical detection, Fluorescent detection

1. Introduction

The completion of the human genome project provides new dimensions for clinical diagnostics and drug development based on an understanding of the role of specific DNA segments. As a result, there is a growing need for DNA-based technology platforms ranging from genotyping to molecular diagnostics. Among these new technologies, there is an increasing level of interest in the development of electrochemical DNA biosensors due to several factors, including (1) their cost-effectiveness coupled with modern semiconductor fabrication processes; (2) their high sensitivity with the possibility of further signal amplification through electrocatalysis (1–4); (3) their rapid and direct electrical detection (5–7) regardless of light-absorbing chemicals; and (4) the ease of manufacturing of portable, robust, low-cost, and easy-to-handle detection instrumentation suitable for field tests and home-care usage.

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One of the key components for an electrochemical biosensor is efficient construction of a conductive biointerface. Construction of self-assembled monolayers (SAMs) based on the gold (Au)–thiol interaction is one of the most popular approaches for the production of a conductive biomaterial interface due to its simplicity, chemical availability, and the ability to produce thin, uniform films (8, 9). However, SAMs suffer from a number of disadvantages, including a limited selection of grafted electrode surfaces, the extended time needed for immobilization, and the potential-dependent instability of thiol–Au bonding (10–12). These drawbacks limit the development and large-scale manufacture of SAM-based electrochemical biosensors. Conducting polymers, on the other hand, are organic materials that have a unique electronic structure responsible for their electrical conductivity and interesting optical properties. Moreover, their electronic and optical properties are highly sensitive to the polymeric chain environment (13–16), which provides a means of generating a signal for binding a target analyte molecule (17). Utilizing conducting polymer films to develop novel DNA biosensors is a promising route due to the following advantages: (1) thin films can be electropolymerized onto electrodes of predetermined areas with a controlled thickness (<100 nm) (18–20), controlled roughness (R_{rms} < 5 nm) (18), and within a short period of time (usually within seconds); (2) they are conductive and therefore act as electrical signal transducers; (3) they are amenable to large-scale manufacturing; and (4) they exhibit very low intrinsic cytotoxicity (18, 21–23).

Herein, we present general procedures for creating DNA-bioconjugated conducting polymer interfaces for both optical and electrochemical DNA detection platforms. The methods described in this chapter have been developed in our two laboratories – at Polymer Electronics Research Centre (PERC) (Auckland, New Zealand) for the approaches based on functionalized polypyrroles (19, 20, 24, 25), and at RIKEN Advanced Science Institute (Japan) for the approaches based on functionalized poly(3,4-ethylenedioxythiophenes) (PEDOT) (26, 27). The methodologies used in our two laboratories follow the same basic procedure, but with a number of small variations.

2. Materials

2.1. Synthesis of Monomers

2.1.1. Synthesis of (3-Pyrrolyl)acrylic Acid

1. Pyrrole 98%, reagent grade, is distilled under vacuum before use.
2. Sodium hydride (NaH), 50% NaH dispersion in oil.
3. Triisopropylsilyl chloride, 97%.

4. *N*-Bromosuccinimide, ≥95%.
5. *n*-Butyllithium, 1.6 M in hexane.
6. (Triphenylphosphoranylidene) methyl acetate, 98%.
7. Dimethylformamide (DMF), anhydrous.
8. Sodium hydroxide (NaOH), 10% (w/v) aqueous solution.
9. Solvents: hexane, ethyl acetate, ether, and methanol.
10. Hydrochloric acid (HCl), 10% (w/v) aqueous solution.
11. Drying reagents: sodium sulfate and magnesium sulfate.
12. Silica gel for column chromatography.
13. Neutral alumina.
14. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were acquired at room temperature using a 300-MHz spectrometer (Bruker).
15. Mass spectrometry (MS) instrumentation.

2.1.2. Synthesis of Carboxylic Acid-Functionalized 3,4-Ethylenedioxythiophene

1. Hydroxymethyl-functionalized 3,4-ethylenedioxythiophene (EDOT+OH). This reagent may either be purchased from commercial suppliers or synthesized according to the literature procedure (28).
2. Tetrahydrofuran (THF), anhydrous in a sure-seal bottle.
3. Sodium iodide (NaI).
4. Sodium hydride (NaH), 60% (w/w) suspension in mineral oil.
5. Methyl bromoacetate.
6. HCl solution, 1N.
7. Argon.
8. Ethyl acetate.
9. Sodium hydroxide (NaOH).
10. Magnesium sulfate, used as received.
11. Standard Schlenk lines and glassware.
12. CombiFlash® Companion® flash chromatography system on normal phase silica gel cartridges (ISCO).
13. ^{1}H and ^{13}C NMR data were acquired at 25°C with an AV 400 Spectrometer (Bruker).
14. Finnigan MAT LCQ Mass Spectrometer (Thermo Finnigan, San Jose, CA) fitted with an electrospray ionization (ESI) probe.

2.2. Electrochemical Polymerization of Conducting Polymer Sensing Films

2.2.1. Electrochemical Copolymerization of (3-Pyrrolyl)acrylic Acid and Pyrrole

1. Working electrode: glassy carbon electrode (3.0 mm i.d.) (Bioanalytical Systems, Inc., West Lafayette, IN, No. MF-2012).
2. Reference electrode: silver/silver chloride (Ag/AgCl) (BASi, MF-2052).
3. Auxiliary electrode: platinum (Pt) wire (BASi, No. MW-1032), used as received.
4. Alumina slurry (0.5 μm) (Allied Tech Products, Inc, USA).
5. Cell vials (2–15 mL) (BASi, No. MF-1084).
6. Electrocopolymerization solution: 0.5 M Pyrrole, 6.25 mM (3-Pyrrolyl)acrylic Acid (PAA), 0.2 M $LiClO_4$ in acetonitrile.
7. CH Instruments electrochemical workstation, Model 440 (CH Instruments, USA).

2.2.2. Electrochemical Copolymerization of Hydroxyl-Functionalized and Carboxylic-Acid Functionalized 3, 4-Ethylenedioxythiophene

1. Working electrodes: indium tin oxide (ITO)-coated glass plates (Delta Technologies, Ltd., Stillwater, MN), gold (Au) and platinum (Pt) disk (BASi., No. 002421, 002422).
2. Reference electrode: silver/silver chloride (Ag/AgCl), (BASi, No. RE-1C) used as received.
3. Auxiliary electrode: Pt gauze and Pt wire counter electrode (Aldrich, No. 444685, 298093), used as received.
4. Polishing Kit PK-4 (Bioanalytical Systems, Inc.) and alumina slurry (0.05-μm, Micropolish Gamma) (Buehler, Lake Bluff, IL).
5. Monomer solution: aqueous solution containing 0.01 M total EDOT monomer, 0.1 M lithium perchlorate ($LiClO_4$), 0.1 M hydrochloric acid (HCl), and 0.05 M sodium dodecyl sulfate (SDS).

2.3. Immobilization of Oligonucleotide Probes

1. The immobilization of amino-oligonucleotide (ODN) onto the carboxylic functionalized polymer films is performed using a carbodiimide catalyst either with (Subheading 2.3.1) or without *N*-hydroxysulfosuccinimide (NHS) activating agent (Subheading 2.3.2) (see Note 1).

2.3.1. Immobilization of ODN Probes onto Functionalized Polypyrrole Films

1. Immobilization agent: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), ≥97%.
2. Immobilization buffer: 10 mM phosphate-buffered saline (PBS), pH 5.2.

2.3.2. Immobilization of ODN Probes onto Functionalized PEDOT Films

1. Activation/immobilization reagents: *N*-Hydroxysulfosuccinimide (sulfo-NHS) (Pierce) and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) (Pierce) solution.
2. Immobilization buffer: PBS consisting of 137 mM NaCl, 2.7 mM KCl, and 10 mM phosphate buffer, pH 7.4.

2.4. ODN Biosensing

2.4.1. ODN Biosensing with Functionalized Polypyrrole Films

1. Hybridization buffer: PBS, pH 7.4 (40 µL).
2. Synthetic, single-stranded, 18-mer ODNs with complementary, noncomplementary sequences (Invitrogen, Carlsbad, CA; or Alpha DNA, Montreal, Canada).
3. PBS (pH 7.4) containing 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1 mol/mol).
4. AC impedance measurement: EG&G potentiostat/galvanostat (Model 280, Princeton Applied Research) with EG&G 1025 Frequency Response Analyzer; cell vials (as in Subheading 2.2.1, item 5); Ag/AgCl reference electrodes (as described in Subheading 2.2.1); Pt sheet (1.5 × 1.5 cm).
5. Data analysis: ZView software (Version 2.80) (Scribner Associates, Inc., Southern Pines, NC).

2.4.2. ODN Biosensing with Functionalized PEDOT Films

1. Electrochemical reporting enzyme solution: glucose oxidase-avidin D (GOD-A) (Vector Laboratories), diluted 1:100 (v/v) in PBS (pH 7.4) to form a 50-µg/mL working solution.
2. Redox polymer: poly(vinylimidazole)-polymer(acrylamide) copolymer partially imidazole-complexed with Os(4,4′-dimethyl-2,2′-bipyridine)$_2Cl^{+/2+}$ (PVA-Os), synthesized as described in ref. (29).
3. Cy3-labeled complementary DNA target (Invitrogen, Carlsbad, CA).

3. Methods

3.1. Synthesis of Carboxylic Acid Group-Functionalized Pyrrole: PAA

The synthetic scheme of PAA is shown in Fig. 1.

3.1.1. Synthesis of N-(Triisopropylsilyl) pyrrole (1)

1. Pyrrole (1.0 mL, 0.96 g, 15 mmol) is added dropwise at 0°C to a stirred suspension of sodium hydride (0.758 g of 50% dispersion in mineral oil, 16 mmol) in anhydrous DMF (20 mL) in a 50-mL round-bottom flask.
2. After hydrogen evolution (foaming) ceases, triisopropylsilyl chloride (3.1 mL, 2.8 g, 15 mmol) is added dropwise, and the solution is stirred continuously at 0°C for 45 min.
3. The reaction mixture is partitioned between ether and water, and the ether phase is washed with water, dried over sodium sulfate, and evaporated with a rotary evaporator under reduced pressure.

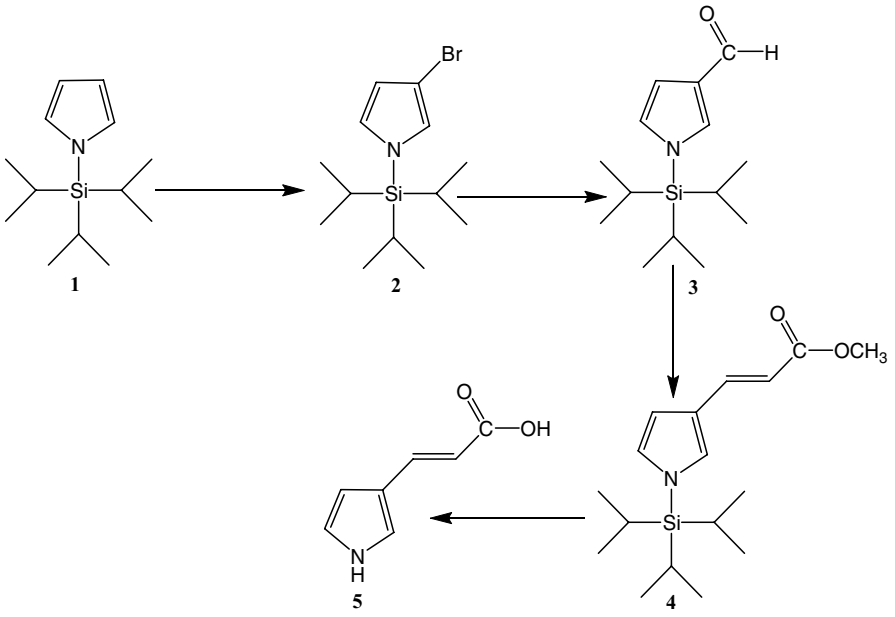

Fig. 1. Schematic representation of the synthesis of (3-pyrrolyl)acrylic acid (PAA).

4. The product is purified by column chromatography on silica gel using hexane/ethyl acetate = 10:1 (by volume) as an eluent to give an oil (1.01 g, 30.2% yield).
5. The compound **1** is characterized by ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (d, 18 H), 1.45 (sept, 3 H), 6.31 (t, 2 H), 6.80 (t, 2 H) and mass spectroscopy (M + H) *m/z*: 224.

3.1.2. Synthesis of 3-Bromo-1-(Triisopropylsilyl)pyrrole (2)

1. *N*-Bromosuccinimide (NBS, 0.8 g, 4.5 mmol) is added to a stirred solution of *N*-(triisopropylsily1)pyrrole (1.0 g, 4.5 mmol) in anhydrous THF (10 mL) at –78°C in a dry ice/acetone bath. The reaction mixture is kept at –78°C for 1–2 h and then left to cool down to room temperature (ca. 1 h).
2. Pyridine (0.1 mL) and hexane (10 mL) are added to the reaction mixture and the resulting suspension is filtered through a plug of neutral alumina.
3. The filtrate is evaporated in vacuum. Purification by column chromatography on silica gel using hexane/ethyl acetate = 10:1 (by volume) as an eluent gives an oil (1.22 g, 90.1% yield).
4. The purity of the compound 2 is checked by ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (d, 18 H), 1.42 (sept, 3 H), 6.26 (dd, 1H), 6.66 (t, 1H), 6.72 (dd, 1H) and MS (M + H) *m/z*: 302.

3.1.3. Synthesis of l-(Triisopropylsilyl) pyrrole-3-Carboxaldehyde (3)

1. A solution of 1.6 M *n*-butyllithium in hexane (1.56 mL, 2.5 mmol) is added, at –78°C in a dry ice/acetone bath, to a solution of 3-bromo-l-(triisopropylsilyl)pyrrole (0.76 g, 2.5 mmol) in anhydrous THF (20 mL).
2. After 15 min at –78°C, DMF (2.5 mmol) is added; 15 min thereafter, the reaction is removed from the cooling bath and left to warm up to room temperature.
3. The reaction mixture is quenched with 20 mL of water and extracted three times with 10 mL of ether. Following this, the extract is dried over $MgSO_4$ and evaporated with a rotary evaporator under reduced pressure.
4. The residue is then purified by column chromatography on silica gel using hexane/ethyl acetate (7:3, v/v) as an eluent to give an oil (0.501 g, yield 80%).
5. The purity of the compound **3** is checked by ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10 (d, 18 H), 1.47 (sept, 3 H), 6.74 (m, 1H), 6.77 (m, 1H), 7.39 (dd, 1H), 9.82 (m, 1H) and MS (M + H) *m/z*: 252.

3.1.4. Synthesis of 3-(l-(Triisopropylsilyl) pyrrol-3-yl)-acrylic Acid Methyl Ester (4)

1. (Triphenylphosphoranylidene) methyl acetate (3 mmol, 1.05 g, 1.5 equiv) is added to a solution of l-(triisopropylsilyl) pyrrole-3-carboxaldehyde (0.502 g, 2.0 mmol) in anhydrous THF (20 mL).
2. The reaction mixture is stirred at 30°C for 2 h and then concentrated with a rotary evaporator under reduced pressure to give an oily residue, which upon purification by column chromatography on silica gel using hexane/ethyl acetate (10:1, v/v) as an eluent gives the product 4.
3. The purity of the compound 4 is checked by ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, 18 H), 1.45 (sept, 3 H), 3.74 (s, 3 H), 6.10 (d, 1H), 6.50 (m, 1H), 6.74 (m, 1H), 6.97 (m, 1H), 7.69 (d, 1H) and MS (M + H) *m/z*: 308.

3.1.5. Synthesis of PAA (5)

1. 3-(l-(Triisopropylsilyl)pyrrol-3-yl)-acrylic acid methyl ester (3 mmol, 0.924 g) is added to an aqueous solution of 10 wt% NaOH (6 mL) and methanol (3 mL), and the mixture is refluxed for 2 h. Methanol is then removed by distillation in vacuum.
2. The aqueous solution is adjusted to pH 4.3 with 10% (w/v) HCl. The resultant precipitate is collected, washed with cold water, and recrystallized from an ethanol–hexane mixture to give **5**.
3. The purity of the compound **5** is checked by ^{1}H NMR (300 MHz, (CH_3)SO-d6): δ 5.95 (d,1H), 6.40 (m, 1H), 6.80 (m, 1H), 7.18 (m, 1H), 7.46 (d, 1H), 11.15 (s, 1H), 11.80 (s, 1H).

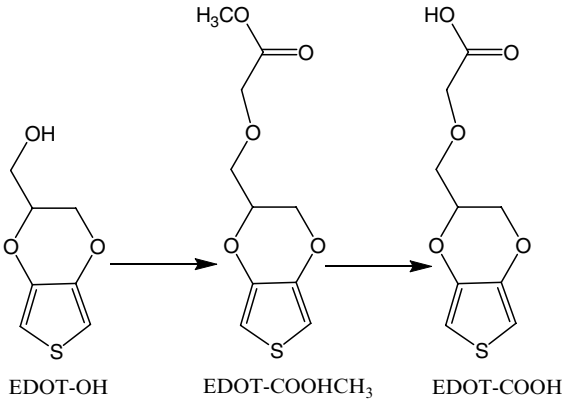

Fig. 2. Schematic representation of the synthesis of carboxylic acid-functionalized 3,4-ethylenedioxythiophene (EDOT-COOH).

3.2. Synthesis of Carboxylic Acid-Functionalized 3,4-Ethylenedioxythiophene

The detailed synthesis scheme is shown in Fig. 2.

3.2.1. Synthesis of Methyl Carboxylate-Functionalized 3,4-Ethylenedioxythiophene

1. A 100-mL round-bottom flask is charged with a stir bar and the reaction mixture, consisting of EDOT-OH (861 mg, 5.0 mmol), NaI (150 mg, 1.0 mmol), and NaH (60% w/w suspension in mineral oil, 240 mg, 6.0 mmol). The flask is evacuated in vacuum and then filled with argon using a dual Schlenk line, with one line connected to vacuum and the other line connected to the argon supply. This procedure needs to be repeated at least three times (see Note 2).
2. Anhydrous THF (stored in an inert gas-filled bottle) is drawn by syringe (20 mL) and introduced into the reaction mixture. After THF is introduced, the suspension is stirred for 15 min and cooled in an ice bath.
3. Methyl bromoacetate (0.57 mL, 0.92 g, 6.0 mmol) is then added dropwise, and the reaction mixture is stirred for 18 h (see Note 3).
4. The majority of THF is removed with a rotary evaporator under reduced pressure; the crude product is partitioned between water and ethyl acetate, and the aqueous layer is extracted with 100 mL of ethyl acetate twice.
5. The combined organic layers are washed with saturated sodium chloride solution. The solution is then dried with anhydrous magnesium sulfate, and the magnesium sulfate drying reagent

is filtered. The filtrate is then evaporated to remove the organic solvent, and the crude product is thereby obtained.

6. The crude product is further purified with a silica gel column using hexane/ethyl acetate (5:1, v/v) as an eluent to give the purified intermediate methyl carboxylate-functionalized 3,4-ethylenedioxythiophene (EDOT-$COOCH_3$) (see Note 4).
7. The purity of the compound EDOT+$COOCH_3$ is checked by ^{1}H NMR (400 MHz, $CDCl_3$): 6.35 (d, 1H, J = 3.6 Hz), 6.33 (d, 1H, J = 3.6 Hz), 4.37 (ddd, 1H, J = 11.6, 7.6, 2.0 Hz), 4.28 (dd, 1H, J = 11.6, 2.0 Hz), 4.19 (s, 2 H), 4.12 (dd, 1H, J = 11.6, 7.6 Hz), 3.84 (dd, 1H, J = 10.4, 5.2 Hz), 3.78 (dd, 1H, J = 10.4, 5.2 Hz), 3.76 (s, 3 H) and ^{13}C NMR (100 MHz, $CDCl_3$): 171.1, 142.0, 141.9, 100.5, 100.4, 73.3, 70.5, 69.5, 69.3, 52.6. Analytically calculated for $C_{10}H_{12}O_5S$: C, 49.17; H, 4.95; found: C, 48.87; H, 4.70.

3.2.2. Synthesis of Carboxylic Acid-Functionalized 3,4-Ethylenedioxythiophene

1. The product EDOT+$COOCH_3$ (610 mg, 2.5 mmol) is dissolved in THF (10 mL) in a 100-mL round-bottom flask.
2. Freshly prepared aqueous NaOH solution (2 M, 10 mL) is added to the reaction mixture, and the mixture is stirred vigorously until the starting material is completely consumed (typically about 3 h). The hydrolysis is monitored by thin layer chromatography (TLC).
3. The mixture is then acidified to pH < 3 by the addition of 1N HCl solution. The reaction mixture is then extracted with ethyl acetate five times and the organic phases are combined (see Note 5).
4. The combined organic layers are washed with water until a neutral pH is obtained. The solution is then dried with anhydrous $MgSO_4$, and the $MgSO_4$ is filtered.
5. The filtrate is then evaporated to remove the organic solvent. The product thereby obtained, carboxylic acid-functionalized 3,4-ethylenedioxythiophene (EDOT-COOH) (480 mg, 83%), is a thick colorless oil that solidifies upon standing overnight.
6. The purity of the compound EDOT-COOH is checked by ^{1}H NMR (400 MHz, $CDCl_3$): 6.36 (d, 1H, J = 3.6 Hz), 6.34 (d, 1H, J = 3.6 Hz), 4.38 (ddd, 1H, J = 11.6, 7.2, 2.4 Hz), 4.26 (dd, 1H, J = 11.6, 2.4 Hz), 4.24 (s, 2 H), 4.12 (dd, 1H, J = 11.6, 7.2 Hz), 3.85 (dd, 1H, J = 10.4, 4.8 Hz), 3.81 (dd, 1H, J = 10.4, 4.8 Hz) and ^{13}C NMR (150 MHz, $CDCl_3$): 175.2, 141.3, 141.1, 100.0, 99.9, 72.6, 70.0, 68.4, 65.8. HR-MS (FAB): [M + H] calculated for $C_9H_{11}O_5S$: 231.0237; found: 231.0237.

3.3. Electrochemical Polymerization of Conducting Polymer Sensing Films

3.3.1. Electrochemical Copolymerization of PAA and Pyrrole

1. A polymerization solution containing 0.5 M pyrrole, 6.25 mM PAA, and 0.2 M $LiClO_4$ in 2 mL of acetonitrile is charged into a three-electrode cell setup comprising of a precleaned glassy carbon working electrode (3.0 mm i.d., 7.07 mm^2 geometric surface area) (see Note 6), an Ag/AgCl (3 M KCl) reference electrode and a Pt wire counter electrode.
2. The electrochemical copolymerization of PAA with pyrrole is carried out using a CH Instruments electrochemical workstation (Model 440) at a constant potential of 1.0 V (vs. Ag/AgCl) until a total charge of 2.12 mC/cm^2 is reached that approximates to a 10 nm film thickness (see Note 7).
3. After polymerization, the film on the electrode is washed with acetonitrile and PBS buffer (pH 7.4), and then stored in PBS, pH 7.4.

3.3.2. Electrochemical Copolymerization of EDOT–COOH

1. A three-electrode cell with a precleaned ITO-coated glass electrode (see Note 8) as the working electrode, a silver/silver chloride (Ag/AgCl) reference electrode, and Pt wire or gauze as an auxiliary electrode is set up. The polymerization solution contains 10 mM total EDOT monomer concentration, with 0.1–1% EDOT-COOH and 99.9–99% EDOT-OH, and other electrolytes as described in Subheading 2.2.2, item 5.
2. The polymer films are electrochemically copolymerized onto the working electrode by applying a constant potential of 1.0 V until a total charge of 60 mC/cm^2 is reached, at which point the film is approximately 30-nm in thickness.
3. The polymer films are rinsed with deionized (DI) water two times before oligonucleotide probe conjugation (see Subheading 3.4).

3.4. Immobilization of Oligonucleotide Probes

3.4.1. Immobilization of ODN Probes onto Carboxylic Acid-Functionalized Polypyrrole Films

1. 40 µl of phosphate buffer (pH 5.2) containing 20 nmol of amine-modified ODN probe and 400 nmol of EDC is injected onto the surface of the copolymer-coated electrode (see Note 9).
2. The electrode is kept at 28°C for 1 h.
3. The modified electrode is thoroughly washed using PBS (pH 7.4) in order to remove any unattached ODN probes, and blown dry with nitrogen.

3.4.2. Immobilization of ODN Probes onto Carboxylic Acid-Functionalized PEDOT Films

1. A solution containing 20 mM EDC and 80 mM *N*-hydroxysulfosuccinimide (sulfo-NHS) in deionized water is mixed, vortexed for 10 s, and then immediately drop-cast onto the conducting polymer films.
2. After incubation at room temperature for 15–20 min, excess sulfo-NHS and EDC are rinsed and removed by washing with deionized water.

3. Amine-modified ODN probes are immobilized onto the films by immersing the activated conducting polymer films in PBS buffer (pH 7.4) containing 1 μM ODN for 5 h.
4. After immobilization, the conducting polymer films are rinsed with PBS (pH 7.4) and water two times, and then blown dry with nitrogen to remove nonspecifically adsorbed DNA probes.

3.5. ODN Biosensing

In this section, we demonstrate (1) the direct, label-free electrochemical detection of target ODNs complementary to the probe ODN using ODN-conjugated polypyrrole films and (2) both fluorescent- and enzyme-catalyzed electrochemical-based target ODN detection using ODN-conjugated PEDOT films.

3.5.1. Electrochemical ODN Detection with ODN-Conjugated Polypyrrole Films

1. Hybridization of target ODNs is performed by injecting 40 μL of PBS solution (pH 7.4) containing ODN target onto the surface of the ODN-probe conjugated polymer film and incubating the film at 42°C in a moisture-saturated chamber for 1 h. The film is then washed three times with PBS to remove any nonybridized ODNs.
2. Electrochemical impedance measurements are undertaken using a conventional three-electrode cell comprised of a polymer film-modified glassy carbon electrode as working electrode, Pt foil as a counter electrode, and an Ag/AgCl (in saturated KCl) reference electrode.
3. Electrochemical impedance spectra (EIS) are measured in 10 mL of PBS solution (pH 7.4) containing 5.0 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ (1:1 mol/mol) as the redox couple. Prior to taking measurements, the solution is purged with nitrogen for 5 min.
4. EIS measurements are taken before ODN probe immobilization, after ODN probe immobilization, and after hybridization with the target ODNs. EIS are measured at an applied bias potential of +230 mV at 20°C, using the $[Fe(CN)_6]^{3-/4-}$ redox couple, and with a 10-mV sinusoidal excitation amplitude over the frequency range of 1 Hz to 2 MHz at 12 steps per decade by means of an EG&G potentiostat/galvanostat coupled with an EG&G 1025 Frequency Response Analyzer.
5. The experimental data are analyzed by using ZView software.
6. A modified Randles equivalent circuit (Fig. 3) consisting of a solution resistance (R_s) in series with a constant phase element (CPE) parallel with a charge-transfer resistance (R_{ct}) and Warburg impedance (W) is used to model the experimental data.
7. A typical result is shown in Fig. 4, which presents the EIS of a polymer film-coated electrode before and after immobilization

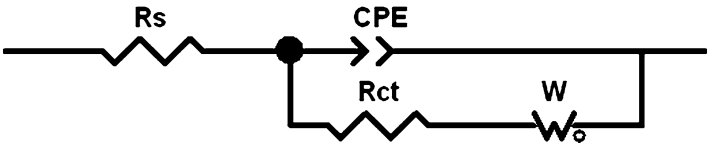

Fig. 3. Equivalent circuit model consisting of a solution resistance, R_s, a charge transfer resistance, R_{ct}, a Warburg impedance, W, and a constant phase element, CPE.

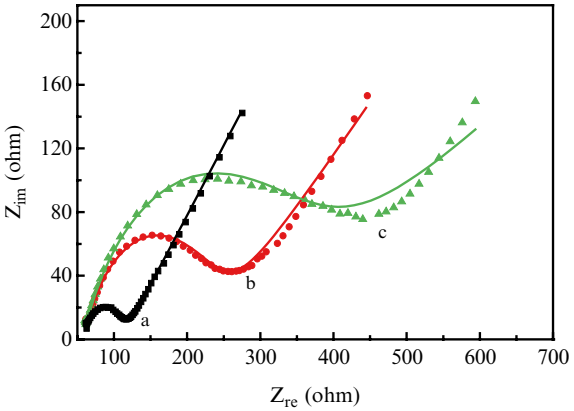

Fig. 4. Electrochemical impedance spectra (presented as Nyquist plots ($-Z_{im}$ vs. Z_{re})) for an electrode coated with poly(Py-*co*-PAA) in PBS solution (pH 7.4) containing 5.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$: (**a**) before immobilization of ODN probes; (**b**) after immobilization of ODN probes; and (**c**) after hybridization with 20.2 nM of complementary ODNs. Reproduced with permission from ref. 25 © 2007 Elsevier.

Table 1
Values of the fitted equivalent circuit parameters derived for the curves shown in Fig. 4

	R_s (Ω)	R_{ct} (Ω)	CPE (μF)	*n*	χ^2
Before probe immobilization	57.8 ± 0.4	55.0 ± 0.8	5.04 ± 0.38	0.77 ± 0.01	2.31×10^{-4}
After probe immobilization	59.5 ± 0.4	173.8 ± 2.7	4.89 ± 0.25	0.77 ± 0.007	4.02×10^{-4}
After hybridization	57.6 ± 0.4	225 ± 6.3	4.21 ± 0.64	0.72 ± 0.008	5.4×10^{-4}

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of ODN probes, and after hybridization with 20.2 nM of fully complementary ODN target. The fitted values for the parameters of the equivalent circuit (Fig. 3) are given in Table 1. The very low chi-squared values suggest that this model fits the experimental data well. After immobilization of the ODN probes, the value of R_{ct} increased from 55 to 174 Ω; and after hybridization with 20.2 nM of fully complementary ODN target, R_{ct} increased significantly further to 255 Ω.

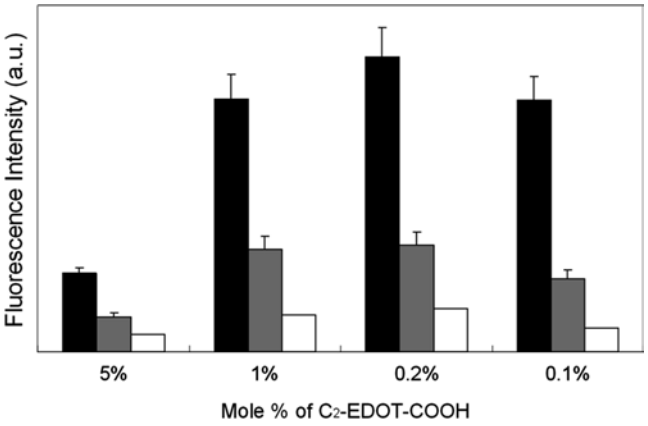

Fig. 5. Fluorescence intensity levels measured on a ODN-bioconjugated PEDOT conducting polymer biointerface in the presence of 1 μM Cy3-labeled complementary target DNA (*black*), one-base mismatch target DNA (*gray*), and noncomplementary target DNA (*white*) in fluorescence labeling experiments. The mol% of carboxylic acid-functionalized 3,4-ethylenedioxythiophene (EDOT-COOH) monomer polymerized on different indium tin oxide (ITO) substrates is indicated. Reproduced with permission from ref. 27 © 2009 American Chemical Society.

3.5.2. Fluorescent and Electrochemical ODN Detection with ODN-Conjugated PEDOT Films

3.5.2.1. Demonstration of Fluorescence-Based Detection Using Cy3-Labeled Target DNA

1. PEDOT–ODN immobilized films are immersed in a PBS solution (pH 7.4) containing 1 μM Cy3-labeled target DNA and incubated overnight in a moisture-saturated chamber maintained at room temperature.
2. After hybridization of the target ODNs, the polymer films are washed with PBS (pH 7.4) three times and blown dry. The polymer films are stored in the dark until fluorescence measurements are taken.
3. Fluorescence measurements are performed with a fluorescence microscope (BX-51, Olympus) with a CCD camera (DP70, Olympus). Images are then analyzed with Image-Pro 3D Suite software (Media Cybernetics) to calculate the fluorescence intensity. The summarized results are shown in Fig. 5.

3.5.2.2. Demonstration of Electrochemical Detection Using Biotin-Labeled Target ODNs

1. The conducting polymer sensing films are immersed in a PBS solution (pH 7.4) containing 1 μM biotin-labeled target ODNs overnight.
2. After rinsing thoroughly with PBS (pH 7.4) and deionized water two times, the electrodes (containing hybridized biotin-labeled target ODNs) are immersed in GOD-A solution for 30 min.
3. The substrates are washed with PBS (pH 7.4) three times and kept in PBS solution before electrochemical detection.
4. The glucose electrooxidation current is measured amperometrically in a PBS solution (pH 7.4) containing 40 mM glucose at a potential of 0.4 V (vs. Ag/AgCl) in a Faraday cage. The summarized results are shown in Fig. 6.

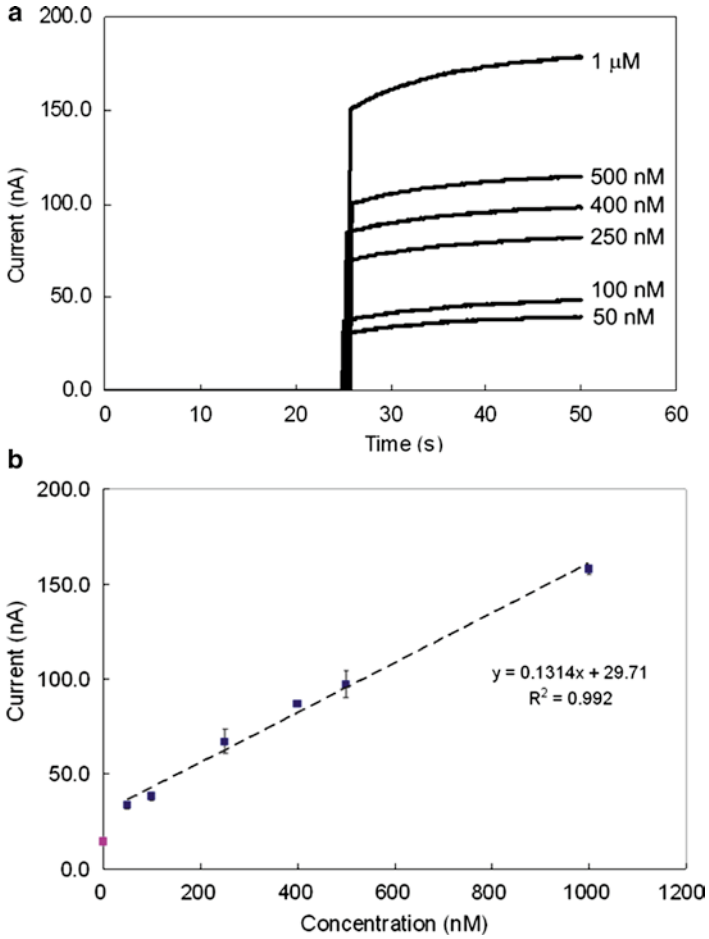

Fig. 6. (**a**) In situ amperometric response of glucose oxidation on poly(EDOT-OH)-co-poly(EDOT-COOH) films (prepared from 0.2 mol% of C2-EDOT-COOH solutions) after hybridization with complementary target DNA at different concentrations. (**b**) Dynamic range and detection limit for the PEDOT-based electrochemical platform. Reproduced with permission from ref. (27) © 2009 American Chemical Society.

4. Notes

1. The NHS route may provide more efficient coupling of amine-ODNs onto carboxylic acid-functionalized conducting polymer films.
2. The weighing paper or container for NaH must be quenched by methanol or acetone before being thrown away.
3. Larger scale synthesis usually takes longer than 24 h.
4. Because the end product is difficult to purify by chromatography, the purification of this intermediate product is crucial

to the purity of the end product. This step must be handled carefully.

5. The acidic solution may cause polymerization of monomers. Therefore, the pH of the solution must be carefully controlled.
6. The glassy carbon electrode is polished with a 0.5-μm alumina slurry, then sonicated in 5 mL of acetonitrile containing 0.2 g of active carbon powder, then in 5 mL of chloroform and 10 mL of Milli-Q water for 10 min, respectively.
7. The thickness of the film is controlled by the total charge passed during polymerization. The relationship between the film thickness and the charge is as follows: Film thickness (nm) = 4.76 × C (mC)/electrode area (cm^2) (30).
8. Indium tin oxide (ITO)-coated glass plates are cleaned by immersion in a detergent solution, acetone, dichloromethane, and finally in isopropanol with ultrasonic agitation for a period of 30 min prior to use. Gold (Au) and platinum (Pt) disk working electrodes are polished by using a PK-4 Polishing Kit (BASi) and with 0.05-μm alumina (Micropolish Gamma) (Buehler) before use.
9. EDC solutions should be freshly prepared and used only once.

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