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(71) Applicant (for all designated States except US): **THE UNIVERSITY OF LIVERPOOL** [GB/GB]; Research and Business Services, Senate House, Abercromby Square, Liverpool L69 3BX (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HIGGINS, Simon, J.** [GB/GB]; Department of Chemistry, The University of Liverpool, Crown Street, Liverpool L69 72D (GB). **MOUFFOUK, Fouzi** [DZ/GB]; Department of Chemistry, The University of Liverpool, Crown Street, Liverpool L69 72D (GB).

(74) Agent: **W. P. THOMPSON & CO.**; Coopers Building, Church Street, Liverpool L1 3AB (GB).

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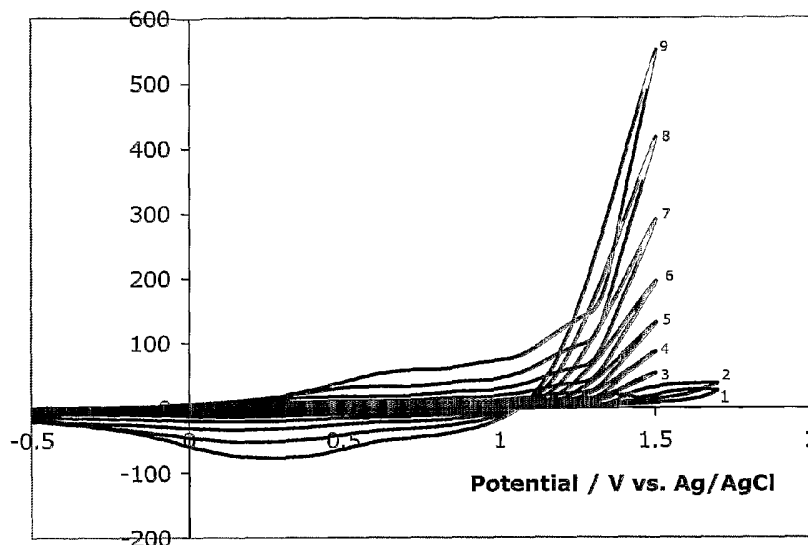
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(54) Title: ELECTROCHEMICAL SENSORS



(57) Abstract: The present invention relates to an electrochemical sensor comprising at least one conjugate having a ligand attached to 3,4-ethylenedioxythiophene (EDOT) or derivative or polymer thereof by means of a spacing element, said ligand being capable of binding a target molecule in a sample, and said EDOT being attached to an electrode such that the binding of a target molecule to the ligand results in a detectable change in the electrochemical properties of the conjugate on the electrode. The sensor is particularly useful for testing biological and chemical material within samples.

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## DESCRIPTION

### ELECTROCHEMICAL SENSORS

The present invention relates to electrochemical sensors and in particular those produced from 3,4-ethylenedioxythiophene (EDOT) and derivatives thereof conjugated to a ligand by means of a functional chain.

The area of multiplex biological sensors which utilise electrochemical interactions of molecules to detect molecules in samples has attracted much attention in recent years from both the press and academia, as it has been suggested that these new biological sensors hold the promise of being able to quickly and inexpensively diagnose a range of human conditions in a single device. Applications for such biological sensors are wide ranging and for example, may be used in such diverse applications as “gene chips” for assessing pharmaco-genetic profiles of individuals prior to administering pharmaceuticals, and to microchips capable of detecting bio-terrorist attacks.

Whilst the technology behind the development of these biological sensors has been rapidly advancing, there still remain a number of problems associated with the technology upon which biological sensors are currently based. For example, many of the methods for electrochemical detection of biological molecules require the biological sample to be processed or mixed with non-physiological compounds (such as organic solvents) prior to testing which can greatly affect the properties of the molecules within the sample and provide incorrect results. Prior

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art methods also rely upon solvents to wash the sample from the sensor and such solvents can also affect the physiology of the molecules within the sample. Additionally, problems have also been reported due to sensitivity of these sensors, which can be attributed to a number of variables.

A number of sensors have been described in the literature, such as a DNA hybridisation electrochemical sensor which uses a conducting polymer comprising poly(thiophen-3-yl-acetic acid 1,3-dioxo-1,3-dihydroisindol-2-yl ester) (PTAE) (*Cha, J. et al., (2003), Biosensors and Bioelectronics, vol. 118 (10) 1241-7?*).

The sensor is produced in the form of a polymer film laid on chip electrodes, and the specificity of the immobilization and the ability of the probe oligonucleotide linked to the polythiophenyl compound to be hybridised is also described in this paper. The redox properties of the polymer used in this paper are not well-defined (they change randomly with cycle number, for instance), the material is not robust to repeated electrochemical cycling, and the nature of the interaction between the polymer and the DNA is not understood. Moreover, this being a simple derivative of polythiophene, it is necessary to conduct the required electrochemical experiments in non-aqueous electrolytes, which may be incompatible with biological systems and may denature the DNA attached thereto and/or affect the hybridisation of the DNA to the chip.

Conjugated polymers derived from 3-4-ethylenedioxythiophene (EDOT) have attracted the intention of many scientists in the last few years due to their great stability under various conditions, such as their ability to retain conductivity even

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after storage at 125°C for 1000 hours. Additionally, EDOT has a moderate band gap with and high visible transparency in its oxidized state and is highly stable in aqueous solutions. Poly-(3-4-ethylenedioxythiophene) has been shown to be useful as an antistatic material and as solid electrolytes in capacitors.

Glucose sensors have also been developed due to the need for accurate measurements for individuals suffering from diabetes. To this end, Kros and colleagues have developed sensors based upon the specific recognition of glucose by the enzyme glucose oxidase (GOx) (*Kros, A. et al., (2001) Advanced Materials, 12, (20), 1555-1557*). These sensors were produced through the polymerisation of 3,4-ethylenedioxythiophene (EDOT) inside the pores of a track-etched membrane in the presence of poly(*N*-methyl-4-pyridine) (PMVP) and the GOx was immobilised inside the polymer-coated pores of the membranes by physical absorption. A similar sensor that utilises an EDOT analogue with a functional group that is used for covalently linking GOx, has also been developed by the same research group (*Kros, A. et al., (2001) Journal of Polymer Science, 40, 738-747*). Whilst these sensors are able to detect amperometrically the glucose in a sample under aerobic and anaerobic conditions, it is not possible to identify single molecules or small quantities of molecules within a sample by using such sensors which would be most desirable in a number of situations where there may be numerous target molecules to identify. Furthermore, the sensors are susceptible to changes in the sample environment, such as changing the atmosphere from inert to oxygen rich, led to difference in the levels of signal, which is unacceptable. The basis of this sensor is the response of the enzyme to

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glucose and the PEDOT polymer is used simply as a conducting 'scaffold' to anchor the enzyme to the electrode, and to carry the charge.

It is therefore an object of the present invention to alleviate one or more of the problems associated with prior art electrochemical based biological sensors. It is also an object of the present invention to provide a multiplex electrochemical sensor for use in biological assays that has a high specificity and sensitivity for identifying target molecules in biological samples that can be operated at normal physiological conditions. It would also be most desirable to provide a sensor that could be redox-cycled in the same medium (*i.e.* aqueous buffer) in which it is exposed to the target molecule.

In accordance with the present invention, there is provided an electrochemical sensor comprising at least one conjugate having a ligand attached to 3,4-ethylenedioxythiophene (EDOT) or derivative or polymer thereof by means of a spacing element, said ligand being capable of binding a target molecule in a sample, and said EDOT being attached to an electrode such that the binding of a target molecule to the ligand results in a detectable change in the electrochemical properties of the conjugate on the electrode.

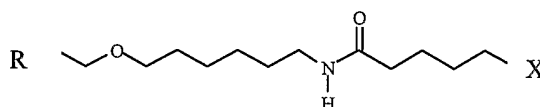
Therefore, the present invention provides a sensor that is robust enough to be used for the testing of biological and chemical samples and does not requires organic solvents for the electrochemical detection of target molecules. Furthermore, the

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present invention provides for a sensor that can detect multiple target molecules in a single sample along with the quantity of each target molecule if appropriate.

Preferably, the spacing element is a long chained spacing element and may comprise 4 or more carbons which are optionally substituted. The spacing element may also have one or more functional groups incorporated therein.

Preferably, the spacing element has two or more functional groups incorporated therein. In a preferred embodiment of the present invention, the spacer element has the structure of:



wherein R is EDOT or a derivative thereof and X is the ligand.

The conjugate(s) may be laid on one or more electrodes so as to form a film and/or matrix of conjugates on the electrode(s). Furthermore, the film and/or matrix may further comprise EDOT or derivatives or polymers thereof that are not associated with a functional spacing element or ligand. The conjugates may therefore provide a more planer film substrate upon which a sample may be tested, or alternatively a more 3-D matrix through which a sample may pass. It will be apparent to one skilled in the art that the configuration of the conjugates will largely be dictated by the design and requirements of a given sensor.

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The ligand and/or target molecule may be selected from one or more of the following: nucleic acid (including nucleic acid analogues), an antibody, a peptide, a protein, a receptor or receptor target molecule, a saccharide, a polysaccharide, a metal-complexing ligand, a lipid and a chemical compound. It will be apparent to one skilled in the art that a number of other ligands and/or target molecules can also be employed and these will largely be dependent upon the application for which the sensor is being used. For example, should the sensor be used to identify whether an individual has taken an illegal substance, the target molecule may be a composition for which an antibody has been raised against.

Alternatively, a sensor may be used to establish whether an individual has a predisposition to genetic condition and a single stranded oligonucleotide may be employed as the ligand so as to bind with DNA from the individual. Due to the width of applications, the sensor can also be used in conjunction with various protocols, such as heating a sample to denature double stranded DNA into single stranded DNA.

The electrode can also be produced from a number of materials and they will commonly comprise a material already used as electrodes. Preferably, the electrode is selected from one or more of the following materials: platinum, indium tin oxide, gold and glassy carbon, highly-oriented pyrolytic graphite (HOPG).

The electrode may comprises a microelectrode. Microelectrodes have been widely-employed for solution electrochemical studies, as their sensitivity is much

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higher owing to the enhanced, three-dimensional diffusion obtained in the size range  $\leq 20 \mu\text{m}$  (*S. Pons and M. Fleischmann, Anal. Chem., 1987, 59, 1391A*). Microband electrodes have often been used in the measurement of conjugated polymer conductivities (*D. Ofer, R. M. Crooks, and M. S. Wrighton, J. Am. Chem. Soc., 1990, 112, 7869*), and arrays of microelectrodes (addressed as a single entity) have been used in sensor design (*A. C. Barton, et al., Biosens. Bioelectron., 2004, 20, 328*). In contrast, the present invention can be used to produce a sensor having a single microelectrode to deposit a functionalised EDOT polymers, thus increasing the sensitivity of the system compared with the same polymers deposited on a normal-sized (of the order  $\text{mm}^2$ ) electrodes.

The EDOT may be attached on an electrode by being polymerised thereon. It will also be apparent to one skilled in the art that EDOT may be attached to the electrode by other methods, such as chemical oxidation, electrochemical deposition and spin coating etc.

Co-polymerisation of EDOT may be used to obtain a favourable compromise between the desirable electrochemical properties of non-functionalised PEDOT (low oxidation potential; excellent stability of the oxidised form) with the presence of the required covalently-anchored receptor sites. The ratio may vary from 100:1 (EDOT:functionalised EDOT) to 3:1. It is preferred that the ratio of EDOT to conjugate in a film is in the order of 5:1, should the ligand be biotin, the ratio may be less for other types of ligand such as nucleic acid.

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The sensor may comprise a plurality of conjugates having the same ligand for quantifying the amount of target molecule in a sample and the plurality of conjugates may be received on one or more electrodes.

Alternatively, the sensor may comprise a plurality of conjugates with different ligands or a plurality of groups of conjugates with different ligands for identifying individual target molecules in a sample. The provision of identical conjugates and non-identical conjugates within the sensor permits the identification and quantification of one or more molecules in a sample. This will be particularly relevant for the development of sensors that have the capability of detecting multiple target molecules and providing an indication as to the levels of the molecules in the sample. For example, the level of expression of a certain protein in an individual may dictate a certain course of therapy with a pharmaceutical.

As stated previously, the sensor may be used in a number of fields such as for testing biological and chemical samples. The sensor may be incorporated into or be operably connected to a semi-conducting chip so that the identification/quantification of a target molecule can be analysed by a computer and the correct molecule or quantity calculated. For example, the semi-conducting chip may be in the form of a matrix with a different ligand attached to a conjugate(s) on a single electrode located on the matrix and the specific electrode corresponds to a given target molecule. In this way, a vast array of targets can be analysed for at the same time and the electrical detection mode has

many advantages over existing techniques such as fluorescence, *in situ* hybridisation etc. A matrix may be in the region of 3 – 5 mm<sup>2</sup> in width.

The sensor as herein above described may advantageously be operable in an aqueous buffer solution, without the requirement for electrochemical cycling with non-aqueous solvents.

A reference solution may be employed to calibrate the sensor before and in between sample testing and this will allow background signal to be almost eliminated from the final testing. The sensor may additionally comprise a target molecule bound to a ligand. This may be advantageous, should the target molecule be required for further analysis for example in high throughput screening of compounds or analysis of variants in proteins etc.

In accordance with a further aspect of the present invention, there is provided a method of identifying a target molecule in a sample by using an electrochemical sensor as herein described above. The method may also be used to quantify the presence of a target molecule in a sample. The sample will preferably be a biological or chemical sample.

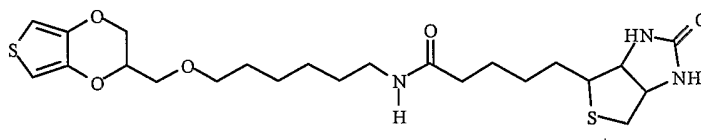
In accordance with yet a further aspect of the present invention, there is provided a method of producing an electrochemical sensor for detecting the presence and/or quantity of a target molecule in a sample comprising the steps of:

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- (a) providing a monomer conjugate comprising EDOT attached to a ligand by means of long chained functional spacing element;
- (b) contacting the conjugate with an electrode under conditions effective to deposit the conjugate on the surface of the electrode; and
- (c) repeating step (b) to form produce one or more layers of electrochemical sensors on an electrode.

The non-conjugate EDOT may also contacted with the electrode in step (b).

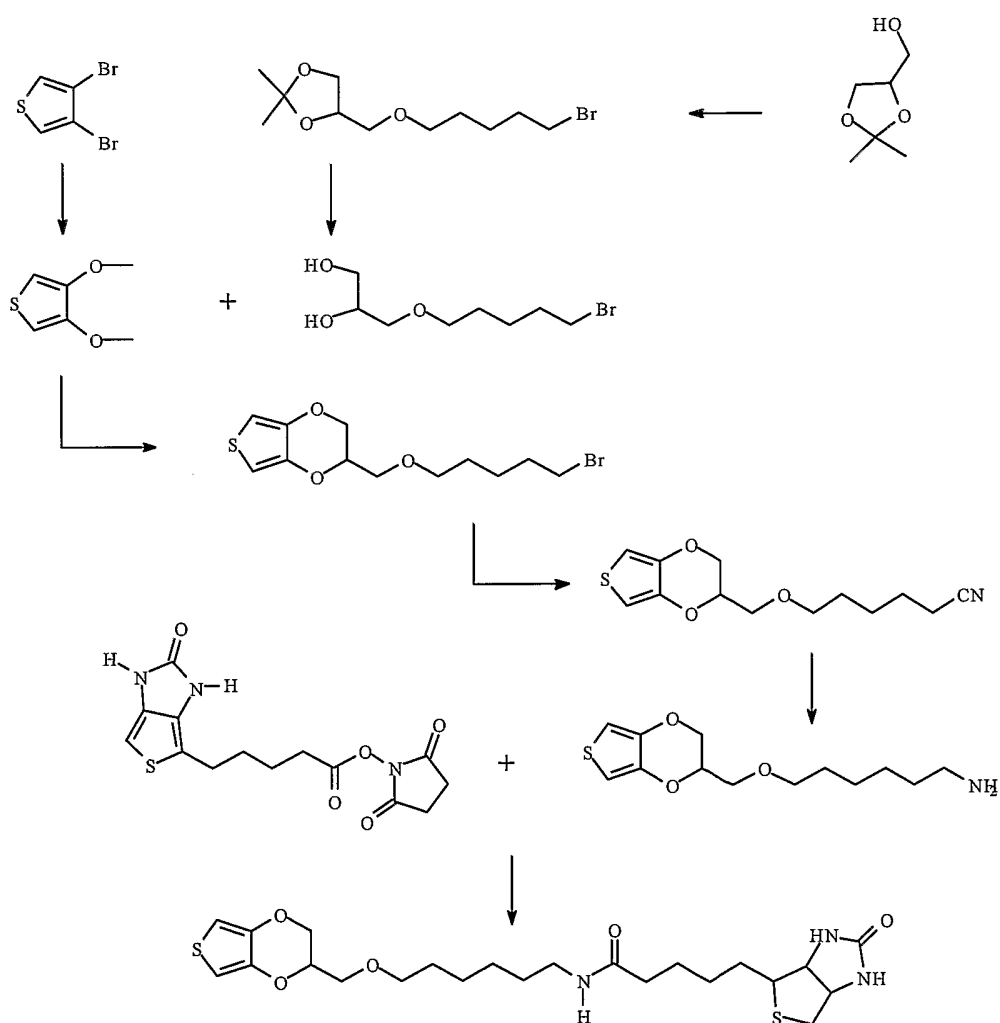
According to yet a further aspect of the present invention, there is provided a conjugate for use with an electrochemical sensor comprising biotin attached to 3,4-ethylenedioxythiophene (EDOT) or derivative or polymer thereof by means of a long chain functional spacing element, said conjugate having the following structure:



As the avidin-biotin interaction has been developed for isolation (affinity chromatography), localization (affinity cytochemistry, cell cytometry, and blotting technology), diagnostics (immunoassay, histopathology, and gene probes), hybridoma technology, bioaffinity sensors, affinity targeting, and drug delivery, the development of a good biotin:avidin – based sensing system may have applications in its own right.

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In accordance with yet another aspect of the present invention, there is provided a method of producing a conjugate for use with an electrochemical sensor comprising biotin attached to 3,4-ethylenedioxythiophene (EDOT) or derivative or polymer thereof by means of a long chain functional spacing element, said method having the following scheme:



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The present invention will now be described by way of example only with reference to and as illustrated in the following example and associated figures:

Fig. 1 is a graph showing the first nine scans for the electrodeposition of the copolymer in Example 1;

Fig. 2 is a graph showing the response of a 10  $\mu\text{m}$  Pt disk modified with a poly(4-EDOT) copolymer to avidin in aqueous buffer in to Example 1;

Fig. 3 is a graph showing the growth of an acid-functionalised PEDOT copolymer of 5 with EDOT itself in Example 2;

Fig. 4 is a graph showing a comparison of the electrochemistry of the PEDOT-acid film before and after derivitisation with the aminoalkyl oligo in Example 2;

Fig. 5 is a graph showing the response of oligonucleotide-functionalised PEDOT film to hybridisation in Example 2; and

Fig. 6 is a graph showing the onset of electrochemical response to minimum amount of complementary oligonucleotide in Example 2.

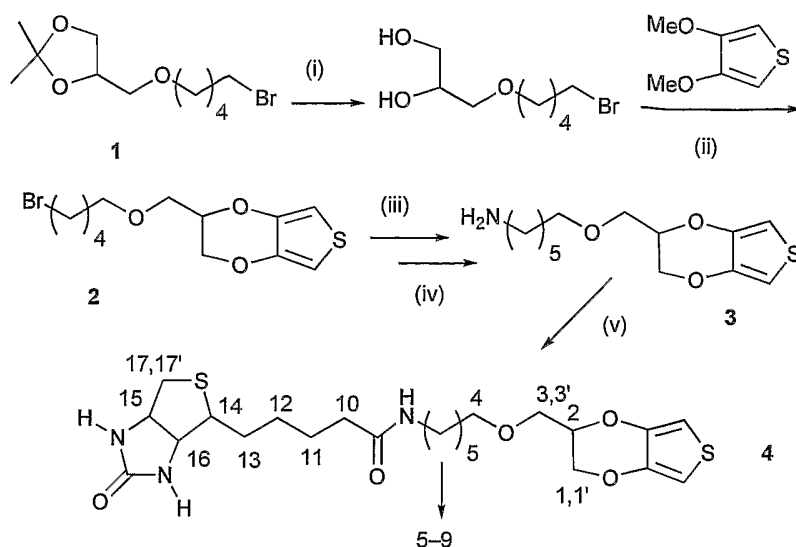
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EXAMPLE 1

An electrochemical conjugate sensor was produced incorporating biotin as the test ligand for the binding of avidin. The sensor was produced by first synthesising the appropriate monomers that formed the sensing layer, followed by the fabrication of this layer (electro-polymerisation). When the sensor had been produced, its sensing ability of avidin was assessed alongside that of a control.

A biotinylated EDOT monomer **4** was prepared by the route shown in Scheme 1 below. Glycerine acetone ketal (2,2-dimethyl-1,3-dioxolane-4-methanol) was reacted with excess 1,5-dibromopentane to afford **1**, which was then deprotected, and the diol reacted with 3,4-dimethoxythiophene in an acid-catalysed transesterification (*Caras-Quintero, D.; Bauerle, P. Chemical Communications 2004, 926*) to give **2**. The presence of a small amount of water in this reaction was found to be necessary to suppress a pinacol rearrangement of the diol, which otherwise resulted in a very low yield of **2**. Treatment of **2** with sodium cyanide, followed by  $\text{LiAlH}_4$  reduction of the nitrile, gave amine **3**, and reaction of **3** with *N*-hydroxysuccinyl-activated biotin, gave monomer **4**. This was purified by preparative-scale tlc on silica gel, and was fully characterised by microanalyses,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy (including  $^1\text{H}$ - $^1\text{H}$  and  $^1\text{H}$ - $^{13}\text{C}$  COSY and  $^{13}\text{C}$  DEPT experiments) and mass spectrometry.

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**Scheme 1.** (i) thf, 1M HCl, 12 h. (ii) toluene, cat. *p*-tosH, 16 h reflux. (iii) KCN, CH<sub>3</sub>CN, reflux, 24 h. (iv) LiAlH<sub>4</sub>, thf, 5 h. (v) *N*-hydroxysuccinylbiotin, dmsO, 12 h. The numbering scheme for **4** refers to the assignment of the NMR signals as described later.

Electropolymerisations involving **4** had to be conducted in CH<sub>2</sub>Cl<sub>2</sub> electrolyte, since it was insufficiently soluble in CH<sub>3</sub>CN. Redox-active polymer films could not be obtained by the electrochemical oxidation of **4** alone, on either macro- or microelectrodes. It was previously found not to be possible to electropolymerise a biotinylated terthiophene alone, and therefore copolymerisation with 2,2':5',2''-terthiophene was used (*Mouffouk, F.; Brown, S. J.; Demetriou, A. M.; Higgins, S. J.; Nichols, R. J.; Rajapakse, R. M. G.; Reeman, S. Journal Of Materials Chemistry* **2005**, *15*, 1186). The same strategy was also used here; copolymerisation of **4** with EDOT itself was employed.

Figure 1 shows the first nine scans for the electrodeposition of the copolymer by repetitive scan cyclic voltammetry on a microelectrode (10 μm diameter Pt disk).

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A monomer ratio of 8:1 EDOT: **4** was optimum. It was necessary to initiate polymerisation by cycling up to +1.7 V for the first two scans; subsequent scans were to +1.5 V. The voltammogram in Figure 1 is unusual for the growth of a PEDOT film. In particular, in the positive-going sweeps, the first oxidation wave is positive of that usually found for functionalised PEDOTs (*Groenendaal, L.; Zotti, G.; Aubert, P. H.; Waybright, S. M.; Reynolds, J. R. Advanced Materials 2003, 15, 855*), and it also moves to more positive potentials with scan number. This behaviour is also similar to that seen for the electrochemical growth of our biotinylated polyterthiophene (*Mouffouk, F.; Brown, S. J.; Demetriou, A. M.; Higgins, S. J.; Nichols, R. J.; Rajapakse, R. M. G.; Reeman, S. Journal Of Materials Chemistry 2005, 15, 1186*), and may be due to the presence of the strongly H-bonding biotin units. It is worthy to note that monomer **4** was >99 % pure by <sup>1</sup>H NMR and GC, and therefore, it is believed that the electropolymerisation behaviour is characteristic of **4** rather than due to an impurity.

The electrochemistry of the polymer (grown using 15 scans; first 9 scans shown in Figure 1) after transfer to an aqueous buffer (0.1 M NaCl, 10 mM EDTA) electrolyte is shown in Figure 2. In spite of the unusual film growth CV, the electrochemistry of the film in water resembles that of other PEDOT derivatives (*Caras-Quintero, D.; Bauerle, P. Chemical Communications 2004, 926*), with a first oxidation process centred at *ca.*  $E = -0.3$  V (Ag/AgCl reference electrode), and a second anodic wave at positive potentials. The peak current for the first wave was linearly dependent upon scan rate, as expected for a surface-localised

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redox couple. After exposure to excess avidin, the electrochemistry of the polymer film was almost completely suppressed. It was believed that this was due to the severe restriction on ion ingress/egress posed by the presence of a layer of avidin on the polymer surface. The fact that this effect is due to the specific binding of biotin by avidin is shown by the fact that, when the electrode was previously exposed to a solution of avidin which had already been exposed to excess free biotin, almost no change was seen in the voltammogram after transfer back to fresh buffer. Exposure to solutions of bovine serum albumin (BSA) also caused almost no change to the voltammogram of the polymer.

Figure 2 shows the response of a 10  $\mu\text{m}$  Pt disk modified with a poly(4-EDOT) copolymer to avidin in aqueous buffer. (1) Pristine polymer, cycled in 0.1 M aq. NaCl / 10 mM EDTA. (2) Polymer after exposure to avidin-biotin complex. (3) A second electrode, after exposure to the minimum amount of avidin to cause a change in the voltammetry ( $10^{-16}$  moles). (4) The first electrode again, after exposure to excess avidin.

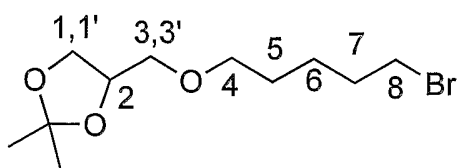
We then exposed a fresh electrode to increasing concentrations of avidin in 1  $\text{cm}^3$  aliquots of buffer, to examine at what point the voltammogram began to change significantly. This occurred when the electrode was exposed to  $10^{-16}$  moles avidin (*i.e.* a  $10^{-13}$  M solution), whereupon the first oxidation wave moved positive, and the peak current diminished. This detection limit is superior to that achieved in our biotinylated polyterthiophene study ( $5 \times 10^{-14}$  moles) with a 0.1  $\text{cm}^2$  electrode

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(Mouffouk, F.; Brown, S. J.; Demetriou, A. M.; Higgins, S. J.; Nichols, R. J.; Rajapakse, R. M. G.; Reeman, S. *Journal Of Materials Chemistry* **2005**, *15*, 1186), and also to that of a system in which a diminution in the redox wave of a monolayer of an electrostatically-bound, biotinylated polythiophene polyelectrolyte (area  $0.01 \text{ cm}^2$ ) was employed (Kumpumbu-Kalemba, L.; Leclerc, M. *Chemical Communications* **2000**, 1847). In the latter studies, macroelectrodes were used. We attribute the greater sensitivity of the PEDOT-modified electrode to the fact that this is a microelectrode. From the charge under the oxidation wave for the pristine polymer in Figure 2 (*ca.*  $1.14 \times 10^{-5} \text{ C}$ ), it is possible to calculate the approximate volume of the polymer layer ( $5 \times 10^{-8} \text{ cm}^3$ ). Assuming that hemispherical diffusion applies (Pons, S.; Fleischmann, M. *Anal. Chem.* **1987**, *59*, 1391A.), and that the polymer forms as a hemisphere centered at the electrode surface (examination of a polymer-modified electrode under magnification suggested that this is reasonable), this corresponds to a radius of  $29 \text{ }\mu\text{m}$ , and the outer surface would have area of  $5.7 \times 10^3 \text{ }\mu\text{m}^2$ . A monolayer of close-packed avidin (approximate dimensions  $40 \times 50 \times 56 \text{ }\text{\AA}$ ) (Rosano, C.; Arosio, P.; Bolognesi, M. *Biomol. Eng.* **1999**, *16*, 5) over this area corresponds to  $4.7 \times 10^{-16}$  moles. Given the gross approximations in this calculation, it is nevertheless interesting that the polymer-modified electrode showed a response at the level of  $10^{-16}$  moles of avidin.

Synthesis and characterisation of monomer 4, the biotinylated EDOT

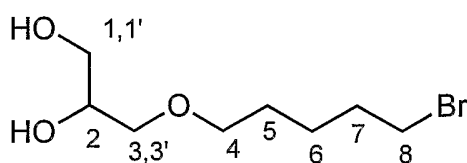
Reagents were purchased from Aldrich Chemical Company and were reagent grade unless otherwise stated. 3,4-dimethoxythiophene was prepared by a literature method from 3,4-dibromothiophene. General methods were as previously published.



*4-(5-Bromo-pentylloxymethyl)-2,2-dimethyl-[1,3]dioxolane, 1*:- To NaH (3 g as 60 % dispersion in oil; 76 mmol) in anhydrous THF (80 cm<sup>3</sup>) was added isopropylideneglycerol (10 g, 76 mmol) slowly over 20 minutes. Once the evolution of gas had ceased, a THF solution (25 cm<sup>3</sup>) of 1,5-dibromopentane (51.7 g, 225 mmol) was added portionwise with mechanical stirring. During the addition the almost solid suspension dissolved. The reaction was then stirred at room temperature for 12 hours before TLC analysis showed it had gone to completion. The solvent was removed *in vacuo* and the brown residue was dissolved in DCM (300 cm<sup>3</sup>). This solution was then washed with distilled water (2 x 200 cm<sup>3</sup>). The organic layer was dried over MgSO<sub>4</sub>, filtered and the solvent removed to yield the product as a viscous yellow oil. This was heated under vacuum to remove the excess of 1,5-dibromopentane and unreacted isopropylideneglycerol. The crude product was pure enough to use in the next step. Yield 15.3 g, 71.5 %. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm (*J* Hz): 4.22 (m, 1H, H<sup>2</sup>), 4.04 (dd, 1H, *J*<sub>1,2</sub> 6.7, *J*<sub>1,1'</sub> 8.2, H<sup>1</sup>), 3.79 (dd, 1H, *J*<sub>1,2</sub> 6.25, *J*<sub>1,1'</sub> 8.0, H<sup>1'</sup>),

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3.70 (overlapping m, 4H, H<sup>3</sup>, H<sup>4</sup>), 3.41 (t, 2H,  $J_{8,7}$  6.8, H<sup>8</sup>), 1.88 (q, 2H,  $J_{5,4}$  6.82, H<sup>5</sup>), 1.70 (s, 6H, 2 CH<sub>3</sub>), 1.60 – 1.45 (m, 4H, H<sup>6</sup>, H<sup>7</sup>). <sup>13</sup>C NMR (161 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 109.4 (C(CH<sub>3</sub>)<sub>2</sub>), 73.2 (C<sup>2</sup>), 72.7 (C<sup>1</sup>), 71.0 (C<sup>2</sup>), 64.4 (C<sup>4</sup>), 34.1 (C<sup>8</sup>), 32.8 (C<sup>5</sup>), 29.3 (CH<sub>3</sub>), 27.0 (C<sup>7</sup>), 25.1 (C<sup>6</sup>). MS (ES<sup>+</sup>): 282 [M+H].

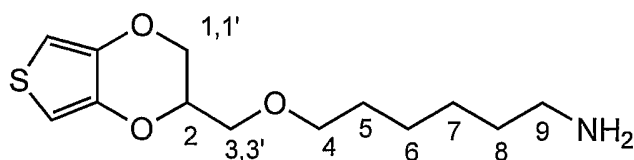


*3-(5-bromopentyloxy)propane-1,2-diol (1)*:- 4-(5-Bromo-pentyloxymethyl)-2,2-dimethyl-[1,3]dioxolane (1 gram, 0.3 mmol) was dissolved in THF (20 cm<sup>3</sup>) and 1 M aq. HCl (10 cm<sup>3</sup>) was added to the solution. The mixture was stirred for 12 hours at room temperature. Most of the THF was removed in *vacuo* and the residue was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The extract was dried over MgSO<sub>4</sub>, filtered and the solvent removed to yield the product as a yellow oil. Flash chromatography (silica, CH<sub>2</sub>Cl<sub>2</sub>) afforded a clear oil. Yield 0.80 g, 93%.

Elemental analysis: calc. for C<sub>8</sub>H<sub>17</sub>BrO<sub>3</sub>: C, 39.85%; H, 7.11%. Found: C, 39.81%; H, 7.17%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm ( $J$  Hz): 3.86 (dt, 1H,  $J_{2,3}$ ,  $J_{2,1}$  5.9, H<sup>2</sup>), 3.68 (dq, 2H,  $J_{1,2}$  5.9,  $J_{1,1}$  11.4, H<sup>1</sup>, H<sup>1'</sup>), 3.48 (overlapping m, 4H, H<sup>3</sup>, H<sup>4</sup>), 3.41 (t, 2H,  $J_{8,7}$  6.8, H<sup>8</sup>), 1.88 (q, 2H,  $J_{5,4}$  6.8, H<sup>5</sup>), 1.65 – 1.45 m, 4H, H<sup>6</sup>, H<sup>7</sup>). <sup>13</sup>C NMR (161 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 72.6 (C<sup>2</sup>), 71.7 (C<sup>1</sup>), 71.0 (C<sup>3</sup>), 64.4 (C<sup>4</sup>), 34.1 (C<sup>8</sup>), 32.8 (C<sup>5</sup>), 29.0 (C<sup>7</sup>), 25.1 (C<sup>6</sup>). MS (CI, NH<sub>3</sub>) 259 [M+NH<sub>4</sub>], 242 [M+H].

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*2-(5-Bromopentyloxymethyl)-2,3-dihydro-thieno[3,4-b]dioxine, 2*:- To a mixture of 3,4-dimethoxythiophene (0.500 g, 3.47 mmol) and the above diol (0.927 g, 3.85 mmol) in toluene (20 cm<sup>3</sup>) was added a catalytic amount of *p*-toluenesulfonic acid, and water (50 µL), and the mixture was refluxed for 16 h. The solvent was removed in *vacuo*, and the residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> (20 cm<sup>3</sup>). The solution was washed with water (5 cm<sup>3</sup> × 3), and the organic layer was dried over MgSO<sub>4</sub> and concentrated. The crude product was purified by column chromatography using hexane as eluant to yield compound **2** (0.61 g, 55%) as a yellow oil. When dry toluene was employed, the diol decomposed as a result of a pinacol rearrangement. The addition of water suppresses this. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm (*J* Hz): 6.33 (m, 2H, thienyl H); 4.35–4.20 (m, 2H, H<sup>2</sup>, H<sup>1</sup>), 4.06 (dd, 1H, *J*<sub>1,2</sub> 6.45, *J*<sub>1,1'</sub> 11.6, H<sup>1'</sup>), 3.63 (dq, 2H, *J*<sub>3,1</sub> 5.9, *J*<sub>3,3'</sub> 10.4, H<sup>3,3'</sup>), 3.52 (t, 2H, *J*<sub>4,5</sub> 6.2, H<sup>4</sup>), 3.45 (t, 2H, *J*<sub>8,7</sub> 6.8, H<sup>8</sup>), 1.85 (q, 2H, *J*<sub>5,4</sub> 7.3, H<sup>5</sup>), 1.64 – 1.41 (m, 4H, H<sup>7</sup>, H<sup>6</sup>). <sup>13</sup>C NMR (161 MHz, CDCl<sub>3</sub>) δ ppm: 142.0, 141.95 (thiophene β-C), 100.0, 99.95 (thiophene α-C), 73.0 (C<sup>2</sup>), 72.0 (C<sup>1</sup>), 69.6 (C<sup>3</sup>), 66.6 (C<sup>4</sup>), 33.9 (C<sup>8</sup>), 32.9 (C<sup>5</sup>), 29.1 (C<sup>7</sup>), 25.1 (C<sup>6</sup>). MS (CI, NH<sub>3</sub>): 322 [M+H], 241 [M-Br].



*6-(2,3-Dihydro-thieno[3,4-b][1,4-dioxin-2-ylmethoxy)-hexylamine, 3*:-To a dried 25 cm<sup>3</sup> Schlenk tube was added potassium cyanide (0.08 grams, 1.23 mmol) and dry acetonitrile (20 cm<sup>3</sup>). To this suspension was added **2** (0.20 grams, 0.62

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mmol). The reaction was stirred vigorously for 24 hours under reflux after which time it had gone to completion (tlc). Solvent was removed in *vacuo* and the residue was treated with water (20 cm<sup>3</sup>) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 50 cm<sup>3</sup>). The organic extracts were combined, dried over MgSO<sub>4</sub>, filtered and the solvent was removed to yield a dark brown oil. To a dried 25 cm<sup>3</sup> Schlenk tube was added lithium aluminium hydride (0.057 g, 1.50 mmol). The tube was fitted with a condenser before dry THF (10 cm<sup>3</sup>) was added. To this suspension was added, dropwise, the crude nitrile in anhydrous THF (2 cm<sup>3</sup>). The reaction was monitored by tlc and after 5 hours appeared to have gone to completion. It was quenched by dropwise addition of dilute NaOH solution. The solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 50 cm<sup>3</sup>), the organic layers were combined, dried over MgSO<sub>4</sub>, filtered and the solvent was removed to yield the product as a clear oil. The crude product was purified by column chromatography using hexane. Yield 0.12 g, 70 %. Elemental analysis calc. for C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>S: C, 57.54; H, 7.80; N, 5.16 %. Found: C, 57.19; H, 7.92; N, 5.04 %. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm (*J* Hz): 6.31 (m, 2H, thiophene α-H), 4.20 – 4.32 (m, 2H, H<sup>2</sup>, H<sup>1'</sup>), 4.05 (dd, 1H, *J*<sub>1,2</sub> 5.6, *J*<sub>1,1'</sub> 11.44, H<sup>1</sup>), 3.65 (dq, 2H, *J*<sub>3,1</sub> 5.02, *J*<sub>3,3'</sub> 10.44, H<sup>3</sup>, H<sup>3'</sup>), 3.48 (t, 2H, *J*<sub>4,5</sub> 6.24, H<sup>4</sup>), 2.67 (t, 2H, *J*<sub>8,7</sub> 7.05, H<sup>9</sup>), 1.90 (br s, 2H, NH), 1.85 (m, 2H, H<sup>5</sup>), 1.59 – 1.34 (m, 6H, H<sup>8</sup>, H<sup>7</sup>, H<sup>6</sup>). <sup>13</sup>C NMR (161 MHz, CDCl<sub>3</sub>) δ ppm: 142.0, 141.95 (thiophene β-C), 100.0, 99.95 (thiophene α-C), 73.0 (C<sup>2</sup>), 72.3 (C<sup>1</sup>), 69.5 (C<sup>3</sup>), 66.6 (C<sup>4</sup>), 33.9 (C<sup>9</sup>), 32.9 (C<sup>5</sup>), 29.85 (C<sup>8</sup>), 25.1 (C<sup>7</sup>, C<sup>6</sup>). MS (CI, NH<sub>3</sub>): 272 [M+H].

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5-(2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoic acid [6-(2,3-dihydro-thieno[3,4-b][1,4]dioxin-2-ylmethoxy)-hexyl]-amide, 4:— To amine 3 (0.10 g, 0.37 mmol) in DMSO (5 cm<sup>3</sup>) was added 1 equivalent of biotin *N*-succinimidyl ester (0.12 g, 0.37 mmol). The mixture was allowed to stir at room temperature for 12 hours under argon. The DMSO was removed via distillation in *vacuo* and the crude product was purified by chromatography on silica gel plates (100 % CH<sub>2</sub>Cl<sub>2</sub> eluant). Yield: 90 %. Elemental analysis calc. for C<sub>23</sub>H<sub>35</sub>O<sub>3</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub> • 0.1CH<sub>2</sub>Cl<sub>2</sub> C, 54.82; H, 7.00; N, 8.30 %. Found: C, 55.05; H, 6.98; N, 8.38 %. <sup>1</sup>H NMR (400.1 MHz, d<sup>6</sup>-DMSO; see Scheme for numbering) δ ppm (*J*Hz): 6.33 (s, 2H, thiophene *CH*), 6.03 (m, 1H, amide NH), 5.47 (s, 2H, biotin NH), 4.53 (m, H<sup>15</sup>), 4.34 – 4.20 (overlapping m, 3H, H<sup>2</sup>, H<sup>1'</sup>, H<sup>16</sup>), 4.05 (dd, 2H, *J*<sup>1,2</sup> = 7.47 Hz, *J*<sup>1,1'</sup> = 11.61 Hz, H<sup>1</sup>), 3.65 (dq, 2H, *J*<sup>3,1</sup> = 5.88 Hz, *J*<sup>3,3'</sup> = 10.49 Hz, H<sup>3</sup>, H<sup>3'</sup>), 3.48 (t, 2H, *J*<sup>4,5</sup> = 6.68 Hz, H<sup>4</sup>), 3.25 – 3.10 (overlapping m, 2H, H<sup>14</sup>, H<sup>9</sup>), 3.00 – 2.60 (m, 2H, H<sup>17</sup>, H<sup>17'</sup>), 2.19 (t, 2H, *J*<sup>10,11</sup> = 7.47 Hz, H<sup>10</sup>), 1.59 – 1.34 (overlapping m, 14H, H<sup>5,6,7,8</sup>, H<sup>11,12,13</sup>). <sup>13</sup>C{<sup>1</sup>H} NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 173.5 (s, HN-CO-NH biotin), 164.2 (amide C=O), 142.0 (thiophene C-β), 100.0 (thiophene C-α), 73.0 (C<sup>2</sup>), 72.3 (C<sup>1</sup>), 69.5 (C<sup>3</sup>), 66.6 (C<sup>4</sup>), 62.2 (C<sup>15</sup>), 60.6 (C<sup>16</sup>), 55.95 (C<sup>9</sup>), 40.9 (C<sup>14</sup>), 39.8 (C<sup>17</sup>), 36.4 (C<sup>10</sup>), 32.9 (C<sup>5</sup>), 29.95 (C<sup>8</sup>), 29.8, 28.6, 28.5, 28.3, 27.1, 26.0, 24.9 (7C, C<sup>5</sup>–C<sup>8</sup> and C<sup>11</sup>–C<sup>13</sup>). Mass spectra (ES<sup>+</sup>): 521 [M+Na], 539 [M+K].

#### Electrochemical formation of sensing copolymer films

*General notes:* Tetrabutylammonium tetrafluoroborate was recrystallised and dried under high vacuum for several hours prior to use. CH<sub>2</sub>Cl<sub>2</sub> was distilled from

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CaH<sub>2</sub> under Ar immediately before use. Solvents and electrolyte were handled under Schlenk conditions. A Ag/AgCl reference electrode, 10  $\mu$ m diameter Pt disk working electrode and Pt gauze counter electrode were used, in a standard 2-compartment electrochemical cell. For some experiments, as outlined in the Results, a 0.1 cm<sup>2</sup> Pt disk macroelectrode was employed.

*Preparation of biotinylated PEDOT films from 4:* A solution of monomer 4 together with EDOT itself (1:8 mole ratio; 0.01 M total monomer concentration) in 0.1 M Bu<sub>4</sub>NBF<sub>4</sub> in dry CH<sub>2</sub>Cl<sub>2</sub> was employed. Consecutive scans at 50 mV s<sup>-1</sup> from -0.5 to +1.7 V (after 2 scans the positive limit was restricted to 1.5 V) were used to grow the polymer. Normally, 15 scans were used.

After growth, the polymer-coated electrode was removed from solution at 0 V, washed with CH<sub>2</sub>Cl<sub>2</sub> and stored in a dry, dust-free environment prior to electrochemical studies in background electrolyte.

Copolymer electrochemistry experiments in aqueous electrolytes.

We first cycled the polymer in background aqueous buffer electrolyte (1 M NaCl, 10 mM EDTA). The potential was cycled between -1 V and +0.5 V at 10 mV s<sup>-1</sup>. To examine the electrochemistry after exposure to excess avidin, the microelectrode was then incubated in avidin buffer solution (1 M NaCl, 10 mM EDTA, 4.04  $\times 10^{-9}$  mol. of avidin) for a period of 15 min. After incubation, the

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microelectrode was rinsed in clean buffer, then cycled again as before in 1 M NaCl, 10 mM EDTA.

To determine the minimum amount of avidin to which the electrode would respond, a second polymer-coated electrode was exposed to successively larger amounts of avidin in 1 cm<sup>3</sup> of buffer, then re-examined by cyclic voltammetry. At the level of 10<sup>-16</sup> moles avidin (*i.e.* 1 cm<sup>3</sup> of a 10<sup>-13</sup> M solution), a change in the voltammogram was observed.

A control experiment was done in which a PEDOT:4 copolymer was exposed to 4.04 × 10<sup>-9</sup> mol. of avidin which had previously been treated with free biotin (1.7 × 10<sup>-8</sup> mol). No change in the electrochemistry of this polymer on transfer back to fresh buffer was observed, illustrating that adventitious adsorption does not occur. Similarly, there was no change in the voltammetry on exposure of the electrode to bovine serum albumin (BSA).

It will be apparent to one skilled in the art, that the sensing layer as herein described above need not be limited to the detection of the biotin-avidin binding, but will also be applicable for the detection of a whole range of biological molecules. The application of this method was then use to develop a sensor capable of detecting the presence of a DNA sequence as outlined in Example 2, although the method can also be extended to many other biological assays.

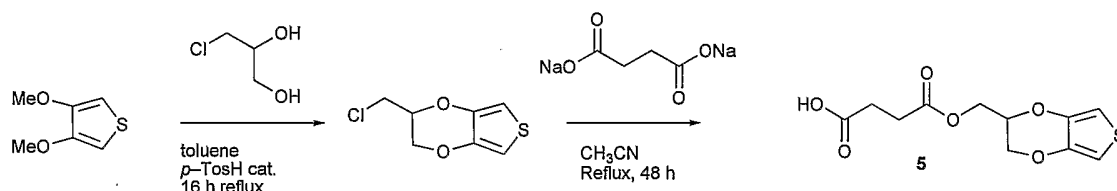
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EXAMPLE 2

An electrochemical conjugate sensor was produced in order to detect the presence of DNA sequence in a sample, by means of 23-base pair oligonucleotide ligand. The protocol for the production of the conjugate sensor in this Example is the same as for Example 1 unless otherwise stated, and therefore the sensor was produced by first synthesising the appropriate monomers that formed the sensing layer, followed by the fabrication of this layer with a oligonucleotide rather than a biotin ligand.

DNA-functionalised PEDOT films

Firstly, a simple acid-functionalised EDOT monomer was synthesised, **5**, using the method in Scheme 2.



**Scheme 2.** Synthesis of monomer **5**.

*Synthesis of monomer 5*

**2-Chloromethyl-2,3-dihydro-thieno[3,4-b][1,4]dioxine:**— To a mixture of 3,4-dimethoxy-thiophene (2.0 g, 0.014 mol) and 3-chloro-1,2-propanediol (1.5 g, 0.014 mol), dissolved in 20 mL of toluene, was added 50  $\mu$ L of water and a catalytic amount of *p*-toluene sulfonic acid, and the mixture was refluxed for 16 h. The solvent was removed *in vacuo*, and the residue was taken up in dichloromethane. The resulting solution was subsequently extracted with water (3

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times), and the organic layer was dried over  $\text{MgSO}_4$  and concentrated. The crude product was purified by column chromatography using hexane as an eluant to yield 50% of the compound as yellow oil. In the absence of added water, 3-(benzyloxy)-1,2-propanediol decomposed overnight at reflux temperatures as a result of a pinacol rearrangement.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  p.p.m.: 6.35 (s, 2H, thiophene  $\alpha$ -H), 4.4 – 4.0 (m, 3H,  $-\text{OCH(R)CH}_2\text{O}-$ ), 3.63 (t, 2H,  $\text{CH}_2\text{Cl}$ ).  $^{13}\text{C}$  NMR (161 MHz,  $\text{CDCl}_3$ )  $\delta$  p.p.m.: 142.0 (thiophene  $\beta$ -C), 100.0 (thiophene  $\alpha$ -C), 73.0 (s, 1C,  $-\text{OCH}-$ ), 72 ( $-\text{OCH}_2-$ ), 42.6 ( $\text{ClCH}_2$ ). MS  $\text{Cl}^+$  ( $\text{NH}_3$ ):  $[\text{M}+\text{H}] = 191$ .

*Succinic acid mono-(2,3-dihydro-thieno[3,4-b][1,4]dioxin-2-ylmethyl) ester, 5:–*

To a dried 25  $\text{cm}^3$  Schlenk tube was added disodium succinate (5 g, 0.03 mol) and 40 ml of dry acetonitrile. To this suspension was added the chloromethyl EDOT derivative (1.0 g, 5.2 mmol). The reaction was stirred vigorously for 48 hours under reflux after which time it had gone to completion. The reaction was quenched with water (20  $\text{cm}^3$ ) before being extracted with DCM (3 x 50  $\text{cm}^3$ ). The organic extracts were combined, dried over  $\text{MgSO}_4$ , filtered and the solvent removed to yield a dark brown oil. The crude product was purified by column chromatography using  $\text{CH}_2\text{Cl}_2$  as an eluant, to yield 0.71 g (50 %) of **5**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  p.p.m.: 6.35 (thiophene  $\alpha$ -H), 4.4 – 4.0 (overlapping m's, 5H,  $-\text{OCH}(\text{CH}_2\text{Cl})\text{CH}_2\text{O}-$ ), 2.72- 2.60 (m, 4H,  $-(\text{O})\text{CCH}_2\text{CH}_2\text{COOH}$ ).  $^{13}\text{C}\{^1\text{H}\}$  NMR (161 MHz,  $\text{CDCl}_3$ )  $\delta$  p.p.m.: 171.9, 168.2 (COO), 142.0 (thiophene  $\beta$ -C),

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100.0 (thiophene  $\alpha$ -C), 73.0, 72.5, 72.0 ( $-\text{OCH}_2\text{CH}(\text{CH}_2\text{O})\text{O}-$ ), 28.9, 28.8 (succinyl  $\text{CH}_2$ ). MS ( $\text{CI}^+$ ,  $\text{NH}_3$ ):  $[\text{M}+\text{H}] = 273$ .

*Preparation of acid-functionalized PEDOT film from 5:* A solution of **5**, together with EDOT itself (1:6 mole ratio; 0.01 M total monomer) in 0.1 M  $\text{Bu}_4\text{NBF}_4$  in dry  $\text{CH}_2\text{Cl}_2$  was used as the electrolyte. Consecutive scans at either 50 or 100  $\text{mV s}^{-1}$  from  $-0.5$  to  $+1.6$  V were employed. With microelectrodes, the positive potential limit was set to  $+1.4$  V after the first 3 scans.

Once again, copolymers of **5** with EDOT itself were then generated (on both 0.1  $\text{cm}^2$  Pt disk electrodes and on a 10  $\mu\text{m}$  diameter Pt disk microelectrode). Care was taken to optimise this process. A variety of monomer mole ratios and concentrations were tried, and different solvents and electrolytes were also examined. For microelectrodes, it was sometimes found that polymer grew in a fairly wide radius, not only on the electrode surface but over a significant area of the surrounding inert support. Such polymer layers were subsequently found to have poor redox switching kinetics when cycled in pure background electrolyte (either 0.1 M  $\text{Et}_4\text{NBF}_4/\text{CH}_3\text{CN}$  or 0.1 M  $\text{NaCl}/\text{H}_2\text{O}$ ).

The optimum conditions were found to be the use of  $\text{CH}_2\text{Cl}_2/0.1$  M  $\text{Bu}_4\text{NBF}_4$  electrolyte, 0.01 M total monomer concentration, 1:6 **5**:EDOT mole ratio.

Repetitive scan cyclic voltammetry was used to grow the films. A typical growth experiment is illustrated in Figure 3 (for a macroelectrode).

Figure 3 shows the growth of an acid-functionalised PEDOT copolymer of **5** with EDOT itself.

After film growth, the polymer films were found to have reversible and stable redox waves on cycling in either organic (0.1 M Et<sub>4</sub>NBF<sub>4</sub>/CH<sub>3</sub>CN) or aqueous buffer (0.1 M NaCl, 10 mM EDTA) electrolyte solutions.

These films were then derivatised with aminoalkyl-terminated oligonucleotides. Coupling reactions were carried out using microelectrodes coated with the polymer, dipped into a solution of a large excess (92  $\mu$ mol) of 5'-NH<sub>2</sub>C<sub>6</sub>H<sub>12</sub>-CGGATAACAATTTTCACACAGGAGG in phosphate buffer (pH 7.4) in the presence of 1-(dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride coupling reagent. The anchoring of the oligo was checked by reflectance infrared spectroscopy, and by the observed change in electrochemistry of the polymer. After modification with the DNA, the polymer film showed a slight positive shift in its redox potential (Figure 4), and the capacitive charging element in the voltammogram increased relative to the Faradaic peak currents. This did not happen when the film was similarly exposed to oligos that did not bear an aminoalkyl terminal function.

Figure 4 shows a comparison of the electrochemistry of the PEDOT-acid film before and after derivitisation with the aminoalkyl oligo.

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The oligonucleotide-functionalised polymer was then exposed to a non-complementary sequence (5'-ATAATAGGTTCCCTTGGGATAAG) and the electrochemistry examined again. No significant change could be seen. However, after exposure to a large excess (69 nmol in 1 cm<sup>3</sup> buffer) of the complementary sequence (5'-CCTCCTGTGTGAAATTGTTATCCG), a large positive shift in the redox wave was observed (see Figure 5).

Figure 5 shows the response of oligonucleotide-functionalised PEDOT film to hybridisation.

In a separate experiment with a second polymer-modified microelectrode, the polymer was derivatised with aminoalkyl-terminated oligo as before. The electrode was then exposed to successively greater amounts of the complementary oligo until a change in its electrochemistry was observed. This occurred at the level of 10<sup>-13</sup> M complementary DNA in 1 cm<sup>3</sup> of buffer.

Figure 6 shows the onset of electrochemical response to minimum amount of complementary oligonucleotide. This occurred at the level of 10<sup>-13</sup> M concentration (10<sup>-16</sup> moles total DNA).

For the DNA hybridization experiments, the aminoalkyl oligo-grafted PEDOT polymer-modified electrode was first cycled in background aqueous buffer electrolyte (1 M NaCl, 10 mM EDTA) between -1 V and +0.5 V at varying scan

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rates. Next, the electrode was placed in a solution of either the complementary oligo, 5'-CCT CCT GTG TGA AAT TGT TAT CCG) (502  $\mu\text{g}$ ,  $68.7 \times 10^{-9}$  mol) in 1  $\text{cm}^3$  buffer solution (1 M NaCl, 10 mM EDTA) for 20 minutes, or into a similar solution of a completely non-complementary oligo, 5'-ATA ATA GGT TCC CTT GGG ATA AGT) (502  $\mu\text{g}$ ,  $67 \times 10^{-9}$  mol). To find the minimum amount of complementary DNA that caused a distinct electrochemical response, solutions of the complementary oligo were prepared by sequential dilution in the buffer solution, and a fresh aminoalkyl oligo-grafted PEDOT polymer-modified electrode was first exposed to the lowest concentration as before, then transferred to fresh buffer and tested by cyclic voltammetry. A distinct change in the electrochemistry (positive shift of the first PEDOT redox process) was seen when the electrode was exposed to  $10^{-16}$  moles of complementary DNA in 1  $\text{cm}^3$  buffer (*i.e.*  $10^{-13}$  M solution).

*In situ DNA grafting to acid-functionalised PEDOT-5 copolymer films:* The polymer-coated microelectrode was characterised electrochemically as described below, and then incubated with 500  $\mu\text{g}$  of 5'- $\text{NH}_2\text{C}_6\text{H}_{12}\text{CGG ATA ACA ATT TCA CAC AGG AGG}$  in 1  $\text{cm}^3$  of phosphate buffer (pH 7.4) containing 1-(dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (10 mg) for a period of 5 hours. The microelectrode was then rinsed in clean buffer and characterised electrochemically as before.

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The experiments therefore show that the sensor of the present invention can be used amongst other assays as a DNA sensor for detecting a single or multiple DNA sequences in a sample. Figure 6 clearly shows the onset of electrochemical response to minimum amount of complementary oligonucleotide. Furthermore, as owing to the low redox potential of poly(3,4-ethylenedioxythiophene) derivatives, and their robustness in the p-doped state, both the binding and electrochemical detection of avidin can be performed in aqueous buffers and prove their suitability for the production of a range of sensors such as gene chips etc.

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CLAIMS

1. An electrochemical sensor comprising at least one conjugate having a ligand attached to 3,4-ethylenedioxythiophene (EDOT) or derivative or polymer thereof by means of a spacing element, said ligand being capable of binding a target molecule in a sample, and said EDOT being attached to an electrode such that the binding of a target molecule to the ligand results in a detectable change in the electrochemical properties of the conjugate on the electrode.
2. A sensor as claimed in claim 1, wherein the spacing element is a long chained spacing element.
3. A sensor as claimed in either claim 1 or 2, wherein the spacing element comprises 4 or more carbons which are optionally substituted.
4. A sensor as claimed in any preceding claim, wherein the spacing element is has one or more functional groups incorporated therein.
5. A sensor as claimed in any preceding claim, wherein the conjugate is laid on one or more electrodes so as to form a film and/or matrix.

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6. A sensor as claimed in claim 5, wherein the film and/or matrix further comprises EDOT or derivatives or polymers thereof that is not associated with a functional spacing element or ligand.
7. A sensor as claimed in any preceding claim, wherein the ligand is selected from one or more of the following: nucleic acid, an antibody, a peptide, a protein, a receptor or receptor target molecule, a saccharide, a polysaccharide, a metal-complexing ligand, a lipid and a chemical compound.
8. A sensor as claimed in any preceding claim, wherein the electrode comprises one or more of the following materials: platinum, indium tin oxide, highly-oriented pyrolytic graphite, glassy carbon and gold.
9. A sensor as claimed in any preceding claim, wherein the electrode comprises a microelectrode.
10. A sensor as claimed in any preceding claim, wherein the sensor comprises a plurality of conjugates having the same ligand for quantifying the amount of target molecule in a sample.
11. A sensor as claimed in claim 10, wherein the plurality of conjugates are received on one or more electrodes.

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12. A sensor as claimed in any of claims 1 to 8, wherein the sensor comprises a plurality of conjugates with different ligands or a plurality of groups of conjugates with different ligands for identifying individual target molecules in a sample.
13. A sensor as claimed in claim 12, wherein the provision of identical conjugates and non-identical conjugates within the sensor permits the identification and quantification of one or more molecules in a sample.
14. A sensor as claimed in any preceding claim, wherein the sample is a biological sample or a chemical sample.
15. A sensor as claimed in any preceding claim, wherein the sensor is incorporated into or operably connected to a semi-conducting chip.
16. A sensor as claimed in any preceding claim, wherein the sensor can be washed with an acid solution to remove bound target molecules.
17. A sensor as claimed in any preceding claim, wherein a reference solution is used to calibrate the sensor before and in between samples.
18. A sensor as claimed in any preceding claim, wherein sensor additionally comprises a target molecule bound to a ligand.

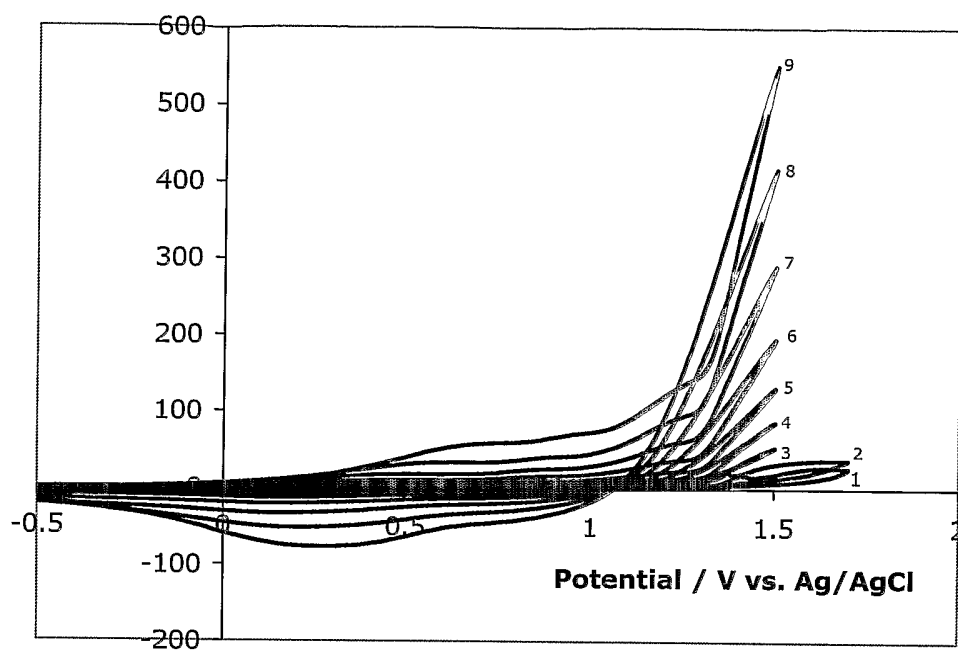
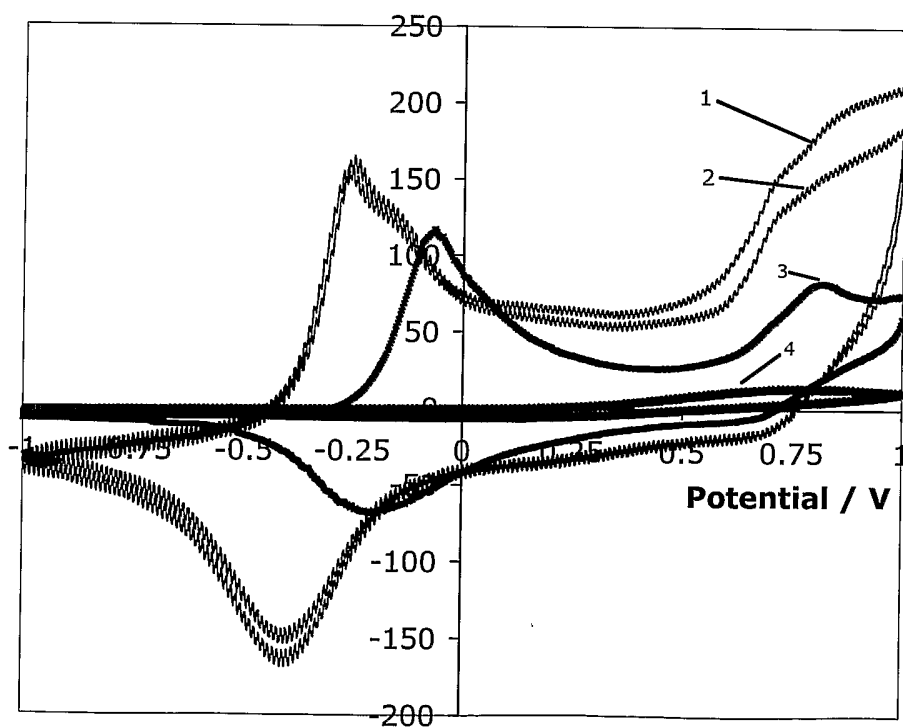
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19. A sensor as claimed in any preceding claim, wherein EDOT is attached to the electrode by being polymerised thereon.
20. A sensor as claimed in any of claims 1 to 18, wherein EDOT is attached to the electrode by chemical oxidation or electrochemical deposition.
21. A method of identifying a target molecule in a sample by using an electrochemical sensor as claimed in any preceding claim.
22. A method of identifying a target molecule in a sample as claimed in claim 21, wherein the method is used to quantify the presence of target molecule in a sample.
23. A method of producing an electrochemical sensor for detecting the presence and/or quantity of a target molecule in a sample comprising the steps of:
  - (a) providing a monomer conjugate comprising EDOT attached to a ligand by means of long chained functional spacing element;
  - (b) contacting the conjugate with an electrode under conditions effective to deposit the conjugate on the surface of the electrode; and
  - (c) repeating step (b) to form produce one or more layers of electrochemical sensors on an electrode.

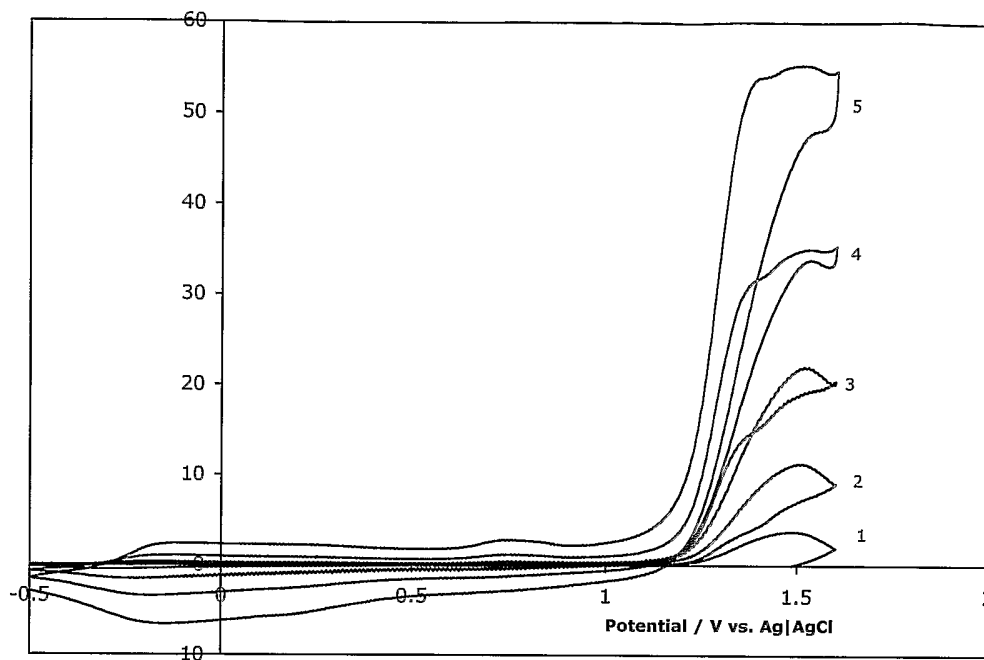
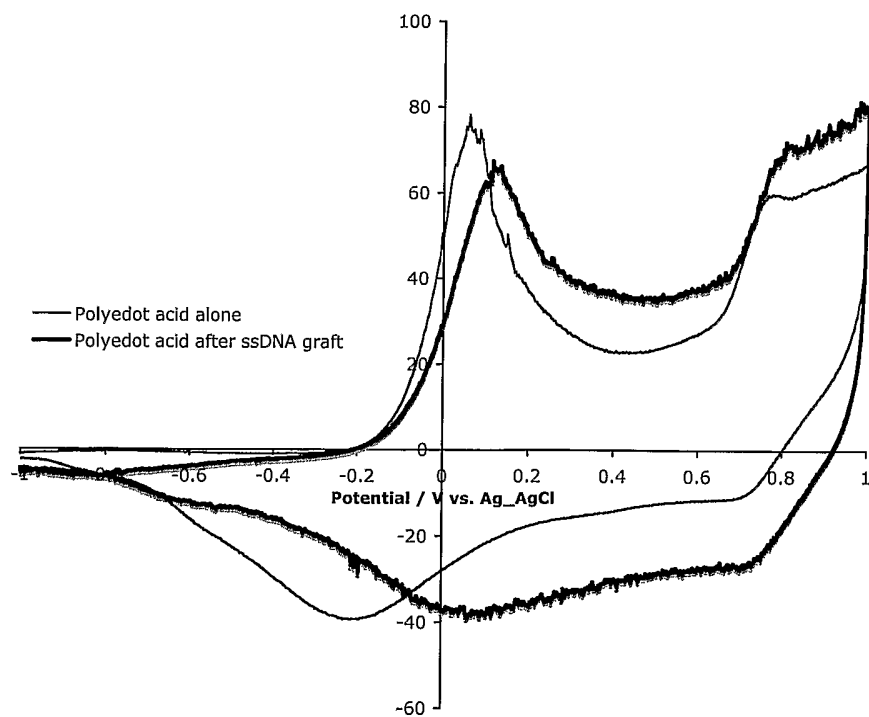
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24. A method as claimed in claim 23, wherein non-conjugate EDOT is also contacted with the electrode in step (b).

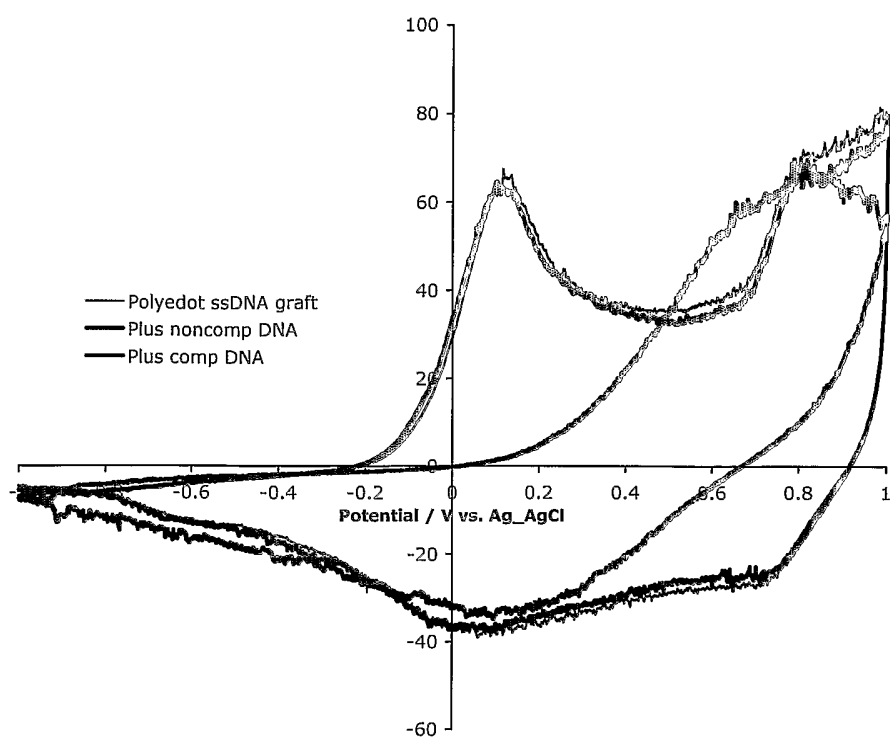
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Figure 1Figure 2

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Figure 3Figure 4

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Figure 5Figure 6