Title: METHODS OF ELECTRICALLY DETECTING A NUCLEIC ACID BY MEANS OF AN ELECTRODE PAIR

Abstract: Methods of electrically detecting a target nucleic acid molecule. A pair of electrodes is arranged at a distance from one another and within a sensing zone. A PNA capture molecule, with a nucleotide sequence that is at least partially complementary to a portion of the target nucleic acid, is immobilised upon an immobilisation unit. The target nucleic acid molecule hybridises and forms a complex with this capture molecule. A polymerisable positively chargeable precursor with an electrostatic net charge complementary to the target nucleic acid is then added. These precursor associates to the complex formed between the PNA capture molecule and the target nucleic acid. Upon addition of a suitable reactant molecule polymerisation is initiated. The electroconductive polymer that is formed influences the electrical characteristics of the region between the electrodes allowing detection of the presence of the target nucleic acid molecule.
METHODS OF ELECTRICALLY DETECTING A NUCLEIC ACID BY MEANS OF AN ELECTRODE PAIR

[0001] The present invention relates to methods of electrically detecting a target nucleic acid molecule by means of an electrode pair.

[0002] The detection and quantification of nucleic acids is a fundamental method not only in analytical chemistry but also in biochemistry, food technology or medicine. The most frequently used methods for determining the presence and concentration of nucleic acids include the detection by autoradiography, fluorescence, chemiluminescence or bioluminescence as well as electrochemical and electrical techniques.

[0003] Different from electrochemical detection methods, electrical detection of nucleic acid molecules relies on the conversion of the base pairing events into detectable electrical signals such as capacitance (Moreno-Hagelsieb, L., et al., Sens. Actuat. B-Chem. (2004) 98, 269-274) and resistance (Park, S. J., et al., Science (2002) 295, 1503-1506). Benefited from the inherent superiorities of electrical transduction methods, nucleic acid biosensors based on the electrical detection are able to provide high performance in terms of accuracy and sensitivity as well as low-cost, miniaturised readout unit and thus exempt from the problems encountered in the optical detection system. In addition, combining with microfabrication technology and microelectronics, microscale electrical detection-based biosensors can be easily constructed, such as silicon nanowire biosensors [1]. Mirkin and co-workers also put forward a successful sample of electrical detection of DNAs with paired microelectrodes separated by a microgap and gold nanoparticle-conjugated oligonucleotide detection probes (Park et al., 2002, supra). The hybridisation of the detection probes to target DNAs led to the aggregation of Au nanoparticles in the gap between the electrodes. A further deposition of silver facilitated by the gold nanoparticles bridged the gap and produced measurable conductivity changes. It was demonstrated that the target DNAs are detected at 500 fM.

[0004] The deposition of electrically active materials on biomacromolecules such as DNA has led to impressive results such as nano-scaled biofunctional matrices as well as highly selective biosensors. An example is the DNA templated deposition of conducting nanoparticles (Torimoto, T., et al., J. Phys. Chem. B (1999) 103, 8799-8803). Benefited from enzymatic polymerisation methods, which provide mild and controllable reaction conditions and allow using delicate biomolecules as templates, electroconductive polymers such as polyaniline have also been fabricated along oligonucleotides and λ-DNA to form nanowires
(Ma, Y., et al., J. Am. Chem. Soc. (2004) 126, 7097-7101), where the phosphate groups of the nucleic acid molecules served as templates, guiding the deposition of polyaniline. An increase of conductivity of the polyaniline could be achieved by a simple doping process with HCl vapour.

[0005] A technique for the specific detection of a selected nucleic acid well established in the art is based on the hybridisation between a nucleic acid capture probe and a target nucleic acid. Typically the respective nucleic acid capture probe is immobilised onto a solid support, and subsequently one of the above mentioned detection methods is employed.

[0006] Sensitivity and selectivity are the two important issues being constantly addressed in the evaluation of nucleic acid detection systems. The most popular methods are performed using fluorescent tags in array formats in conjunction with solution phase (off-chip) pre-amplification/labelling approaches employing polymerase chain reactions. They offer the highest degree of sensitivity, the highest throughput, and the widest dynamic range. However, the amplification power of polymerase chain reactions may be dramatically affected by small variations in experimental conditions and sample compositions. Optimisation of the complicated primers and experimental conditions for each specific gene is a formidable task. Often, the finalised amplification protocols are not optimal for many genes being studied. PCR-based amplification methods therefore do not faithfully reproduce the relative concentrations of genes in complex matrixes, due to selective and nonlinear target amplifications (Ho, H.A., et al., J. Am. Chem. Soc. (2005) 127, 12673-12676). Moreover, optical transduction engages not only highly precise and expensive equipment but also complex algorithms to interpret the data. Furthermore, off-chip target amplifications also significantly increase the cost of the procedures and often lead to sequence-dependent quantification bias.

[0007] Consequently, in situ signal amplification strategies, such as rolling circle amplification (RCA), T7 DNA polymerase, branched DNA technology, catalysed reporter deposition, dendritic tags, enzymatic amplification and chemical amplification, have been proposed for both optical and electrochemical systems. Among them, the inherent miniaturisation of electrochemical devices, excellent compatibility with advanced semiconductor technology, and low cost make electronic transduction-based biosensors very promising candidates for analysing nucleic acids with reduced cost and size of the read-out unit in comparison with the more conventional optical systems. It has been shown that the sensitivity of those assays is comparable to that of PCR-based fluorescent assays. As few as
10^3 copies of genes can be directly detected (Zhang, Y., et al., Anal. Chem. (2003) 75, 3267-3269; Xie, H., et al., Anal. Chem. (2004) 76, 1611-1617). While such improvements may enhance the sensitivity of nucleic acid detection, inherent disadvantages are additional costs for amplification reagents such as primers, as well as the additional time required for signal amplification.

[0008] Thus, there remains a need for an alternative method for the detection of nucleic acids, which overcomes the above limitations and allows the detection even of picomolar levels of a nucleic acid with high sensitivity.

[0009] Accordingly it is an object of the present invention to provide methods for the detection and/or quantification of a nucleic acid, which avoids the discussed signal amplification strategies.

[0010] According to a first aspect, the invention provides methods of electrically detecting a target nucleic acid molecule by means of a pair of electrodes. The electrodes are arranged at a distance from one another. Furthermore the pair of electrodes is arranged within a sensing zone.

[0011] A first respective method includes immobilising on an immobilisation unit a peptide nucleic acid (PNA) capture molecule. The PNA capture molecule has a nucleotide sequence that is at least partially complementary to at least a portion of a strand of the target nucleic acid molecule. The immobilisation unit is arranged within the sensing zone. The method further includes contacting the immobilisation unit with a solution suspected to comprise the target nucleic acid molecule. The method also includes allowing the target nucleic acid molecule to hybridise to the PNA capture molecule on the immobilisation unit. Thereby the method includes allowing the formation of a complex between the PNA capture molecule and the target nucleic acid molecule. Furthermore the method includes adding a polymerisable positively chargeable precursor. The polymerisable positively chargeable precursor has an electrostatic net charge that is complementary to the electrostatic net charge of the target nucleic acid molecule. The polymerisable positively chargeable precursor associates to the complex formed between the PNA capture molecule and the target nucleic acid molecule. Furthermore, the polymerisation of the precursor can be carried out by means of a suitable reactant molecule. The method further includes adding a suitable reactant molecule, thereby initiating the polymerisation of the polymerisable positively chargeable precursor. Thereby an electroconductive polymer (i.e. generally a conducting polymer) is formed from the polymerisable precursor. This electroconductive polymer is associated with
the complex formed between the PNA capture molecule and the target nucleic acid molecule. Further, the method includes determining the presence of the target nucleic acid molecule based on an electrical characteristic of a region in between the electrodes. The electrical characteristic is influenced by the electroconductive polymer.

[0012] According to a particular embodiment, the method includes exposing the reactant molecule to a suitable catalyst. The catalyst may be light, a metal chloride, a metal bromide, a metal sulphate or an enzyme. In such embodiments the reactant molecule may be a substrate molecule for the catalyst.

[0013] A second respective method includes immobilising on an immobilisation unit a nucleic acid capture molecule. The nucleic acid capture molecule has a nucleotide sequence that is at least partially complementary to at least a portion of a strand of the target nucleic acid molecule. The immobilisation unit is arranged within the sensing zone. The method further includes contacting the immobilisation unit with a solution suspected to comprise the target nucleic acid molecule. The method also includes allowing the target nucleic acid molecule to hybridise to the nucleic acid capture molecule on the surface of the immobilisation unit. Thereby the method includes allowing the formation of a complex between the nucleic acid capture molecule and the target nucleic acid molecule. Furthermore, the method includes adding a polymerisable positively chargeable precursor. The polymerisable positively chargeable precursor has an electrostatic net charge that is complementary to the electrostatic net charge of the target nucleic acid molecule. The polymerisable positively chargeable precursor associates to the complex formed between the nucleic acid capture molecule and the target nucleic acid molecule. Furthermore, the polymerisation of the precursor can be carried out by means of a suitable reactant molecule. The method further includes adding a suitable substrate molecule. The method also includes adding an enzyme attached to a probe nucleic acid molecule. The probe nucleic acid molecule is at least partially complementary to at least a portion of the target nucleic acid molecule. The detection probe hybridises to a portion of the target nucleic acid that is different from the portion to which the nucleic acid capture molecule hybridises. The method thereby includes allowing the probe nucleic acid molecule to hybridise to the target nucleic acid molecule. The method thereby also includes catalysing the polymerisation of the polymerisable positively chargeable precursor. Thereby an electroconductive polymer (i.e. generally a conducting polymer) is formed from the polymerisable precursor. This electroconductive polymer is associated with the complex formed between the nucleic acid capture molecule and the target nucleic acid molecule. Further, the method includes
determining the presence of the target nucleic acid molecule based on an electrical characteristic of a region in between the electrodes. The electrical characteristic is influenced by the electroconductive polymer.

[0014] According to a further aspect, the invention provides a kit for electrically detecting of a target nucleic acid molecule.

[0015] In a first embodiment a kit includes a pair of electrodes. The electrodes are arranged at a distance from one another. Furthermore the pair of electrodes is arranged within a sensing zone. The respective kit further includes an immobilisation unit. The immobilisation unit is arranged within the sensing zone. The kit also includes a PNA capture molecule. This PNA capture molecule has a nucleotide sequence that is at least partially complementary to at least a portion of the target nucleic acid molecule. The kit also includes a polymerisable positively chargeable precursor. The electrostatic net charge of the polymerisable positively chargeable precursor is complementary to the electrostatic net charge of the target nucleic acid molecule. Furthermore the kit includes a suitable reactant molecule. In some embodiments the kit also includes a catalyst.

[0016] In a second embodiment a kit includes a pair of electrodes. The electrodes are arranged at a distance from one another. Furthermore the pair of electrodes is arranged within a sensing zone. The respective kit further includes an immobilisation unit. The immobilisation unit is arranged within the sensing zone. The kit also includes a nucleic acid capture molecule. This nucleic acid capture molecule has a nucleotide sequence that is at least partially complementary to at least a portion of the target nucleic acid molecule. The kit also includes a polymerisable positively chargeable precursor. The electrostatic net charge of the polymerisable positively chargeable precursor is complementary to the electrostatic net charge of the target nucleic acid molecule. Furthermore the kit includes a suitable substrate molecule. Furthermore, the kit includes an enzyme attached to a probe nucleic acid molecule. The probe nucleic acid molecule is at least partially complementary to at least a portion of the target nucleic acid molecule.

[0017] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0018] Figure 1 depicts a (nano)gapped microelectrodes array that may be used in a method of the present invention. The microarray of Fig. IA includes 10x10 pairs of nanogapped microelectrodes fabricated on 1.2 x 1.2 cm² silicon wafer. It was fabricated as a
10x10 array on a silicon chip with 500 nm coating of SiC by standard photolithography.

Fig. 1B depicts a schematic illustration of the pairs of nanogapped microelectrodes. A power source can be connected via contacting pads (11) so that by applying an electric current an electric field between the microelectrodes can be generated. Fig. 1C shows a SEM (JEOL-6000 Field emission scan electron microscope) image of the nanogapped microelectrodes.

The chip included 100 pairs of interlocking comb-like microelectrodes (gold 15 nm, titanium 10 nm) with 150-200 fingers, each 700 nm wide and 200 µm long, and with a 300-nm gap (appearing dark on the photo). Parallel connection potentiates the sensitivity of e.g. resistance measurements.

[0019] Figure 2 depicts a schematic representation of an exemplary method of the present invention. A PNA capture molecule (3) is immobilised on an immobilisation unit (5). The immobilisation unit is contacted with a solution suspected or known to include a target nucleic acid molecule (2). The two nucleic acid molecules hybridise. The polymerisable positively charged precursor molecule aniline and the reactant molecule hydrogen peroxide, as well as an optional catalyst, are added. The polymerisable positively charged precursor molecule associates to the complex of PNA capture molecule (3) and target nucleic acid molecule (2). The positively charged precursor polymerises, forming an electroconductive polymer. An electrical characteristic of a region in between a pair of electrodes (1) can be used to determine the presence of the target nucleic acid molecule (2).

[0020] Figure 3 depicts a schematic representation of a further exemplary method of the present invention. A nucleic acid capture molecule (4) is immobilised on an immobilisation unit (5). The immobilisation unit is contacted with a solution suspected or known to include a target nucleic acid molecule (2). The two nucleic acid molecules hybridise. An enzyme attached to a probe nucleic acid molecule (6) is added. The probe nucleic acid molecule (6) hybridises to the target nucleic acid molecule (2) at a portion different from the portion where the nucleic acid capture molecule (4) hybridises. The polymerisable positively charged precursor molecule aniline and the reactant molecule hydrogen peroxide are added. The polymerisable positively charged precursor molecule associates to the complex of nucleic acid capture molecule (4), target nucleic acid molecule (2) and probe nucleic acid molecule (6). The positively charged precursor polymerises, forming an electroconductive polymer. An electrical characteristic of a region in between a pair of electrodes (1) can be used to determine the presence of the target nucleic acid molecule (2).

[0021] Figure 4 illustrates examples of an electrode arrangement (1) and an immobilisation unit (5), on which a nucleic acid capture molecule (4) is immobilised. Fig.
4A: Three ring-shaped electrodes (1) are provided. The immobilisation unit (5) is arranged in such a way that its location partly overlaps with the region between two of the electrodes (1). Fig. 4B: Two interdigital electrodes (1) are provided. The immobilisation unit (5), on which a nucleic acid capture molecule (4) is immobilised, is arranged in vicinity to the electrodes (1) such that the electrical characteristics of the region within electrode areas opposing each can be influenced by a complex formed between an immobilised nucleic acid molecule and the electrically conducting polymer. Fig. 4C depicts in top view an arrangement of two interdigital electrodes (1) that resembles the arrangement depicted in Fig. 4B. The immobilisation unit (5), on which a nucleic acid capture molecule (4) is immobilised, is arranged below the electrodes (1). Fig. 4D: An array of electrodes (1) is provided. The array of electrodes (1) defines a region in between them. The immobilisation unit (5) is arranged in vicinity to the electrodes (1), so that the nucleic acid capture molecule (4) is capable of taking an orientation in which its location partly overlaps with the region between two of the electrodes (1). A nucleic acid molecule (not shown) hybridising with the nucleic acid capture molecule (4) will therefore typically take an orientation in which it is essentially located in the region defined by the array of electrodes (1).

[0022] Figure 5 shows examples of aromatic amines that may serve as a substrate molecule: A: Aniline, Chemical Abstracts No. 62-53-3; B: 3-methylaniline, Chemical Abstracts No. 108-44-1; C: 3,4-pyridinediamine, CAS-No. 54-96-6; D: 5-(5-oxazolyl)-3-pyridinamine, CAS-No. 893566-28-4; E: 1-aminodibenzofuran, CAS-No. 50548-40-8; F: 4-amino-fluoren-9-one, CAS-No. 4269-15-2; G: 4-amino-2-phenyl-indene-1,3(2H)-dione, CAS-No. 6795-96-6; H: 2-acetyl-4-amino-indene^1,3(2H)-dione, CAS-No. 25125-06-8; I: fluorine-1,9-diamine, CAS-No. 15824-95-0; J: 1-amino-fluoren-9-ol, CAS-No. 6957-58-0; K: 9-anthraceneamine, CAS-No. 779-03-3; L: lO-phenyl-9-anthraceneamine, CAS-No 1718-54-3; M: 4-[[4-(aminomethyl)phenyl]methoxy]-2-pyrimidinamin, CAS-No. 913817-37-5; N: 4,Il-diamino-naphth[2,3-f]isoindole-1,3,5,10(2H)-tetrone, CAS-No. 128-81-4; O: pyrrole, CAS-No. 109-97-7; P: 3-methanamine-pyrrol, CAS-No. 888473-50-5; Q: napht[2,3-f]isoindole, CAS-No. 259-05-4; R: 2-(lH-indol-5-yl)-pyrrolo[2,3-b]pyridin-4-ol, CAS-No. 913698-79-0; S: 2-(1,3-dihydro-3-oxo-mdol-2-ylidene)-l,2-dihydro-indol-3-one, CAS-No. 482-89-3; T: 1,2,3,4-tetrahydro-6-methyl-cyclopent[b]indole, CAS-No. 887122-89-6; U: imidazole, CAS-No. 288-32-4; V: 2-(benzimidazol-2-yl)-N-methyl-3-pyridinamine, CAS-No. 500857-92-1; W: 3-(4-pyridinyl)-indol-7-amine, CAS-No. 887615-82-9; X: 3’,4’,5-triethyl-4-methyl-[2,2’-bi-pyrrole]-3-carboxylic acid ethyl ester, CAS-No. 6798-16-79-6.

[0023] Figure 6 depicts a comparison of the conductivity of the space defined by the
distance between a pair of electrodes used in the method of the present invention. Blank: A PNA capture molecule was immobilised on an immobilisation unit, but not exposed to a target nucleic acid molecule, and neither to the positively chargeable precursor aniline; Control: A PNA capture molecule was immobilised on an immobilisation unit and a solution that included a non-complementary DNA molecule contacted therewith. The positively chargeable precursor aniline, horseraddish peroxidase and hydrogen peroxide were added. The formed electroconductive polyaniline polymer was contacted with the enhancer reagent HCl (doped) before determining the presence of the target nucleic acid molecule. Target DNA: A PNA capture molecule was immobilised on an immobilisation unit and a solution that included a complementary DNA molecule contacted therewith. The positively chargeable precursor aniline, horseraddish peroxidase and hydrogen peroxide were added. The formed electroconductive polyaniline polymer was contacted with the enhancer reagent HCl (doped) before determining the presence of the target nucleic acid molecule.

[0024] Figure 7 depicts scanning electron microscope (SEM) images of chip surface for (A), hybridisation with non-complementary DNA and deposition with polyaniline and (B), hybridisation with complementary DNA and deposition with polyaniline. SEM was performed with JEOL 4000 field emission scan electron microscope.

[0025] Figure 8 illustrates the influence of the concentration of the polymerisable positively charged precursor molecule aniline on conductivity of polyaniline. DNA was used as the target nucleic acid molecule for hybridisation at 10 pM. Horse raddish peroxidase was used as a catalyst, and H₂O₂ as the reactant molecule. The concentration of horse raddish peroxidase was 1 μg/mL. H₂O₂ was used in the same concentration as aniline. The incubation time was set to 40 min.

[0026] Figure 9 illustrates the influence of the concentration of the catalyst horse radish peroxidase (HRP) on the conductivity of polyaniline. DNA was used as the target nucleic acid molecule for hybridisation at 10 pM and the concentration of aniline and H₂O₂ was 1 μg/mL. The incubation time was set to 40 min.

[0027] Figure 10 depicts an optimisation of the incubation time for polyaniline deposition. DNA was used as the target nucleic acid molecule for hybridisation at 10 pM. The catalyst horse radish peroxidase was used at 1 μg/mL and the concentration of aniline was 2 mM.

[0028] Figure 11 shows a linear relation between conductance and the concentration of the target nucleic acid molecule DNA. DNA concentration ranged from 50 fM to 100 pM.
Polyaniline deposition was performed under the optimised conditions.

[0029] Figure 12 depicts discriminating between complementary and single base mismatched DNA. The hybridisation with complementary and single base mismatched DNA sample was carried out at 10 pM and 100 pM. Polyaniline deposition was performed with the same procedure for both samples.

[0030] The methods of the present invention allow for the detection of any target nucleic acid molecule. As used herein, the term "detecting", "detect" or "detection" refers to measurements which provide an indication of the presence or absence, either qualitatively or quantitatively, of a target nucleic acid molecule. Accordingly, the term encompasses quantitative measurements of the concentration of a target nucleic acid molecule in a sample, as well as qualitative measurements in which for instance different types of target nucleic acid molecules in a given sample are identified, or, as a further example, the behaviour of a particular nucleic acid molecule in a given environment is observed. A target nucleic acid is typically considered to be detected if a value of a measurement of an electrical characteristic exceeds a given threshold value to a reference measurement. If the threshold value is reached in the measurement, then it is typically concluded that the target nucleic acid is absent (which however means detected in the context of the present invention) from the analyte that is investigated.

[0031] For obtaining a value of the measurement of a characteristic of the electrical field in a method of the invention (either when detecting the target nucleic acid or when carrying out the reference measurement) typically a first measurement is carried out at the electrodes before adding the polymerisable positively chargeable precursor, and a second measurement is carried out after having added the reactant molecule that initiates the polymerisation of the polymerisable positively chargeable precursor. The first and the second measurement are then compared and the value of the measurement is the result of this comparison.

[0032] The term "nucleic acid molecule" as used herein refers to any nucleic acid in any possible configuration, such as single stranded, double stranded or a combination thereof. Nucleic acids include for instance DNA molecules, RNA molecules, analogues of the DNA or RNA generated using nucleotide analogues or using nucleic acid chemistry, locked nucleic acid molecules (LNA), and protein nucleic acids molecules (PNA). DNA or RNA may be of genomic or synthetic origin and may be single or double stranded. Such nucleic acid can be e.g. mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, a copolymer of DNA and RNA, oligonucleotides, etc. A respective nucleic acid may furthermore contain
non-natural nucleotide analogues and/or be linked to an affinity tag or a label.

[0033] Many nucleotide analogues are known and can be used in nucleic acids used in the methods of the invention. A nucleotide analogue is a nucleotide containing a modification at for instance the base, sugar, or phosphate moieties. As an illustrative example, a substitution of 2'-OH residues of siRNA with 2'F, 2'0-Me or 2'H residues is known to improve the in vivo stability of the respective RNA. Modifications at the base moiety include natural and synthetic modifications of A, C, G, and T/U, different purine or pyrimidine bases, such as uracil-5-yl, hypoxanthin-9-yl, and 2-aminoadenin-9-yl, as well as non-purine or non-pyrimidine nucleotide bases. Other nucleotide analogues serve as universal bases. Universal bases include 3-nitropyrrrole and 5-nitroindole. Universal bases are able to form a base pair with any other base. Base modifications often can be combined with for example a sugar modification, such as for instance 2'-O-methoxyethyl, e.g. to achieve unique properties such as increased duplex stability.

[0034] The target nucleic acid molecule may be included in any analyte/sample of any origin. It may for instance, but not limited to, be derived from human or non-human animals, plants, bacteria, viruses, spores, fungi, or protozoa, or from organic or inorganic material of synthetic or biological origin. Accordingly, any of the following samples selected from, but not limited to, the group consisting of a soil sample, an air sample, an environmental sample, a cell culture sample, a bone marrow sample, a rainfall sample, a fallout sample, a sewage sample, a ground water sample, an abrasion sample, an archaeological sample, a food sample, a blood sample, a serum sample, a plasma sample, an urine sample, a stool sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, a nasopharyngeal wash sample, a sputum sample, a mouth swab sample, a throat swab sample, a nasal swab sample, a bronchoalveolar lavage sample, a bronchial secretion sample, a milk sample, an amniotic fluid sample, a biopsy sample, a cancer sample, a tumour sample, a tissue sample, a cell sample, a cell culture sample, a cell lysate sample, a virus culture sample, a nail sample, a hair sample, a skin sample, a forensic sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, a space sample, an extraterrestrial sample or any combination thereof may be processed in a method of the invention. Where desired, a respective sample may have been pre-processed to any degree. As an illustrative example, a tissue sample may have been digested, homogenised or centrifuged prior to being used with the device of the present invention. The sample may furthermore have been prepared in form
of a fluid, such as a solution. Examples include, but are not limited to, a solution or a slurry of a nucleotide, a polynucleotide, a nucleic acid, a peptide, a polypeptide, an amino acid, a protein, a synthetic polymer, a biochemical composition, an organic chemical composition, an inorganic chemical composition, a metal, a lipid, a carbohydrate, a combinatorial chemistry product, a drug candidate molecule, a drug molecule, a drug metabolite or of any combinations thereof. Further examples include, but are not limited to, a suspension of a metal, a suspension of metal alloy, and a solution of a metal ion or any combination thereof, as well as a suspension of a cell, a virus, a microorganism, a pathogen, a radioactive compound or of any combinations thereof. It is understood that a sample may furthermore include any combination of the aforementioned examples. As an illustrative example, the sample that includes the target nucleic acid molecule may be a mammal sample, for example a human or mouse sample, such as a sample of total mRNA.

[0035] In some embodiments the sample is a fluid sample, such as a liquid or a gas. In other embodiments the sample is solid. In case of a solid or gaseous sample, an extraction by standard techniques known in the art may be carried out in order to dissolve the target nucleic acid molecule in a solvent. Accordingly, the target nucleic acid molecule, or the expected target nucleic acid molecule, is provided in form of a solution for the use in the present invention. As an illustrative example, the target nucleic acid molecule may be provided in form of an aqueous solution.

[0036] Where desired, further matter may be added to the respective solution, for example dissolved or suspended therein. As an illustrative example an aqueous solution may include one or more buffer compounds. Numerous buffer compounds are used in the art and may be used to carry out the various processes described herein. Examples of buffers include, but are not limited to, solutions of salts of phosphate, carbonate, succinate, carbonate, citrate, acetate, formate, barbiturate, oxalate, lactate, phthalate, maleate, cacodylate, borate, N-(2-acetamido)-2-amirio-ethanesulfonate (also called (ACES), N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (also called HEPES), 4-(2-hydroxyethyl)-1-piperazine-propanesulfonic acid (also called HEPPS), piperazine-1,4-bis(2-ethanesulfonic acid) (also called PIPES), 2-[Ms(hydroxymemyl)-memylamino]-1-ethansulfonic acid (also called TES), 2-cyclohexylamino-ethansulfonic acid (also called CHES) and N-(2-acetamido)-iminodiacetate (also called ADA). Any counter ion may be used in these salts; ammonium, sodium, and potassium may serve as illustrative examples. Further examples of buffers include, but are not limited to, triethanolamine, diethanolamine, ethylamine, triethylamine, glycine, glycylglycine, histidine, tris(hydroxymethyl)aminomethane (also
called TRIS), bis-(2-hydroxyethyl)-imino-tris(hydroxymethyl)methane (also called BIS-TRIS), and N-[Tris(hydroxyriietliyl)-methyl]-glycine (also called TRICINE), to name a few. A respective buffer may be an aqueous solution of such buffer compound or a solution in a suitable polar organic solvent. One or more respective solutions may be used to accommodate the suspected target nucleic acid as well as other matter used, throughout an entire method of the present invention.

[0037] Further examples of matter that may be added, include salts, detergents or chelating compounds. As yet a further illustrative example, nuclease inhibitors may need to be added in order to maintain a nucleic acid molecule in an intact state. While it is understood that for the purpose of detection any matter added should not obviate the formation of a complex between the PNA capture molecule (or other nucleic acid capture molecule used in another method of the invention, see below) and the target nucleic acid molecule, for the purpose of carrying out a control measurement a respective agent may be used that blocks said complex formation.

[0038] The methods of the present invention allow detecting a target nucleic acid molecule by means of an electrode arrangement such as a pair of electrodes. The term "electrode" as used herein is employed in its conventional sense, thereby referring to an object that is capable of serving as an electric conductor, through which an electrical current or voltage may be brought into and/or out of a medium in contact with the electrode. Typically an electrode is one of at least two terminals of an electrically conducting medium. The term "electrode arrangement" or "pair of electrodes" as used herein refers to any number of electrodes of two or higher. Accordingly, two or more electrodes are provided in the method (as well as the kits, see below) of the invention. The electrodes are arranged at a distance from one another. In embodiments where two electrodes are provided, the two electrodes may for instance be separated by a gap. In such embodiments the two electrodes of this pair of electrodes may face each other across the gap. In some embodiments the two electrodes are at least essentially parallel. The electrodes may be of any desired dimension and shape. They may for example have the shape of a flat, arched, concave or convex slab. In some embodiments they may have the shape of a ring (for an example see Green, BJ, & Hudson, J.L., Phys. Rev. E (2001), 63, 026214; see also Fig. 4A). In some embodiments interdigital electrodes are provided, which typically include a digitlike or fingerlike pattern of parallel in-plane electrodes (see Mamishev, A.V., Proc. IEEE (2004), 92, 5, 808-845, or Matsue, T., Trends Anal. Chem. (1993), 12, 3, 100 - 108 for examples; see also Fig. 4B and Fig. 4C). hi some embodiments an array of electrodes may be provided. If desired, one or
more floating electrodes may be used. In some embodiments the electrodes that are provided are of similar size, for example of identical size.

[0039] The distance between the two or more electrodes (to which is also referred herein as gap) may be of any dimension, as long as the change of an electrical characteristic of the respective region can be determined in the method of the present invention (see below), so that a detection of a target nucleic acid molecule can be carried out. In some embodiments where more than two electrodes are provided, the distance at which the electrodes are arranged may be identical between each of the respective electrodes. In other such embodiments the distance at which the electrodes are arranged may be identical between some of the respective electrodes. In yet other embodiments where more than two electrodes are provided, each distance at which two electrodes are arranged may be different from another distance at which two electrodes are arranged.

[0040] As an illustrative example the distance at which the electrodes are arranged, for instance a gap between two electrodes, may be in a range that corresponds to the length of the target nucleic acid molecule. It is noted in this regard that for instance a linearised chromosome may have a length of up to 1.5 m (http://hypertextbook.com/facts/1998/StevenChen.shtml). As a further illustration, already Watson and Crick were able to determine the distance between the two strands of DNA as 2 nanometres. From their DNA model the vertical rise per base pair along the axis of a DNA molecule can be calculated to be 0.34 nm. Typical DNA molecules in human blood plasma have furthermore been reported to be of a length of 100 to 900 nm (http://cat.inist.fr/?aModel=afficheN&cpsidt=2324077). In some embodiments the distance at which the electrodes are arranged is of the same or a smaller length than the length of the target nucleic acid molecule. In such embodiments the target nucleic acid molecule is capable of spanning the respective gap. A respective distance, e.g. a gap, may for instance have a with selected in the range of about 0.5 nm to about 10 µm, such as a range of about 1 nm, or about 10 nm to about 200 nm, about 300 nm, about 500 nm, about 700 nm, about 800 nm or about 1 µm or 2 µm. As two illustrative examples, a distance of 30 nm may be selected, which would roughly correspond to a length of a linear nucleic acid of about 100 bp. (Such an estimate can be made based on the known helical pitch of ideal A, B and Z DNA for example. B DNA, for example, has a height of 0.34 nm per helical turn and base pair so that 10 base pairs (bp) bridge a distance of 3.4 nm). Alternatively, the distance with can be determined empirically for longer non linear nucleic acids; a distance of 200 nm, may roughly correspond to a length of a nucleic acid of about 2000 to 5000 bp.
[0041] As an illustrative example, a target nucleic acid molecule may be of for instance 100 - 500 nm, which is for example of a sufficient size of a nucleic acid molecule to includes exemplary genes. The PNA capture molecule may in such an embodiment be immobilised in vicinity to the region in between the electrodes, or even within the respective region. In embodiments where this region in between the electrodes is defined by a small distance separating the electrodes, such as e.g. about 20 - about 30 nm, the size of such a target nucleic acid molecule will allow the target nucleic acid molecule to bridge the respective distance between the electrodes (e.g. a gap). Thus, the present invention provides a method by which a single target nucleic acid molecule can be detected.

[0042] The sensing zone is usually a region or aperture into which the target nucleic acid molecule is caused to be located. As two illustrative examples, the sensing zone may be a region or aperture to which the target nucleic acid molecule is caused to flow or into which the target nucleic acid molecule is disposed. In typical embodiments the sensing zone is defined by the zone in which an electric field of the pair of electrodes is effective.

[0043] In the method of the invention a capture molecule (see below) is used that is capable of associating with the target nucleic acid molecule. The capture molecule is immobilised on the immobilisation unit, generally on a surface or a part of a surface of an immobilisation unit. The respective surface (or surface part) of the immobilisation unit is arranged within the sensing zone. In some embodiments at least a part of the respective surface of the immobilisation unit is arranged in a zone where an electric field of the pair of electrodes is effective. In some embodiments upon immobilisation of the capture molecule at least a part thereof is included in the region defined by the distance between the (or some of the) electrodes (see e.g. Fig. 2 or Fig 4D).

[0044] The surface of the immobilisation unit may be of any material as long as an electrical detection can be carried out. As an illustrative example, the surface of the immobilisation unit may include or consist of an electric insulator. It maybe desired to select the material of the immobilisation unit in order to immobilise a nucleic acid thereon (see also below). The surface of the immobilisation unit, or a part thereof, may also be altered, e.g. by means of a treatment carried out to alter characteristics of the solid surface. Such a treatment may include various means, such as mechanical, thermal, electrical or chemical means. As an illustrative example, the surface properties of any hydrophobic surface can be rendered hydrophilic by coating with a hydrophilic polymer or by treatment with surfactants. Examples of a chemical surface treatment include, but are not limited to exposure to hexamethyldisilazane, trimethylchlorosilane, dimethyldichlorosilane, propyltrichlorosilane,
tetraethoxysilane, glycidoxypropyltrimethoxy silane, 3-aminopropyltriethoxysilane, 2-(3,4-epoxy cyclohexyl)ethyltrimethoxysilane, 3-(2,3-epoxy propoxy)propyltrimethoxysilane, polydimethylsiloxane (PDMS), \( \gamma \)-3,4-epoxy-cyclohexyl)ethyltrimethoxysilane, poly(methyl methacrylate) or a poly(methylacrylate) co-polymer, urethane, polyurethane, fluoropolyacrylate, poly(methoxy polyethylene glycol raethacrylate), poly(dimethyl acrylamide), poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA), \( \alpha \)-phosphorylcholine-o-(N,N-diethylthiocarbamyl)vindecyl oligoDMAAm-oligo-STblock co-oligomer (cf. e.g. Matsuda, T., et al., *Biomaterials*, (2003), 24, 4517-4527), poly(3,4-epoxy-1-butene), 3,4-epoxy-cyclohexyl-methylmethacrylate, 2,2-bis[4-(2,3-epoxy propoxy) phenyl] propane, 3,4-epoxy-cyclohexyl-methylacrylate, (3',4'-epoxycyclohexylmethyl)-3,4-epoxycyclohexyl carboxylate, di-(3,4-epoxycyclohexylmethyl)adipate, bisphenol A (2,2-bis-(p-(2,3-epoxy propoxy) phenyl) propane) or 2,3-epoxy-1-propanol.

[0045] In some embodiments the surface may for instance be coated with an electroconductive polymer, such as polypyrrole (Wang, J., et al., *Anal. Chem.* (1999) 71, 18, 4095-4099; Wang, J., et al., *Anal. CMm. Acta* (1999) 402, 7-12), polystyrene, polyaniline, polyacetylene, poly(N-vinyl carbazole), or a copolymer such as a copolymer of pyrrole and thiophene or a copolymer of juglone and 5-hydroxy-3-thioacetic-1,4-naphthoquinone (Reisberg, S., et al., *Anal. Chem.* (2005) 77, 10, 3351 -3356). In embodiments where the surface is a surface of a carbon paste electrode, it may for example be modified with carboxyl groups by mixing stearic acid with the paste. The linking molecule ethylenediamine may for instance be immobilised on a respective electrode in order to facilitate the subsequent immobilisation of a capture molecule (see below).

[0046] For detecting a target nucleic acid, the electrical characteristic of the region in between the electrode arrangement must be influenced by the electrically conducting polymer associated with the target nucleic acid, should this polymer be formed. For this it is sufficient that that the immobilisation unit, or at least the surface or a part of the surface thereof, is located in vicinity to the electrodes of the electron pair. In these embodiments, the (immobilised) complex of the nucleic acid molecule (in particular a nucleic acid molecule with a size of several thousands or more base pairs) with the electrically conducting polymer may, for example, swing by Brownian motion with their flexible parts into the distance in between the electrodes. Alternatively, the electrical interaction between the electrically conducting polymer and an electrical field applied at the electrodes can alone also be sufficient to influence the electrical characteristics in the gap in between the electrodes in a detectable manner. In alternative embodiments the respective immobilisation surface of the
immobilisation unit is arranged within the respective region defined by the distance between
the (or some of the) electrodes. In some further embodiments the immobilisation surface is
included on one of the electrode (e.g. a detection electrode). A respective detection electrode
may for example be used for the detection of an electric signal in the method of the present
invention (see below). As an illustrative example, a respective detection electrode may be
used for the generation of an electric field. In some embodiments the surface is conductively
connected to an electrode.

[0047] One method of the invention includes immobilising on the immobilisation
unit, or at least the surface or a part of the surface thereof, a peptide nucleic acid (PNA)
capture molecule. As indicated above, a PNA molecule is a nucleic acid molecule in which
the backbone is a pseudopeptide rather than a sugar. Accordingly, PNA generally has a
charge neutral backbone, in contrast to DNA or RNA. Nevertheless, PNA is capable of
hybridising at least complementary and substantially complementary nucleic acid strands,
just as e.g. DNA or RNA (to which PNA is considered a structural mimic).

[0048] The PNA capture molecule, as well as other nucleic acid capture molecules
used in other methods according to the present invention, may be of any suitable length. In
some embodiments the PNA capture molecule or other nucleic acid capture molecule has a
nucleic acid sequence of a length of about 7 to about 30 bp, for example a length of about 9
to about 25 bp, such as a length of about 10 to about 20 bp.

[0049] The PNA capture molecule used in the present invention has a nucleotide
sequence that is at least partially complementary to at least a portion of the target nucleic
acid molecule. The respective nucleotide sequence of the PNA capture molecule may for
example be 70, for example 80 or 85, including 100 % complementary to another nucleic
acid sequence. The higher the percentage to which the two sequences are complementary to
each other (i.e. the lower the number of mismatches), the higher is typically the sensitivity of
the method of the invention. In typical embodiments the respective nucleotide sequence is
substantially complementary to at least a portion of the target nucleic acid molecule.
"Substantially complementary" as used herein refers to the fact that a given nucleic acid
sequence is at least 90, for instance 95, such as 100 % complementary to another nucleic
acid sequence. The term "complementary" or "complement" refers to two nucleotides that
can form multiple favourable interactions with one another. Such favourable interactions
include Watson-Crick base pairing. As an illustrative example, in two given nucleic acid
molecules (e.g. DNA molecules) the base adenosine is complementary to thymine, while the
base cytosine is complementary to guanine. A nucleotide sequence is the complement of
another nucleotide sequence if all of the nucleotides of the first sequence are complementary to all of the nucleotides of the second sequence. Accordingly, the respective nucleotide sequence will specifically hybridise to the respective portion of the target nucleic acid molecule under suitable hybridisation assay conditions, in particular of ionic strength and temperature. Where desired, more than one PNA capture molecule may be immobilised. This may for instance be desired in order to broadly screen for the presence of any of a group of selected target nucleic acid sequences. The use of more than one PNA capture molecule may also be desired for the detection of the same target nucleic acid molecule via different recognition sequences, e.g., the 5'- and 3'-termini thereof, which enhances the likelihood to detect even a few copies of a target nucleic acid molecule in a sample.

[0050] Any target nucleic acid molecule that is capable of hybridising to at least a part of the PNA-capture molecule can be detected by the method of the present invention. The skilled artisan will appreciate that the method of the present invention allows the detection of small numbers of target nucleic acid molecules, including a single target nucleic acid molecule (see below). The present method thus redundantises the need of conventional nucleic acid detection methods of amplifying nucleic acid molecules prior to detection, in cases where it is desired to detect such low numbers of target molecules. The target nucleic acid molecule that can be detected may be of any length. In some embodiments it may be selected to be of a size of about 50 bp to about 5 \times 10^6 bp, such as a size of about 50 bp to about 1 \times 10^5 bp or about 50 bp to about 5 \times 10^3 bp. In other embodiments it may be selected to be of a size of about 100 bp to about 5 \times 10^6 bp, such as a size of about 100 bp to about 1 \times 10^4 bp or about 100 bp to about 1 \times 10^3 bp.

[0051] In typical embodiments the target nucleic acid molecule includes a predefined sequence, hi some embodiments the target nucleic acid molecule furthermore includes at least one single-stranded region. In such embodiments it may be desirable to select a single-stranded region as the predefined sequence. In this case the PNA capture molecule can directly form Watson-Crick base pairs with the target nucleic acid molecule, without the requirement of separating complementary strands of the target nucleic acid molecule. Where the target nucleic acid molecule, or a region thereon that includes a predefined sequence, is provided or suspected to be in double strand form, the respective nucleic acid duplex may be separated by any standard technique used in the art, for instance by increasing the temperature (e.g. 95 °C, see also the Examples below). In embodiments where multiple sequences may be included in the target nucleic acid molecule, multiple respective PNA capture molecules may be used, each of which being at least partially
complementary to e.g. a selected portion of the target nucleic acid molecule (see also below).
It is of course also possible to detect a plurality of nucleic acid molecules by using a plurality
(that means at least two) different capture molecules each being complementary to a specific
target nucleic acid. As an illustrative example, if dengue virus nucleic acid is to be detected
using the present invention, four different capture molecules each of which is specific for
each of the four dengue virus sub-strains can be immobilised on sensing units being arranged
in different locations of an interdigitated electrode arrangement.

[0052] The PNA capture molecule may be immobilised on the immobilisation unit at
any stage during the present method of the invention. As two examples, it may be
immobilised at the beginning of the method or before adding a polymerisable positively
charged precursor (see below). In typical embodiments it is immobilised before performing
an electrical measurement (see below). The PNA capture molecule may be immobilised by
any means. It may be immobilised on the entire surface, or a selected portion of the surface
of the immobilisation unit. In some embodiments the PNA capture molecule is provided first
and thereafter immobilised onto the surface of the immobilisation unit. An illustrative
example is the mechanical spotting of the PNA capture molecule onto the surface of the
immobilisation unit. This spotting may be carried out manually, e.g. by means of a pipette,
or automatically, e.g. by means of a micro robot. As an illustrative example, the polypeptide
backbone of the PNA capture molecule may be covalently linked to a gold surface via a thio-
ether-bond.

[0053] The surface of the immobilisation unit may be activated prior to immobilising
the PNA capture molecule thereon, for instance in order to facilitate the attachment reaction
(see also above). Where an immobilisation unit of glass is used, the respective surface may
for example be modified with aminophenyl or aminopropyl silanes. 5'-succinylated PNA
capture molecules (or in other methods of the invention other nucleic acid capture
molecules) may be immobilised thereon by carbodiimide-mediated coupling. In some
embodiments the surface may for instance be coated with an electroconductive polymer,
such as polypyrrole (Wang, J., et al., Anal. Chem. (1999) 71, 18, 4095-4099; Wang, J., et al,
carbazole), or a copolymer such as a copolymer of pyrrole and thiophene or a copolymer of
jeglone and 5-hydroxy-3-thioacetic-1,4-naphthoquinone (Reisberg, S., et al., Anal. Chem.
(2005) 77, 10, 3351 -3356). In embodiments where a carbon surface is used, it may for
example be modified with carboxyl groups by mixing stearic acid with the paste. A PNA
capture molecule may be immobilised on a respective immobilisation unit by means of
linking molecule ethylenediamine.

[0054] As a further illustrative example, a linking moiety such as an affinity tag may be used to immobilise the PNA capture molecule. Examples of an affinity tag include, but are not limited to biotin, dinitrophenol or digoxigenin, oligohistidine, polyhistidine, an immunoglobulin domain, maltose-binding protein, glutathione-S-transferase (GST), calmodulin binding peptide (CBP), FLAG- peptide, the T7 epitope (Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly), maltose binding protein (MBP), the HSV epitope of the sequence Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp of herpes simplex virus glycoprotein D, the hemagglutinin (HA) epitope of the sequence Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala, the "myc" epitope of the transcription factor c-myc of the sequence Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu, or an oligonucleotide tag the sequence of which typically differs from the sequence of the target nucleic acid molecule to which a portion of the PNA capture molecule is at least partially complementary. These two nucleotide sequences may differ to such an extent that the sequence of the nucleotide tag is not capable of hybridising to the sequence of any portion of the target nucleic acid molecule. Such an oligonucleotide tag may for instance be used to hybridise to an immobilised oligonucleotide with a complementary sequence. A further example of a linking moiety is an antibody. In respective embodiments an antibody-nucleic acid conjugate may be used as the PNA-capture molecule.

[0055] Avidin or streptavidin may for instance be employed to immobilise a biotinylated nucleic acid, or a biotin containing monolayer of gold may be employed (Shumaker-Parry, J.S., et al., Anal. Chem. (2004) 76, 918). As yet another illustrative example, the PNA capture molecule may be locally deposited, e.g. by scanning electrochemical microscopy, for instance via pyrrole-oligonucleotide patterns (e.g. Fortin, E., et al., Electroanalysis (2005) 17, 495). hi other embodiments the PNA capture molecule may be directly synthesised on the surface of the immobilisation unit, for example using photoactivation and deactivation.

[0056] After immobilising the PNA capture molecule on the immobilisation unit in the sensing zone, any remaining PNA capture molecule, or molecules, that were not immobilised may be removed from the immobilisation unit. Removing an unbound PNA capture molecule may be desired to avoid subsequent hybridisation of such PNA molecule with the target nucleic acid molecule, which might reduce the sensitivity of the present method. Removing an unbound PNA capture molecule may also be desired to avoid a non-specific binding of such PNA molecule to any matter present in a sample used, which might for instance alter the conductivity of such matter (e.g., reducible metal cations), which might
interfere with the results of the electrical measurement (see also below). An unbound capture molecule may for instance be removed by exchanging the medium, e.g. a solution that contacts the surface of the immobilisation unit.

[0057] Where desired, a blocking agent may be immobilised on the surface of the immobilisation unit. This blocking agent may serve in reducing or preventing non-specific binding of matter included in the solution expected to include the target nucleic acid molecule. It may also serve in reducing or preventing non-specific binding of any other matter, such as a molecule or solution that is further added to the immobilisation unit when carrying out the method of the invention.

[0058] The blocking agent may be added together with the PNA capture molecule or subsequently thereto. Any agent that can be immobilised on the immobilisation unit and that is able to prevent (or at least to significantly reduce) the non-specific interaction between molecules, the detection of which is undesired, and the PNA capture molecule is suitable for that purpose, as long as the specific interaction between the PNA capture molecule and the target nucleic acid molecule is not prevented. Examples of such agents are thiol molecules, disulfides, thiophene derivatives, and polythiophene derivatives. An illustrative example of a useful class of blocking reagents include thiol molecules such as 16-mercaptophexadecanoic acid, 12-mercaptopdocanoic, 11-mercaptopdocanoic acid or 10-mercaptopdocanoic acid.

[0059] The term "derivative" as used herein thus refers to a compound which differs from another compound of similar structure by the replacement or substitution of one moiety by another. Respective moieties include, but are not limited to atoms, radicals or functional groups. For example, a hydrogen atom of a compound may be substituted by alkyl, carbonyl, acyl, hydroxyl, or amino functions to produce a derivative of that compound. Respective moieties include for instance also a protective group that may be removed under the selected reaction conditions.

[0060] The present method of the invention further includes contacting the immobilisation unit with the solution expected to include the target nucleic acid molecule (cf. Fig. 2). The immobilisation unit may for example be immersed in a solution, to which the solution expected to include the target nucleic acid molecule is added. In some embodiments both such solutions are aqueous solutions, in one embodiment the entire method is carried out in an aqueous solution. The method further includes allowing the target nucleic acid molecule to hybridise to the PNA capture molecule on the immobilisation unit. As already indicated above, thereby the formation of a complex between the PNA capture
molecule and the target nucleic acid molecule is allowed (cf. Fig. 2). If the solution contains a plurality of different target nucleic acid molecules to be detected, the conditions are chosen so that the target nucleic acid molecules can either bind simultaneously or consecutively to their respective capture molecules.

[0061] Where desired, after allowing the target nucleic acid molecule to hybridise to the PNA capture molecule, the analyte/solution expected to include the target nucleic acid molecule may be removed. This may for example be desired in order to remove any negatively charged molecules, such as nucleic acid molecules that were present in the solution expected to include the target nucleic acid molecule. Depending on the origin of the respective sample and any extraction used (if any; cf. above) removing components of the respective solution may improve the signal-to-noise ratio of the electrical measurement performed in the method of the invention (see below). Repeated replacement of a solution that contacts the surface of the immobilisation unit ("washing") or rinsing etc. of the immobilisation unit may also be performed where desired. Any matter known to be present in a sample used may also be removed by a suitable method that does not dissolve the complex formed between PNA capture molecule and target nucleic acid molecule, such as enzymatically. The same applies for any target nucleic acid molecule that did not hybridise to the PNA capture molecule. As an example, this may be accomplished by an enzyme, which selectively breaks down single-stranded DNA, such as mung bean nuclease, nuclease P1 or nuclease SI.

[0062] The method of the present invention also includes providing a polymerisable positively chargeable precursor. The method further includes adding the polymerisable positively chargeable precursor (cf. Fig. 2). The polymerisable positively chargeable precursor has an electrostatic net charge that is complementary to the electrostatic net charge of the target nucleic acid molecule. Typically the target nucleic acid molecule is negatively charged in neutral and acidic conditions, in particular where the target nucleic acid molecule is DNA or RNA, due to the backbone of alternating sugar and phosphate molecules in this type of nucleic acid. Accordingly, conditions are selected where the polymerisable positively chargeable precursor has a positive net charge. Those skilled in the art will be aware of the fact that the pK value of a selected precursor molecule serves as a valuable guidance in choosing a suitable pH range or pH value for a solution, in which the precursor molecule is positively charged. This pH range or value is obtained by way of adjusting (e.g. titrating) or by providing the target nucleic acid —and thus typically the immobilisation unit as well - in a solution of the desired pH. When the polymerisable positively chargeable precursor is
added, the target nucleic acid molecule may for example be included in a solution of a pH value selected in the range of about 1.5 to about 8.0, such as in the range of about 1.7 to about 7.0, in the range of about 1.9 to about 6.0, or in the range of about 2.0 to about 5.5. The pH value may thus for instance be selected to be about 3.0 or about 4.0. In some embodiments the pH may also be adjusted to be in a respective range (e.g. of about 1.5 to about 8.0, such as in the range of about 1.7 to about 7.0, in the range of about 1.9 to about 6.0, or in the range of about 2.0 to about 5.5) at the same time as, or after the addition of the polymerisable positively chargeable precursor.

[0063] Accordingly, the polymerisable positively chargeable precursor associates to the target nucleic acid molecule, and thus to the complex formed between the PNA capture molecule and the target nucleic acid molecule. Therefore, the polymerisation of the polymerisable precursor can be carried out by means of a suitable reactant molecule. The respective polymerisation will accordingly involve, including start at, a precursor that is associated to the target nucleic acid molecule.

[0064] Any positively chargeable precursor may be used as long as it can be polymerised in the presence of the complex between the PNA capture molecule and the target nucleic acid molecule, without dissolving or degrading the respective complex in such a manner that the target nucleic acid molecule is no longer associated to the surface of the immobilisation unit. As an illustrative example, the polymerisable positively chargeable precursor may be an aromatic amine (see e.g. Fig. 5 for examples), such as aniline; pyridineamine (e.g. 2-pyridineamine, 3-pyridineamine or 4-pyridineamine), pyrrole, imidazole or a derivative thereof. As a further illustrative example, an aniline derivative may also be of the general formula

\[
\text{H}_2\text{N}-\text{R}^1\text{-R}^2
\]

wherein \(R^1\) and \(R^2\) may independently selected from the group consisting of H, aliphatic, cycloaliphatic, aromatic, arylaliphatic, and arylecycloaliphatic hydrocarbyl groups, comprising 0 - 5 heteroatoms, i.e. atoms that differ from carbon, for example 0 - 3 heteroatoms, selected from the group N, O, S, and Si. \(R^1\) and \(R^2\) may optionally be linked so as to define an aliphatic, cycloaliphatic, aromatic, arylaliphatic, or arylecycloaliphatic hydrocarbyl bridge. In case that a compound is selected that includes a negatively chargeable moiety (or functional group, e.g. carboxyclic, or sulfonic), it may be desired to verify that the
respectively compound is indeed positively chargeable under the selected conditions. This may for example be done by calculating the respective pK value or by testing whether the compound associates with the selected nucleic acid molecule.

[0065] The term "aliphatic" means, unless otherwise stated, a straight or branched hydrocarbon chain, which may be saturated or mono- or polyunsaturated. An unsaturated aliphatic group contains one or more double and/or triple bonds. The branches of the hydrocarbon chain may include linear chains as well as non-aromatic cyclic elements. The hydrocarbon chain, which may, unless otherwise stated, be of any length, and contain any number of branches. Both the main chain as well as the branches may furthermore contain heteroatoms as for instance N, O, S, Se or Si.

[0066] The term "alicyclic" means, unless otherwise stated, a nonaromatic cyclic hydrocarbon moiety, which may be saturated or mono- or polyunsaturated. The cyclic hydrocarbon moiety may be substituted with nonaromatic cyclic as well as chain elements. The main chain of the cyclic hydrocarbon moiety may, unless otherwise stated, be of any length and contain any number of non-aromatic cyclic and chain elements. Both the cyclic hydrocarbon moiety and the cyclic and chain substituents may furthermore contain heteroatoms, as for instance N, O, S, Se or Si.

[0067] The term "aromatic" means, unless otherwise stated, a planar cyclic hydrocarbon moiety of conjugated double bonds, which may be a single ring or include multiple fused or covalently linked rings. The main chain of the cyclic hydrocarbon moiety may, unless otherwise stated, be of any length and contain any number of heteroatoms, as for instance N, O and S.

[0068] By the term "arylaliphatic" is meant a hydrocarbon moiety, in which one or more aryl groups are attached to or are substituents on one or more aliphatic groups. Thus the term "arylaliphatic" includes for instance hydrocarbon moieties, in which two or more aryl groups are connected via one or more aliphatic chain or chains of any length, for instance a methylene group.

[0069] Each of the terms "aliphatic", "alicyclic", "aromatic" and "arylaliphatic" as used herein is meant to include both substituted and unsubstituted forms of the respective moiety. Substituents may be any functional group, as for example, but not limited to, amino, amido, azido, carbonyl, cyano, isocyano, dithio, halogen, hydroxyl, nitro, seleno, silyl, silano, thio, thiocyano, and trifluoromethyl.

[0070] The present method of the invention further includes providing a suitable
reactant molecule that is capable of undergoing a reaction with the positively chargeable precursor that results in polymerisation of the latter. The reactant molecule may also be able to initiate the polymerisation of the polymerisable positively chargeable precursor. As an illustrative example, the reactant molecule may be an oxidant. Examples of a suitable oxidant include, but are not limited to, a ruthenium tris(bipyridinium) complex, a persulfate (such as ammonium persulphate or tetrabutylammonium persulphate), a peroxide (see also below), hydrogen tetrachloroaurate (auric acid, HAuCl₄), a chromate, a dichromate, a manganate, a permanganate, oxygen, ozone, nitrogen oxide, a halogene, a chlorite, a chloride, a perchloride, a chlorate, a iodate, a nitrate, a sulfoxide and osmium tetroxide.

[0071] The method of the present invention also includes adding the reactant molecule (cf. Fig. 2). The addition of a suitable reactant molecule initiates the polymerisation of the polymerisable positively chargeable precursor (see. Fig. 2). As an illustrative example, if aniline or a suitable derivative thereof is selected as the respective precursor and an oxidant is selected as the reactant molecule, the polymerisation may start according the following reaction scheme (I) and subsequently continue respectively:

![Reaction Scheme (I)]

It may also start along the following reaction scheme (II):

![Reaction Scheme (II)]

as well as along the following reaction scheme (III):

![Reaction Scheme (III)]
A respective electroconductive form of the depicted reaction product polyaniline according to any of schemes (I) to (III) is also termed to be in the "emeraldine" oxidation state in the art (see e.g. Huang, J. et al., Chem. Eur. J. (2004), 10, 1314-1319).

[0072] Without the intent of being bound by theory, it is believed that with aniline as the precursor, in the methods of the invention the polymerisation typically occurs at the para position as shown above (scheme I, scheme III). The hybridised anionic target nucleic acid molecule may serve as a template, providing a local environment that guides para-coupling of aniline molecules. Previous studies on enzymatically catalysed polymerisation of aniline have shown that the polymerisation of aniline is catalysed by peroxidases, horse-radish peroxidase for example, under mild conditions (Liu, W., et al., J. Am. Chem. Soc. (1999) 121, 71-78; Nagarajan, R., et al., Macromolecules (2001) 34, 3921-3927; Caramyshev, A.V., et al., Biomacromolecules (2005) 6, 1360-1366.). However, the products of the horse-radish peroxidase-catalysed polymerisation of aniline are a mixture of highly branched ortho- and para-substituted carbon-carbon and carbon-nitrogen polyaniline together with the desired head-to-tail polymerised polyaniline, which greatly diminishes the degree of conjugation and severely affects the electrical, electrochemical, and optical properties of the resulting electroconductive polymers. On the contrary, in the presence of an anionic polyelectrolyte template, a water-soluble, polyaniline/polyelectrolyte complex is formed, in which the polyaniline is wrapped around the polyelectrolyte template (Liu et al., 1999, supra, Nagarajan et al., 2001, supra, Caramyshev et al., 2005, supra). More importantly, polyaniline in the complex retains its full electrical activity and is mostly the desired head-to-tail structure with minimal branching, paving the way for the development of ultrasensitive electrochemical nucleic acid biosensors. Later, this enzyme-catalysed template-guided synthetic approach was applied to prepare highly ordered polyaniline (Nagarajan et al., 2001, supra) and polyaniline nanowires on silicon substrates by using fully stretched nucleic acid as growing templates.

[0073] As a further illustrative example, if pyrrole or a suitable derivative thereof is
selected as the respective precursor and an oxidant is selected as the reactant molecule, the polymerisation may start according the following reaction scheme (IV) and subsequently continue respectively:

![Reaction Scheme IV](image)

[0074] As already mentioned above, the anionic target nucleic acid molecule, which forms a complex with the PNA capture molecule, typically serves as a template for the polymerisation of the positively chargeable precursor. As a consequence, the polymerisation occurs exclusively at the target nucleic acid molecule.

[0075] In some embodiments, a suitable initiator may be added. An initiator is generally matter such as a molecule that can generate radical species under mild conditions. Usually it can also promote radical polymerisation reactions. Examples of a respective initiator include, but are not limited to a radical initiator, such as a halogen molecule, a azo compound, a persulfate molecule, a peroxysulfate ((SO₃)₂O₂⁻) and a peroxide compound, such as an organic peroxide compound. Two illustrative examples of a halogen molecule are chlorine or bromine. Three illustrative examples of an azo compound are 2,2'-azobis(2-amidinopropane), 2,2'-azobis(2,4-dimethylvaleronitrile) and azobis(isobutryramidine). Two illustrative example of a persulfate molecule are ammonium persulfate and potassium persulfate. Where desired, a persulfate molecule may also be combined with a thiosulfate (e.g. sodium thiosulfate, Na₂S₂O₇), a bisulfite, a dithionite, or an ascorbic acid molecule. Examples of an organic peroxide compound include, but are not limited to, benzyol peroxide, cumenyl peroxide, dicumyl peroxide, cumene hydroperoxide, diphenyl peroxide, bis-(tert-butyl)peroxide, bis(tert-butyloxysuccinyl) peroxide, a lipid peroxide such as lauroyl peroxide, diacetyl peroxide or trifmoroacetyl peroxide, bis(o-iodophenylacetyl) peroxide, ethyl peroxide, 2-naphthyl peroxide, 2-naphthylperoxide, butyl hydroperoxide, tert-butyl hydroperoxide, vinyl hydroperoxide, cyclohexyl hydroperoxide, trifluoromethyl peroxide, 2,5-bis(l-methylethyl)phenyl-hydroperoxide, 1,5-dimethyl-6,8-bis(trimethylsilyl)-2,3-dioxabicyclo[2.2.2]oct-7-en-5-yl hydroperoxide, and ρ-l,l-dimethylethyl-l-methyl-3-phenyl-2-propynyl peroxide. A photoinitiator such as dibenzyl ketone, camphorquinone, 1-(bromoacetyl)pyrene, α-ketoglutaric acid, or an uranyl salt may likewise be employed.

[0076] Where desired, for example to accelerate the polymerisation, or in embodiments where the reactant molecule undergoes a sluggish reaction, a suitable catalyst
may furthermore be provided and the reactant molecule be exposed thereto. Examples of a catalyst include, but are not limited to, light, a metal halide such as a metal chloride or a metal bromide, a metal sulphate, and an enzyme or an enzyme-conjugate. Examples of suitable metal halides include, but are not limited to a ferrous halide, e.g. ferrous chloride, a lithium halide, e.g. lithium chloride, a copper halide, e.g. a copper(I) halide or a copper(II) halide such as CuCl₂ or CuBr₂, a molybdenum halide such as MoCl₅, an indium halide, such as (NEt₄)₂IrCl₆, a manganese chloride such as MnCl₂, a nickel halide such as NiCl₂, a titanium chloride such as titanium trichloride (TiCl₃), and an aluminium halide, such as aluminium chloride. Two examples of a suitable metal sulphate FeSO₄ and CuSO₄. Where the catalyst is e.g. a compound or a powder, a respective catalyst may for example be added to the immobilisation unit, on which the PNA capture molecule is immobilised. It may, for instance, be added after the polymerisable positively chargeable precursor has associated to the complex formed between the PNA capture molecule and the target nucleic acid molecule. It may for instance also be added together with the reactant molecule or subsequently thereto. The catalyst may for instance be selected in such a way that the reactant molecule is a substrate for the catalyst.

[0077] In embodiments where an enzyme or an enzyme-conjugate is used as a catalyst, any enzyme may be used that is capable of catalysing the polymerisation of the positively chargeable precursor. The enzyme may for instance be selected in such a way that the reactant molecule is a substrate for the enzyme. As an illustrative example of an enzyme suitable for the purposes of the present invention, an oxidoreductase enzyme may be selected. Examples of an oxidoreductase enzyme include, but are not limited to, a peroxidase, an oxidase, a dehydrogenase, a monoxygenase, a hydroxylase, a dioxygenase, or a hydrogenase.

[0078] A respective peroxidase enzyme may for instance be a haem peroxidase enzyme. Examples of a haem peroxidase enzyme include, but are not limited to, horseradish peroxidase, cytochrome c peroxidase, glutathione peroxidase, myeloperoxidase, thyroid peroxidase, eosinophil peroxidase, lactoperoxidase, ascorbate peroxidase, peroxidin, prostaglandin H synthase, a bacterial catalase-peroxidase such as E. coli catalase-peroxidase, M. tuberculosis catalase-peroxidase, or Bacteroides fragilis catalase-peroxidase, lignin peroxidase, plant ascorbate peroxidase, Haem chloroperoxidase, manganese peroxidase, stigma specific peroxidase, Euphorbia characias latex peroxidase, Arthropages ramosus peroxidase, sorghum grain peroxidase SPC4, soybean peroxidase, Phanerochaete chrysosporium manganese-dependent peroxidase, lacrimal gland peroxidase, or any
combination thereof. Examples of an oxidase enzyme include, but are not limited to, the oxygen oxidase laccase, glucose oxidase, lactase oxidase, pyruvate oxidase, tyrosinase or any combination thereof. A respective peroxidase enzyme may also be a non-haem peroxidase enzyme such as for instance bromoperoxidases BPO-Al, BPO-A2 or BPO-B, the non-haem chloroperoxidase from *Pseudomonas fluorescens*, or the non-haem extracellular peroxidase from *Thermomonospora fusca* BD25. A secreted mixture or an extract of enzymes may also be used as a catalyst where desired. As an illustrative example, the so-called "white-rot fungi" produces lignin-modifying extracellular enzymes (LME) that include two peroxidase enzymes (lignin peroxidase and Mn peroxidase), an oxidase enzyme laccase and an oxidase enzyme aryl alcohol oxidase. A respective crude extract may be used as a catalyst in a method of the present invention (cf. also Curvetto, N.R., et al., *Biochemical Engineering Journal* (2006) 29, 3, 191-203). Further examples of suitable catalysts include haematin (Curvetto et al., 2006, supra) and haemoglobin.

[0079] In one embodiment the oxidant is a peroxide such as hydrogen peroxide, and the catalyst is horse radish peroxidase. Horse radish peroxidase has previously been reported (Liu, W., et al., *J. Am. Chem. Soc.* (1999) 121, 71-78; Nagarajan, R., et al., *Macromolecules* (2001) 34, 3921-3927) to catalyse the oxidative polymerisation of aniline in the presence of the oxidant hydrogen peroxide (H$_2$O$_2$). This polymerisation can also be carried out in the presence of DNA, which expedites the polymerisation reaction (Nickels, P. et al., *Nanotechnology* (2004) 15, 1524-1529). In another embodiment the oxidant is molecular oxygen (O$_2$) and the catalyst is laccase (e.g. isolated from *Coriolus hirsutus*). Laccase *C. hirsutus* has been reported to possess a higher operational stability than horse radish peroxidase under acidic conditions in the polymerisation of aniline (Karamyshev, A.V. et al., *Enzyme and Microbial Technology* (2003) 33, 5, 556-564).

[0080] In some embodiments the catalyst is in solution or in suspension. In some embodiments the catalyst is coupled to a detection probe. The detection probe may for example be a label that emits light of a certain wavelength upon irradiation (e.g. fluorescein or a fluorescent protein), thus enabling an optical control measurement to verify the presence of the catalyst. The detection probe may also be a probe nucleic acid molecule. In some embodiments the catalyst is an enzyme or an enzyme-conjugate to which a probe nucleic acid molecule is coupled. The probe nucleic acid molecule may be any nucleic acid of any length, for example of about 5 to about 250 bp, such as about 5 to about 100 bp. Such a nucleic acid molecule is at least partially complementary to at least a portion of the target nucleic acid molecule. The detection probe, which is attached to the catalyst, hybridises to a
respective portion of the target nucleic acid different from the portion to which the capture
nucleic acid molecule hybridises. Accordingly, the detection probe directs the catalyst to the
target nucleic acid and associates it thereto.

[0081] Where desired, further methods for detection may be employed. As an
example, an optical detection may also be performed or enhanced by means of an optically
amplifying conjugated polymer, e.g. in a Förster energy transfer system (Gaylord, B.S., et
(2003) 125, 896-900). As a further example, a cationic polythiophene may be added, which
changes its color and fluorescence in the presence of single-stranded or double-stranded

[0082] The polymerisation of the polymerisable positively chargeable precursor
forms an electroconductive polymer. As already explained above, due to the (electrostatic)
association of the polymerisable positively chargeable precursor to the complex formed
between the PNA capture molecule and the target nucleic acid molecule, this
electroconductive polymer is likewise associated with the complex formed between the PNA
capture molecule and the target nucleic acid molecule. The present inventors have found that
the electroconductive polymer is usually robustly bound to the surface of the immobilisation
unit. For example extensive washing and potential cycling thereafter produced no noticeable
changes in subsequent electrical measurements. Where desired, further reactions, washing
steps and additional detection methods etc. may therefore be performed. The electroactivity
of the electroconductive polymer allows ultrasensitive electrochemical detection of target
nucleic acid molecules as explained in the following.

[0083] In typical embodiments the distance between the two electrodes is of a
dimension that is comparable to the length of the target nucleic acid molecule, i.e. some
embodiments the target nucleic acid molecule even spans the entire gap. The
electroconductive polymer formed allows the transfer of electrons along the target nucleic
acid molecule. In some embodiments it even allows the transfer of electrons between the two
electrodes. The electroconductive polymer may for example include moieties such as
functional groups (e.g. amino groups) that are capable of accepting and/or donating electron
density, thus allowing the flow of electrons between or from/to the two electrodes. While the
electroconductive polymer is already partially formed and the polymerisation reaction still
continues, electrons may for instance also be transferred along the polymer chain to the
oxidant during a polymerisation reaction as illustrated by examples in Fig. 2 and Fig. 3.
[0084] The present invention thus uses the dynamic growing process of an electroconductive polymer as a means to both generate and amplify an electrical signal. A longer electroconductive polymer generally includes more moieties, e.g. functional groups such as amino groups that are capable of accepting and/or donating electron density. Accordingly, a longer polymer generally has a higher capacity of allowing the flow of electrons. As mentioned above, the obtained polymer will typically furthermore include branches of various degree rather than form a purely linear product. Such branches also amplify signals, including signals from the respective growing ends of the polymer. Furthermore typically a plurality of polymerisable positively chargeable precursors associates to one target nucleic acid molecule, such that multiple polymer chains start at a single target nucleic acid molecule. The present invention thus includes a highly efficient signal amplification and transduction route.

[0085] Because the polymer associated to the target nucleic acid is electroactive, the current generated from it nevertheless directly correlates to the concentration of nucleic acid in the sample solution. The combination of highly efficient polymerisation, guided-deposition, and electrical detection (see below) thus provides a generic platform for ultrasensitive detection of nucleic acids. As an illustrative example, using horseradish peroxidase as a catalyst, hydrogen peroxide as a reactant molecule and aniline as a positively chargeable precursor, a linear correlation between the number of target nucleic acid molecules and the detected signal was observed (see Fig. 11). The detection limit was in this example about 40 fM.

[0086] Furthermore, the catalytic and cumulative nature of the system causes the signal (see below) to increase with increasing incubation time before sampling. Essentially the signal is only limited by capacity of the selected system in terms of the amounts of reactant molecule and polymerisable precursor, and where applicable, the amount of catalyst in the system. As a result, a longer incubation period produces a higher signal and a lower detection limit (see e.g. Fig. 10).

[0087] In this regard it is also noted that the association of a nucleic acid molecule to an electroconductive polymer such as polypyrrole or polyaniline has previously been found to have an enhancing effect on the capabilities of the electroconductive polymer to generate an electrical or electrochemical signal (see Ramanavicius, A., et al., *Electrochimica Acta* (2006) 51, 6025-6037).

[0088] If desired, an enhancer reagent may furthermore be added. The
electroconductive polymer is in such embodiments contacted with the respective enhancer reagent, typically before determining the presence of the target nucleic acid molecule. A respective enhancer molecule serves in further enhancing a signal (see below) caused by the electroconductive polymer. As an illustrative example, an acid or base may be used as an enhancer molecule. Examples of suitable acids that may be used include, but are not limited to HCl (hydrochloric acid), HBr (hydrobromic acid), H2SO4 (sulphuric acid) and HClO4 (perchloric acid) (Gök, A., et al, Int. J. Polym. Anal. Charact. (2006) 11, 227-238). The following two schemes may serve in depicting an effect of an acid purely for illustrative purposes. As well known to the skilled artisan, polyaniline may be described as being in a redox equilibrium according to scheme (V), depending on its exposure to oxidants:

The different oxidation states are known to those skilled in the art as the "leucoemeraldine" state, "emeraldine" state and the "perniganiline" state (see e.g. Kang, E.T., et al. Macromolecules (1990) 23, 2918-2926; or Ping, Z. et al. J. Chem. Soc. Faraday Trans. (1997) 93, 1 121-129). Without being bound by theory, upon treatment with an acid the nitrogen atoms of the polymer backbone may be protonated and thereby induced to bear charge carriers as illustrated in scheme (VI) (see also e.g. Huang et al, 2004, supra):
[0089] An illustrative example of a suitable base that may be used is NH₃, ammonia. A respective acid or base may for example be contacted with the electroconductive polymer in form of a vapour. As can be inferred from the above, a respective enhancer molecule may also be an oxidant, such as for example a halobenzoquinone (e.g. o-chloranil or o-bromanil). Such an oxidant may cause an oxidation according to scheme (V) or even form a covalent bond with the electroconductive polymer as observed by Kang et al. (1990, supra). The formation of a respective exemplary reaction product may be illustrated by the following scheme (VII):

[0090] Examples of further enhancer molecules include, but are not limited to an organic solvent such as chloroform, ethanol and benzene (Gök et al., 2006, supra), a pesticide such as methylparathion, paraquat, bentazon or glyphosate and a cyclodextrine compound such as β-cyclodextrine acid sulphate.

[0091] The electroconductive polymer changes an electrical characteristic of a region in between the electrodes. As noted above, an enhancer reagent may be used to enhance this change of a respective electrical characteristic. The polymer may for instance change the electric field, change the conductivity or resistance of a medium in the electric field, obtain a charge, transfer charge or conduct a current. The detection technique that may be employed in the method of the present invention is thus selected to be based on the measurement of a respective electrical characteristic. It may thus for instance be based on an impedance or a capacitance measurement. A change of a value of the electrical characteristic, such as a change in electric current, may also be detected as a function of time.
A respective signal of the electroconductive polymer is detected in the method of the present invention. Any detection technique for electric signals may be used in the method of the present invention. A detection according to the invention may or instance include a measurement of a conductance, a voltage, a current, a capacitance or a resistance. As an illustrative example, conductance may be measured by linear cyclic voltammetry, square wave voltammetry, normal pulse voltammetry, differential pulse voltammetry and alternating current voltammetry. As a further example, the immobilisation unit, or at least a part of the surface thereof, may be exposed to an electric field. In this case the electroconductive polymer immobilised thereon via its association to the complex of analyte molecule and capture molecule is likewise exposed to the respective electric field. This results in an electric signal caused by the electroconductive polymer. Accordingly, in some embodiments of the method of the invention an electric field is generated, which may in some embodiments be a symmetric or a homogenous electric field. The electric filed may for example be an external field. It may also be generated at at least one electrode of the pair of electrodes.

In typical embodiments, the result obtained is then compared to that of a reference measurement (or control measurement), i.e. a respective reference measurement PNA capture molecules unable to bind the target nucleic acid to be detected may for instance be used. An example of such a "control" PNA capture molecule is a PNA molecule having a sequence not complementary to any portion of the target nucleic acid molecule (see e.g. Fig. 12). A further example of a reference measurement, which may also be performed on the same sample during analysis, is determining the respective electrical characteristic in the absence (or before) adding the respective polymerisable precursor. Yet a further example of a reference measurement (that may also be performed on the same sample during analysis) is determining the respective electrical characteristic in the absence (or before) adding the respective reactant molecule. If the two measurements of the respective electrical characteristic, i.e. "sample" and "control" measurement, differ in such a way that the difference between the values determined is greater than a pre-defined threshold value (see below), the sample solution contained the relevant target nucleic acid molecule. A respective threshold value may for instance be determined in a calibration experiment, e.g. using different amounts of the target nucleic acid molecule to be detected in a medium that is known or expected to correspond to the sample. As a further example, an internal or external reference electrode may in some embodiments be used that contacts a reference medium. The reference medium may in some embodiments include a known and constant amount of the target nucleic acid molecule.
In some embodiments, the method is designed in such a way that a reference measurement and a measurement for detecting a target nucleic acid molecule are performed simultaneously. This may for instance be done by carrying out a reference measurement only with a control medium and, at the same time, a measurement with the sample solution expected to contain the target nucleic acid to be detected. Likewise, a respective control measurement with a PNA capture molecule that is not complementary to any portion of the target nucleic acid molecule may be carried out in parallel to a measurement for detecting a target nucleic acid molecule.

In typical embodiments a threshold value in relation to a reference measurement is defined (see above). This threshold value may be a fixed value of a respective electrical characteristic, the change of which is expected in the presence of the target nucleic acid molecule. It may also be a fixed value of a parameter that is caused by the electrical characteristic. The presence or absence of a target nucleic acid molecule is then determined by means of comparison of the value detected, typically in relation to the reference measurement, with the threshold value. If the obtained value exceeds the threshold value, then it is inferred that the target nucleic acid molecule is present in the sample, and if appropriate in what concentration. In the case a detected value is below the threshold value it is inferred that no target nucleic acid molecule is present, in the same way the rate of change of a parameter more than a predetermined threshold value can be used to indicate the presence of a target nucleic acid molecule in a sample.

The present method also allows detecting more than one target nucleic acid molecule simultaneously or consecutively in a single measurement. For this purpose, a substrate including a plurality of immobilisation units as described above may for example be used, wherein different types of PNA capture molecules, each of which exhibiting (specific) is capable of hybridising to at least a portion of a particular target nucleic acid molecule, are immobilised on the surfaces of the immobilisation units. Alternatively, it may also be possible to use a plurality of immobilisation units, each of which being provided with only one type of PNA capture molecule.

The method according to the invention may be carried out by using virtually any electrode arrangement known in the art that includes a pair of electrodes. Such an electrode arrangement may include a detecting or working electrode and a reference electrode (see also above). The electrodes may be a conventional metal electrode (gold electrode, silver electrode etc.) or an electrode made from polymeric material or carbon. An electrode arrangement may also include a common silicon or gallium arsenide substrate, to
which a gold layer and a silicon nitride layer have been applied, and which has subsequently been structured by means of conventional lithographic and etching techniques to generate the electrode arrangement(s).

[0098] The method according to the present invention also allows detecting more than one type of analyte simultaneously or consecutively in a single measurement. For this purpose, a substrate comprising a plurality of electrode pairs with immobilisation units as disclosed herein may be used, wherein different types of capture molecules, each of which exhibiting (specific) binding affinity for a particular analyte to be detected, are immobilised on the electrodes of the individual electrode arrangements. Alternatively, it may also be possible to use a plurality of electrode arrangements, each of which being provided with only one type of capture molecules.

[0099] An example of an electrode arrangement, which may be used for carrying out the present method, as well as any other method according to the invention, is a conventional interdigitated electrode. Consequently, an arrangement provided with a plurality of interdigitated electrodes, i.e. an electrode array, can be employed for parallel or multiple determinations. Another usable electrode arrangement is an electrode arrangement in the form of a trench or a cavity, which is formed, for example, by holding regions such as, for example, a gold layer on which the capture molecules capable of binding the analytes are immobilised being located on two opposite side walls.

[0100] Another method of the invention includes immobilising on the immobilisation unit a nucleic acid capture molecule that has a nucleotide sequence that is at least partially complementary to at least a portion of the target nucleic acid molecule. This capture molecule may be any nucleic acid as long as it is capable of hybridising to at least a portion of the sequence of the target nucleic acid molecule. The capture molecule may for example be a DNA, RISfA or PNA molecule. A respective nucleic acid molecule may be immobilised by any means, as long as it can hybridise to the target nucleic acid molecule thereafter. Examples of immobilising a nucleic acid capture molecule have already been explained above. As a further example, in embodiments where an immobilisation unit of gold is used, an ionisable thiol compound such as 2-dimethylaminoethanethiol hydrochloride may be covalently linked to the surface of the immobilisation unit. This modification allows nucleic acid molecules to bind through electrostatic interactions.

[0101] Where desired, more than one nucleic acid capture molecule may be immobilised on the immobilisation unit. This may for instance be desired in order to broadly screen for the presence of any of a group of selected nucleic acid sequences. This may also
be desired to allow for the simultaneous or consecutive detection of different analytes such as two or more genomic DNAs, each of them having binding specificity for one particular type of capture molecule. In some embodiments similar nucleic acid sequences, e.g. a number of nucleic acid sequences that are partially or substantially complementary to a selected target nucleic acid molecule, may be immobilised in order to enhance the likelihood of detecting the respective target nucleic acid molecule. Where desired, a further selectivity may be introduced by the selection of the nucleic acid molecule used that is attached to the enzyme added (see below). Furthermore, in this manner the detection of the same target nucleic acid molecule via different recognition sequences can be achieved, e.g., the 5’- and 3’-termini of a nucleic acid molecule, which enhances the likelihood to detect even a few copies of a target nucleic acid molecule in a sample.

[0102] It is understood that the above explanations with respect to a PNA capture molecule respectively also apply to a nucleic acid molecule used in the present method of the invention, in the light of the detailed explanations of the previous method of the invention, explanations with regard to the present method of the invention are focussed on differences between these two methods.

[0103] The present method also includes contacting the surface of the immobilisation unit with a solution expected to include the target nucleic acid molecule (see Fig. 3), and allowing the target nucleic acid molecule to hybridise to the nucleic acid capture molecule on the immobilisation unit (see Fig. 3), thereby allowing the formation of a complex between the nucleic acid capture molecule and the target nucleic acid molecule (cf. above).

[0104] Similar to the first method described above, the present method also includes adding a polymerisable positively chargeable precursor (see Fig. 3). As this polymerisable positively chargeable precursor has an electrostatic net charge that is complementary to the electrostatic net charge of the target nucleic acid molecule, it associates with the complex formed between the nucleic acid capture molecule and the target nucleic acid molecule (see above).

[0105] The present method further includes adding a suitable substrate molecule (see Fig. 3). Any substrate molecule may be used that is suitable as a substrate for the enzyme used (see above for examples) in the present method. As an illustrative example, the enzyme may be a peroxidase enzyme, for instance a haem peroxidase, in such embodiments the substrate will typically be a peroxide. Any peroxide that can, at least to a certain degree, be dissolved in a selected solution used in this method or the method described above, such as an aqueous solution, may be used in the present invention. Examples of a suitable peroxide
include, but are not limited to, hydrogen peroxide, nitrogen peroxide, magnesium peroxide, calcium peroxide, zinc peroxide, benzoyl peroxide, cumenyl peroxide, dicumyl peroxide, diphenyl peroxide, bis(tert-butylperoxy succinyl) peroxide, a lipid peroxide such as lauroyl peroxide, diacetyl peroxide or trifluoracetyle peroxide, bis(o-iodophenylacetyl) peroxide, ethyl peroxide, 2-naphthyl peroxide, 2-naphthoyl peroxide, butyl hydroperoxide, vinyl hydroperoxide, cyclohexyl hydroperoxide, trifluoromethyl peroxide, 2,5-bis(1-methyl-ethyl)phenyl-hydroperoxide, 1,5-dimethyl-6,8-bis(trimethylsilyl)-2,3-dioxabicyclo[2.2.2]oct-7-en-5-yl hydroperoxide, and p-1,l-dimethylethyl-l-methyl-3-phenyl-2-propynyl peroxide.

As explained above, in some embodiments the enzyme may also be an oxidase enzyme such as for instance laccase. In this case oxygen may for instance conveniently be added together with any aqueous solution used in the method of the invention, in which it is dissolved. In such embodiments, no additional measures need to be taken to add oxygen. Where desired, this may however be carried out by mechanical action, such as stirring, rotating or shaking.

[0106] The enzyme, the addition of which is also included in the present method (see Fig. 3), is attached to a probe nucleic acid molecule. Any nucleic acid molecule may be used for this purpose that is at least partially complementary to at least a portion of the target nucleic acid molecule. Accordingly, the detection probe hybridises to a respective portion of the target nucleic acid different from the portion to which the capture nucleic acid molecule hybridises (cf. also above).

[0107] As indicated above, the catalyst, such as an enzyme used in the method described above, may optionally also be attached to a probe nucleic acid molecule. Where in the present method of the invention a DNA or RNA molecule are selected as the nucleic acid capture molecule, both the respective nucleic acid capture molecule and the target nucleic acid molecule may however be negatively charged under the assay conditions selected. As explained above, upon addition of the polymerisable positively chargeable precursor the target nucleic acid molecule may for instance be included in a solution of a pH value selected in the range of about 1.5 to about 8.0, such as in the range of about 1.7 to about 7.0, in the range of about 1.9 to about 6.0, or in the range of about 2.0 to about 5.5 (including a value of about 3.0 or about 4.0). The pH may also be brought to a desired range or value, such as one of the aforementioned ranges, during or after adding the polymerisable positively chargeable precursor. In any such embodiments various DNA and RNA molecules will typically have a negatively charged backbone. Accordingly, the positively chargeable precursor may bind to either nucleic acid molecule. Additional selectivity is however introduced into the present method by the use of an enzyme, which is attached to a probe
nucleic acid molecule.

[0108] The present method also includes allowing the probe nucleic acid molecule to hybridise to the target nucleic acid molecule (see Fig. 3). By hybridisation of the two nucleic acid molecules, i.e. the capture molecule and the analyte molecule, a complex is formed. It is understood that for the quantification of such a nucleic acid molecule a plurality of the respective capture molecules is usually required. In a suitable concentration range of the analyte molecule, where the method of the invention can be used to quantify a respective analyte molecule, generally an excess of capture molecules in comparison to analyte molecule is required. As a result, one or more single-stranded nucleic acid capture molecules, which do not form a complex with an analyte molecule, may remain. Depending on the polymerisable positively chargeable precursor used, the presence of such a nucleic acid capture molecule may interfere with the detection of the method of the present invention, in particular where the nucleic acid capture molecule is a single-stranded DNA molecule or a single-stranded RNA molecule, such a remaining nucleic acid molecule may be removed from the surface.

[0109] hi such embodiments the surface may be contacted with at least one enzyme with nuclease activity, in order to remove any nucleic acid capture molecule that has not hybridised to an analyte molecule. It may be desired to reduce or block nuclease activity that is directed against double-strands of nucleic acids in order to avoid a reduction of detection signal, caused by the degradation of complexes of capture molecule and analyte. hi some embodiments an enzyme may be selected that selectively degrades single-stranded nucleic acids. Examples of such enzymes include, but are not limited to, mung bean nuclease, nuclease P1 (e.g. from fungi), nuclease SI (e.g. from fungi), CEL I nuclease (e.g. from plants), recJ exonuclease (e.g. from E. coli), and a DNA polymerase that is capable of degrading single-stranded DNA due to its 5'-> 3' exonuclease activity and a DNA polymerase that is capable of degrading single-stranded DNA due to its 3'-> 5' exonuclease activity.

[0110] As already explained above, the present method also includes catalysing the polymerisation of the polymerisable positively chargeable precursor. Since the enzyme, which catalyses the polymerisation, is associated with the complex formed between the nucleic acid capture molecule and the target nucleic acid molecule (via the probe nucleic acid molecule), the polymerisation usually starts at the target nucleic acid molecule. At this location the polymerisation accordingly also progresses. The target nucleic acid/enzyme adduct accordingly catalyses the polymerisation of the precursor and guides the deposition of electroconductive polymer at the immobilisation unit in the sensing zone. The
electroconductive polymer, which is formed from the polymerisable precursor, is thus associated with the complex formed between the nucleic acid capture molecule and the target nucleic acid molecule.

[0111] As for the method described above, the present method further includes determining the presence of the target nucleic acid molecule based on an electrical characteristic of a region in between the electrodes. This electrical characteristic is influenced by the electroconductive polymer (supra). The method may for instance include performing an electrical measurement of an electric signal caused by the electroconductive polymer. Where applicable, the method may include quantifying the amount of target nucleic acid present (supra).

[0112] The methods according to the present invention may be a diagnostic method for the detection of one or more target genes. The target gene may be involved in or associated with a disease or a state of the human or animal body that requires prophylaxis or treatment.

[0113] The method of the invention may be combined with other analytical and preparative methods. As already indicated above, the target nucleic acid may in some embodiments for instance be extracted from matter in which it is included. Examples of other methods that may be combined with a method of the present invention include, but are not limited to isoelectric focusing, chromatography methods, electrochromatographic, electrokinetic chromatography and electrophoretic methods. Examples of electrophoretic methods are for instance free flow electrophoresis (FFE), polyacrylamide gel electrophoresis (PAGE), capillary zone or capillary gel electrophoresis. Furthermore the data obtained using the present invention may be used to interact with other methods or devices, for instance to start a signal such as an alarm signal, or to initiate or trigger a further device or method.

[0114] The present invention also provides kits for electrically detecting a target nucleic acid molecule, which may for instance be diagnostic kits. A respective kit includes a pair of electrodes, which are arranged at a distance from one another, for example separated by a gap. The pair of electrodes is arranged within a sensing zone. A kit according to the present invention furthermore includes an immobilisation unit. The surface of the immobilisation unit is arranged within the sensing zone. As explained above, the sensing zone may for example be defined by the zone in which an electric field of said pair of electrodes is effective.

[0115] One kit according to the present invention also includes a PNA capture
molecule, which has a nucleotide sequence that is at least partially complementary to at least a portion of the target nucleic acid molecule (see above). The kit also includes a polymerisable positively chargeable precursor. Illustrative examples of suitable precursors have been given above and are also found in Fig. 5. As explained above, the electrostatic net charge of the polymerisable positively chargeable precursor is complementary to the electrostatic net charge of the target nucleic acid molecule. The kit also includes a suitable reactant molecule, such as an oxidant. In some embodiments the kit may also include a suitable initiator, for example a halogen molecule, an azo compound, a persulfate molecule, and a peroxide compound. Furthermore, in some embodiments the kit may include a catalyst, e.g. a metal chloride, a metal bromide, a metal sulphate or an enzyme (e.g. a peroxidase enzyme or an oxidase enzyme). The catalyst may for example be in solution or coupled to a probe nucleic acid molecule. As explained above, in embodiments where the kit includes a catalyst, the reactant may be a substrate molecule for the catalyst.

[0116] A respective kit may furthermore include means for immobilising the capture molecule to the surface of the immobilisation unit. As explained above, a nucleic acid capture molecule included in the kit may have a moiety that allows for, or facilitates, an immobilisation on a respective immobilisation unit. The kit may also include a linking molecule. As an illustrative example, 6-mercapto-l-hexanol may be included in the kit. Upon using a respective kit, the PNA capture molecule may then be 5'-C₆H₁₂SH-modified (see above for examples).

[0117] Another kit according to the present invention also includes a nucleic acid capture molecule, which has a nucleotide sequence that is at least partially complementary to at least a portion of the target nucleic acid molecule (see above). The kit further includes a polymerisable positively chargeable precursor, e.g. an aromatic amine. As explained above, the electrostatic net charge of the polymerisable positively chargeable precursor is complementary to the electrostatic net charge of the target nucleic acid molecule. The kit also includes a substrate molecule, such as a peroxide or oxygen dissolved in a solution. As indicated above, oxygen dissolves in aqueous solutions, so that any aqueous solution included in the kit may be the source of this substrate molecule. Furthermore, the kit includes an enzyme attached to a probe nucleic acid molecule (see above for examples). The probe nucleic acid molecule is at least partially complementary to at least a portion of the target nucleic acid molecule. A respective kit may furthermore include means for immobilising the capture molecule to the surface of the immobilisation unit as described above.

[0118] A respective kit may be used to carry out a method according to the present
invention. It may include one or more devices for accommodating the above components before, while carrying out a method of the invention, and thereafter. As an illustrative example, it may include a microelectromedical system (MEMS).

[0119] The present invention is of particular significance in the design and manufacture of ultrasensitive non-labelling nucleic acid biosensors and biosensor arrays. The nucleic acid-guided deposition of an electroconductive polymer such as polyaniline combined with efficient biocatalysis offers a very attractive alternative to hybridisation-based nucleic acid biosensors. The skilled artisan will further appreciate that the present invention also allows for the fabrication of simple, low-cost, and portable electrical nucleic acid detection devices, providing fast, cheap and simple solutions for e.g. molecular diagnosis, particularly for cancer diagnosis, point-of-care, and field uses. For instance, point-of-care applications require systems that are portable, robust and easy to use, coupled with a reliable assay. Electrical systems according to the present invention meet and/or exceed all these requirements. Furthermore, this unique combination of amplification by way of polymerisation and template-guided deposition can be used in conjunction with other detection techniques such as quartz-crystal microbalance, surface plasmon resonance, fluorometry, and colorimetry.

[0120] In order that the invention may be readily understood and put into practical effect, particular embodiments will now be described by way of the following non-limiting examples.

**EXAMPLES**

**Reagents**

[0121] PNA capture probe and oligonucleotides were obtained from Eurogentec and used as received. Amino-terminated peptide nucleic acid (PNA) capture probes (N- » C: NH₂-AAC CAC ACA ACC TAC TAC CTC A) (SEQ ID NO: 1), DNA, 5'-TGA GGT AGT AGG TTG TGT GGT T-3' (SEQ ID NO: 2), Homo sapiens microRNA let-7b, corresponding to of SwissProt-Acc.-No AJ421727; single base mismatched DNA, 5'-TGA GGT AGT AGG ATG TGT GGT T-3' (SEQ ID NO: 3), non-complementary DNA, 3'-ACAAGACA TGGTTTTTCCCCCATCA AAGGAAT-5' (SEQ ID NO: 4).

[0122] 3-Aminopropyl triethoxysilane (APTES, 99 %), aniline (99.5 %) and 1,4-phenylenendiisothiocyanate (PDITC, 98 %) were obtained form Sigma-Aldrich (St. Louis, MO). Horseradish peroxidase (200 units/mg) was purchased from Boehringer Mannheim.
GmbH (Germany). Hydrogen dioxide (31 %) was purchased from Santoku BASF. All other reagents were obtained from Sigma-Aldrich and used without further purification.

**Biosensor fabrication**

[0123] The microelectrodes (Au 15 nm, Ti 10 nm) with 500 nm gaps were fabricated as 10x10 arrays on Si wafer with 500 nm coating of SiO₂ by the photolithography method, as displayed in Fig. 1.

[0124] The surface modification of the chips was performed according to the method described by Liu & Bazan (Proc. Natl. Acad. ScL U.S.A. (2005) 102, 3, 589-593) who used the polymer poly[9,9'-bis(6''-N,N,N-trimethylammonium)hexyl]fluorene-co-alt-4,7-(2,1,3-benzothiadiazole)dibromide] .

[0125] The chips were first cleaned with chloroform and acetone in sequence thoroughly to remove any possible organic contaminants. After rinsing with 1M NaOH, followed by thoroughly washing with water, the chips were activated in oven at 120 °C for 20 min. The silanisation of the chips were carried out by soaking the chips in ethanol containing 2 % 3-aminopropyltriethoxysilane and 1 % water (v/v). Then the chips were washed with ethanol and allowed to dry under a mild stream of nitrogen before aging at 120 °C for 20 min. The bifunctional coupling agent phenylendiisothiocyanate was employed to hyphenate PNA capture molecules to the amino-modified chips. Into a mixture solvent of dimethylformamide and pyridine, 50 mg of phenylendiisothiocyanate was added. Then the chips were allowed to react with the solution for 2h, followed by washing with DMF and dichloromethane and subsequently drying under nitrogen flow. PNA was dissolved in 0.1 % trifluoroacetic acid solution and diluted to a concentration of 1.0x10⁻⁶ M in sodium carbonate buffer (50 mM, pH 9.0). Using a standard micropipette a 100 µl aliquot of the solution was spotted onto the chip and the reaction was carried out in a humid chamber in 37 °C for 5 h. Subsequently a washing step utilising ultrapure water and methanol was applied to remove unreacted probes. The surface was deactivated with a dimethylformamide solution (25 ml) containing aminoethanol (0.1 ml), diisopropylethylamine (0.65 ml) over a period of 2 h. The slides were subsequently washed with dimethylformamide, acetone, and water, and dried with nitrogen. Slides were not fully dried between washing steps.

**Sensing procedure and electrical detection**

[0126] A schematic illustration of the sensing procedure was displayed in Fig. 2 and Fig 3.
[0127] The hybridisation was performed with a 10 mM Tris-HCl, 1.0 mM EDTA and 0.1 M NaCl (TE) buffer at room temperature for 6 min in a commercially available hybridisation chamber. After hybridisation, the chips were thoroughly rinsed with blank hybridisation buffer (see above) to remove the unhybridised DNA.

[0128] Deposition of polyaniline was accomplished as described earlier (Liu, W. et al., J. Am. Chem. Soc. (1999) 121, 71-78) as following: to a solution of 2 mM aniline in 0.1 M NaAc buffer solution (pH 4.0), a 2 µL aliquot of 0.2 mg/mL HRP and stoichiometric amount of H₂O₂ was successively added. To avoid the inhibition of HRP due to excess H₂O₂, a diluted H₂O₂ (0.02 M) was added dropwise, incrementally, over 1.5 h. After the addition of H₂O₂, the reaction was left stirring for at least 1 h, and then the final solution was dialyzed (cutoff molecular weight of 2000) against pH 4.3 deionised water overnight to remove any unreacted monomer, oligomers, and phosphate salts. Thereafter a 200 µL of the mixture was directly applied to the chip and kept for 40 min. Then the chip was thoroughly washed with the buffer solution and water to remove any residual enzyme and aniline monomer, followed by a drying process under nitrogen flow. Finally, a doping process was carried out using the enhancer molecule HCl. Exposure to HCl vapour was performed before resistance measurement.

[0129] The measurement of resistance was performed under ambient conditions with a Alessi REL-6100 probe station (Cascade Microtech.) and an ultrahigh resistance multimeter (R8340A, Advantest). The working voltage was set to 0.3 V for all the measurements.

[0130] In an evaluation experiment the detection capability of a sensor with 10 pM DNA, which was fully complementary to the capture molecule, was analysed (see above, SEQ ID NO: 2, Homo sapiens microRNA let-7b, corresponding to of SwissProt-Acc.-No AJ421727). For comparison, a control sample was also tested by applying a non-complementary DNA (of the sequence of SEQ ID NO: 4, see above) for hybridisation and then followed the same procedure as that for complementary ones. For the complementary DNA sample, an obvious increase in conductivity of the nanogaps with polyaniline deposited could not be immediately obtained since the conductivity of the polyaniline before HCl doping was very low.

[0131] After a simple doping process with HCl vapour, the conductance was measured again and the results were depicted in Fig. 6: a remarkable increase in conductivity is found for the complementary DNA sample; while for the control one, only a slight increase was shown when compared to the blank sample (PNA immobilised chips without undergoing polyaniline deposition and doping). This clearly demonstrates that the formation of polyaniline in between the nanogaps was templated by the hybridised DNA molecules,
and the resulted polymer network bridged the gaps and produced measurable conductivity change. The result for control sample shows that the non-hybridised signal for this biosensor was low, which facilitates the detection of DNAs at ultralow concentrations. This might be at least in part attributed to the use of neutral PNA probes instead of conventional oligonucleotide probes, which greatly reduced the background noise by preventing the aniline monomer from adsorbing onto the negatively charged sites of the probes and thus suppressed the background noise (It should however be note here again that the use of charged capture molecules such as "regular" oligonucleotides with phosphate backbone is of course also within the scope of the present invention). As to the slight increase of conductivity found for control sample, this small variation of conductivity might be caused by the contamination of the chip surface during sensing process such as the polyaniline deposition and HCl exposure.

[0132] As can be seen from Fig. 6, the conductivity achieved for the respective biosensor exposed to 10 pM target DNA was 0.36 nS (7.2×10⁻⁶S·cm⁻¹, considering the width of the gap was 500 nm), which was at the same level as that for DNA-polyaniline pellets [Nagarijan, R., et al., J. Macromol. Sci.-Pure Appl. Chem. (2001) A38, 1519-1537] while was as about one order of magnitude lower then that of polyaniline deposited with L-DNA as the template [Liu & Kumar, 1999, supra]. This could be attributed to the existence of discontinuous areas in the polyaniline network between the gaps and also the agglomeration of the DNA-polyaniline complexing strands occurred in deposition (see Nagarijan, R., et al., J. Macromol. Sci.-Pure Appl. Chem. (2001) 38, 1519-1537.)

[0133] A scanning electron microscope characterisation was performed for the chips exposing to control sample and the complementary DNA sample. The images were shown in Fig. 7. It could be seen that, after polyaniline deposition, the chip surface with complementary DNA hybridised was rough with a polymer network. While the control one showed no visible change. The morphology of the polyaniline deposited was somewhat different from the wire-like polymer found in using aligned long DNA strands as the templates for polymerisation. The possible reason might be the DNA sample used here was relatively short with only 22 bases long (estimated strand length of 7~8 nm) and randomly spread. The agglomeration of the DNA/polyaniline complex occurred during the polymerisation due to the neutralisation of the negative charges in DNA strands would inhibit the complex strands from fully stretching and even result in overwound polymorph [Nagarijan et al., 2001, supra]. Therefore, the deposited polyaniline was likely to take on a network-like look other than independent wires with clear boundary.
Optimisation of polyaniline deposition

[0134] Since the driving force of the formation of the aniline-DNA complex is the electrostatic interaction between the positively charged aniline monomer and the negatively charged phosphate groups in DNA backbone, an acidic buffer solution at pH 4.0 was selected as the background to perform the polyaniline synthesis and deposition in order to facilitate the electrostatic interactions between aniline and the phosphate groups and also provide adequate activity of HRP [Liu et al., 1999, supra; Gaylord, B. S., et al., Proc. Natl. Acad. Sd. U.S.A. (2005) 102, 34-39]. To further optimise the conditions for polyaniline deposition, the influences of concentration of aniline, HRP and also the incubation time on the conductivity of the resulting polyaniline were evaluated.

[0135] The optimisation of aniline concentration was performed by varying the aniline concentration in the mixture solution for polymerisation from 0.5 to 5mM while keeping the HRP concentration and incubation time constant. As can be seen from Fig. 8, as the aniline concentration increased from 0.5 to 5 mM, the conductivity of the resulted polyaniline formed in the nanogaps first increased and then achieved a steady value at 2 mM. It is known that the rate of enzyme catalyzing reactions increases with the increasing substrate concentration until the substrate concentration is high enough to saturate the enzyme. According to Fig. 8, 2 mM of aniline was selected to perform polyaniline deposition, which was also high enough to facilitate the longer polymer chain growth and avoid forming short chain segments [Gaylord et al., 2005, supra].

[0136] The concentration of HRP was also optimised in the range of 0.1 to 2.5µg/mL; the results were depicted in Fig. 9. The conductivity of the resulted polyaniline increased with the increasing of the HRP concentration in the range of 0.1~1.0 µg/mL, and further increasing the HRP concentration did not result in continuous increasing in conductivity. According to the enzymatic catalyzing mechanism, the reaction rate is linearly correlated with the enzyme concentration when the substrate concentration is high enough. However, in the case the HRP concentration was too high, the non target-guided aniline polymerisation taking place in the solution would also be accelerated and favour the production of multiple branched polyaniline [Gaylord et al., 2005, supra], which would result in random deposition of polyaniline onto the chip and deteriorate the conductivity of nanogaps. This could be demonstrated by the appearance of platform in Fig. 9 and a higher background obtained for control samples under high HRP concentration. Therefore, 1.0 µg/mL of HRP was selected for polyaniline deposition.

[0137] The incubation time of polyaniline deposition was also optimised. As
displayed in Fig. 10, the longer the incubation time, the higher the conductivity could be achieved. However, an increase of the background noise was shown after 45 min of incubation. The reason might be the increase of the deposition of the polyaniline synthesised in the solution onto the nanogaps. Thus, an incubation time of 40 min was selected to ensure highest signal/noise ratio.

Linearity

[0138] By using this target-guided deposition of polyaniline as the signal amplification method for DNA electrical detection, it is expected that the conductivity between the nanogapped electrodes will depend on the amount (density) of the polyaniline formed along the target DNA molecules. The more target DNA molecules hybridised, the more polyaniline will deposit templatedly along or wrapping the DNA molecules, thus the higher the conductivity will be. Therefore, the target DNA molecules hybridised in the gaps will be directly correlated to the conductivity. Supposing the hybridisation efficiency was the same for all the chips, the quantitative detection could then be performed with the established method.

[0139] Under the optimised conditions, the complementary DNA can be detected in a dynamic range of 50 fM~100 pM with the regression coefficient $R^2$ as 0.976, as depicted in Fig. 11. The detection limit achieved was 40 fM. Comparing with the gold nanoparticle labeling and silver enhance method for detection of DNAs [Liu & Kumar, 1999, supra], the sensitivity of the present assay was one order of magnitude higher.

Single-base mismatch discrimination

[0140] Identification of genetic mutations or single-nucleotide polymorphisms (SNPs) becomes more and more critical in the field of genetic disease therapy and drug discovery. Therefore, the biosensor arrays capable of high-throughput screening and sensitive detecting with standard laboratory techniques are highly desired. It is reported that specificity in recognising the complementary DNA can be achieved even with short PNA probes due to the higher stability of PNA/DNA duplexes than DNA/DNA duplexes [Brandt, O., Hoheisel, J. D., Trends Biotechnol. (2004) 22, 617-622]. Thus, PNA is regarded as promising probe in SNP detections.

[0141] In order to evaluate the capability of the presented method in discriminating single-base mismatched (SBM) DNA, the oligonucleotide with a "G" replaced by "A" was taken as the sample and tested at two concentrations of 100 pM and 10 pM, respectively. The results are shown in Fig. 12: the increases in conductivity for 100 pM and 10 pM SBM
samples were 4.3 and 5.1% of that for complementary ones, respectively. That is to say, the
detection of the single base mismatched mutations is possible using the presented method
with the one point mutation selectivity factor at about 19:1, therefore, the selective detection
of certain DNA in mixture samples will be possible.

Analysis of microRNA from HeLa cell and lung cancer cell extracts

[0142] The sensitive and high-throughput methods for microRNA assays are desired
since they will be the first step to understand the biofunctions of microRNAs. Thus, the
applicability of the presented method was also evaluated by analyzing microRNA let-7b
(sequence of SwissProt-Acc.-No AJ421727) in total RNA extracted from HeLa cells and lung
cancer cells. The concentrated extracts were redissolved in TE buffer at 0.48 and 0.40 μg/μL,
respectively and direct taken for hybridisation. Both samples gave the positive conductive
changes and the results were normalised with respect to total RNA. As a result, let-7b found
in HeLa cell and lung cancer cell total RNA samples was $2.34 \pm 0.29 \times 10^7$ copies and
$2.53 \pm 0.33 \times 10^7$ copies, respectively. The results were consistent with previously published
detection limits of the established assay, it can be expected that hundreds of cells are able to
provide adequate total RNA for microRNA detection. The precision of the assay was also
satisfactory with the RSDs better than 16%, which provided satisfactory accuracy to
distinguish slight microRNA expression differences. Therefore, this assay is promising in
microRNA analysis.

Conclusions

[0143] Sensitive detection of DNA with the target-guided deposition of conducting
polyaniline as the signal amplification method was demonstrated. By using the phosphate
groups in the DNA backbone as template, polyaniline was directly deposit in the nanogaps
between the paired microelectrodes, which resulted in measurable conductive changes. This
method directly utilised chemical ligation for signal read-out and thus eliminated the use of
labeling probes, which greatly simplified the detection procedure. With the synthesised
22mer oligonucleotide as the target, the presented method provided a detection limit of as
low as 40 fM. It can be expected that much lower detection limits can be obtained when
longer target DNA is tested since the bridge of the nanogapped microelectrodes by the
polyaniline can be realized with fewer DNA molecules. Therefore, sensitive and efficient
gene chips can be developed with this strategy. In addition, the applicability to microRNA
detection was also demonstrated preliminarily, indicating that the method also can be used
for microRNA profiling. Finally, the multiplex detection can be easily realized by introducing different species of probes into the chip, which will make the presented method more versatile for various research purposes.

[0144] The listing or discussion of a previously published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge. All documents listed are hereby incorporated herein by reference in their entirety.

[0145] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by exemplary embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0146] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0147] Other embodiments are within the following claims. hi addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.
Claims

What is claimed is:

1. A method of electrically detecting a target nucleic acid molecule by means of a pair of electrodes, wherein said electrodes are arranged at a distance from one another and wherein said pair of electrodes is arranged within a sensing zone, the method comprising:
   (a) immobilising on an immobilisation unit a peptide nucleic acid (PNA) capture molecule, which has a nucleotide sequence that is at least partially complementary to at least a portion of a strand of the target nucleic acid molecule, wherein said immobilisation unit is arranged within said sensing zone;
   (b) contacting said immobilisation unit with a solution suspected to comprise the target nucleic acid molecule;
   (c) allowing the target nucleic acid molecule to hybridise to the PNA capture molecule on the immobilisation unit, thereby allowing the formation of a complex between said PNA capture molecule and said target nucleic acid molecule;
   (d) adding a polymerisable positively chargeable precursor, wherein said polymerisable positively chargeable precursor has an electrostatic net charge that is complementary to the electrostatic net charge of the target nucleic acid molecule, such that (i) said polymerisable positively chargeable precursor associates to the complex formed between said PNA capture molecule and said target nucleic acid molecule, and (ii) the polymerisation of said precursor can be carried out by means of a suitable reactant molecule;
   (e) adding a suitable reactant molecule, thereby initiating the polymerisation of said polymerisable positively chargeable precursor, wherein an electroconductive polymer is formed from the polymerisable precursor and this electroconductive polymer is associated with the complex formed between the PNA capture molecule and the target nucleic acid molecule;
   (f) determining the presence of the target nucleic acid molecule based on an electrical characteristic of a region in between the electrodes, wherein the electrical characteristic is influenced by the electroconductive polymer.

2. The method of claim 1, wherein (f) comprises comparing the result of the electrical measurement obtained with that of a reference measurement.
3. The method of claims 1 or 2, wherein said immobilisation unit comprises an electric insulator.

4. The method of any one of claims 1 - 3, wherein the electric characteristic influenced by the electroconductive polymer is any one of a conductance, a voltage, a current, a capacitance, and a resistance.

5. The method of any one of claims 1 - 4, wherein (f) further comprises generating an electric field.

6. The method of claim 5, wherein said electric field is generated at at least one electrode of said pair of electrodes.

7. The method of any one of claims 1 - 6, wherein said sensing zone is defined by the zone in which an electric field of said pair of electrodes is effective.

8. The method of any one of claims 1 - 7, wherein the reactant molecule is an oxidant.

9. The method of claim 8, wherein the oxidant is at least one of a ruthenium tris(bipyridinium) complex, a persulfate, a peroxide, a chromate, a dichromate, a manganate, a permanganate, oxygen, ozone, a persulfate, a halogen, a chlorite, a chloride, a perchloride, a chlorate, a iodate, a nitrate, a sulfoxide and osmium tetroxide.

10. The method of any one of claims 1 - 9, wherein (e) comprises exposing the reactant molecule to a suitable initiator.

11. The method of claim 10, wherein the initiator is one of a halogen molecule, an azo compound, a persulfate molecule, and a peroxide compound.

12. The method of claim 11, wherein the persulfate molecule is ammonium persulfate.

13. The method of any one of claims 1 - 12, wherein (e) comprises exposing the reactant molecule to a suitable catalyst.

14. The method of claim 13, wherein the catalyst is selected from the group consisting of a metal chloride, a metal bromide, a metal sulphate and an enzyme.

15. The method of claim 14, wherein the reactant molecule is a substrate molecule for said catalyst.
16. The method of claim 14 or claim 15, wherein said catalyst is in solution or is coupled to a probe nucleic acid molecule, wherein said probe nucleic acid molecule is complementary to at least a portion of the target nucleic acid molecule.

17. The method of claim 16, wherein said probe nucleic acid molecule has a nucleic acid sequence of a length of about 5 to about 50 bp.

18. The method of any one of claims 14—17, wherein said catalyst is an enzyme.

19. The method of claim 18, wherein said enzyme is a peroxidase enzyme or an oxidase enzyme.

20. The method of any one of claims 1—19, wherein upon immobilisation at least a part of said PNA capture molecule is comprised within the region in between said pair of electrodes.

21. The method of any one of claims 1—20, wherein the surface of said immobilisation unit on which the PNA capture molecule is immobilised is arranged within the region in between said pair of electrodes.

22. The method of any one of claims 1—21, wherein said PNA capture molecule is single-stranded.

23. The method of any one of claims 1—22, wherein said PNA capture molecule has a nucleic acid sequence of a length of about 7 to about 30 bp.

24. The method of claim 23, wherein said nucleic acid sequence is of a length of about 10 to about 20 bp.

25. The method of any one of claims 1—24, wherein the distance at which the electrodes are arranged from one another is selected in the range between about 0.5 nm to about 10 µm.

26. The method of claim 25, wherein said distance is selected in the range between about 1 nm to about 800 nm.

27. The method of any of claims 1—26, wherein the target nucleic acid molecule has a size of about 50 bp to about 5 x 10^6 bp.
28. The method of claim 27, wherein the target nucleic acid molecule has a size of about 100 bp to about $1 \times 10^4$ bp.

29. A method of electrically detecting a target nucleic acid molecule by means of a pair of electrodes, wherein said electrodes are arranged at a distance from one another and wherein said at least two electrodes are arranged within a sensing zone, the method comprising:

(a) immobilising on an immobilisation unit a nucleic acid capture molecule, which has a nucleotide sequence that is at least partially complementary to at least a portion of a strand of the target nucleic acid molecule,

wherein said immobilisation unit is arranged within said sensing zone;

(b) contacting the immobilisation unit with a solution suspected to comprise the target nucleic acid molecule;

(c) allowing the target nucleic acid molecule to hybridise to the nucleic acid capture molecule on the immobilisation unit, thereby allowing the formation of a complex between said nucleic acid capture molecule and said target nucleic acid molecule;

(d) adding a polymerisable positively chargeable precursor, wherein said polymerisable positively chargeable precursor has an electrostatic net charge that is complementary to the electrostatic net charge of the target nucleic acid molecule, such that (i) said polymerisable positively chargeable precursor associates with the complex formed between said nucleic acid capture molecule and said target nucleic acid molecule, and (ii) the polymerisation of said precursor can be carried out by means of a suitable enzyme and a substrate molecule;

(e) adding a suitable substrate molecule;

(f) adding an enzyme attached to a probe nucleic acid molecule, wherein said probe nucleic acid molecule is at least partially complementary to at least a portion of the target nucleic acid molecule, wherein the detection probe hybridises to a portion of the target nucleic acid different from the portion to which the nucleic acid capture molecule hybridises,

thereby (i) allowing the probe nucleic acid molecule to hybridise to the target nucleic acid molecule, and (ii) catalysing the polymerisation of said polymerisable positively chargeable precursor, wherein an electroconductive polymer is formed from the polymerisable precursor and this electroconductive
polymer is associated with the complex formed between the nucleic acid capture molecule and the target nucleic acid molecule;

(g) determining the presence of the target nucleic acid molecule based on an electrical characteristic of a region in between the electrodes, wherein, wherein the electrical characteristic is influenced by the electroconductive polymer.

30. The method of claim 29, wherein (g) comprises comparing the result of the electrical measurement obtained with that of a reference measurement.

31. The method of claims 29 or 30, wherein the electric characteristic influenced by the electroconductive polymer is any one of a conductance, a voltage, a current, a capacitance, and a resistance.

32. The method of any one of claims 29 - 31, wherein (g) further comprises generating an electric field.

33. The method of claim 32, wherein said electric field is generated at at least one electrode of said pair of electrodes

34. The method of any one of claims 29 - 33, wherein said immobilisation unit comprises an electric insulator.

35. The method of any one of claims 29 - 34, wherein said sensing zone is defined by the zone in which an electric field of said pair of electrodes is effective.

36. The method of any one of claims 29 - 35, wherein the substrate molecule is an oxidant.

37. The method of claim 36, wherein the oxidant is at least one of a persulfate, a peroxide, a chromate, a dichromate, a manganate, a permanganate, oxygen, ozone, a halogen, a chlorite, a chloride, a perchloride, a chlorate, a iodate, a nitrate, a sulfoxide and osmium tetroxide.

38. The method of any one of claims 29 - 37, wherein said enzyme is a peroxidase enzyme or an oxidase enzyme.

39. The method of any one of claims 29 - 38, wherein said probe nucleic acid molecule has a nucleic acid sequence of a length of about 5 to about 50 bp.
40. The method of any one of claims 29 - 39, wherein said nucleic acid capture molecule is single-stranded.

41. The method of any one of claims 29 — 40, wherein said nucleic acid capture molecule has a nucleic acid sequence of a length of about 7 to about 30 bp.

42. The method of claim 41, wherein said nucleic acid sequence is of a length of about 10 to about 20 bp.

43. The method of any one of claims 29 - 42, wherein said nucleic acid capture molecule is one of DNA, RNA and PNA.

44. The method of any one of claims 29 - 43, wherein upon immobilising said nucleic acid capture molecule at least a part of the same is comprised within the region in between said pair of electrodes.

45. The method of any one of claims 29 - 44, wherein the distance at which the electrodes of said pair of electrodes are arranged from one another is selected in the range between about 0.5 nm to about 10 µm.

46. The method of claim 45, wherein said distance is selected in the range between about 1 nm to about 800 nm.

47. The method of any of claims 29 - 46, wherein the target nucleic acid molecule has a size of about 50 bp to about 5 x 10^6 bp.

48. The method of claim 47, wherein the target nucleic acid molecule has a size of about 100 bp to about 1 x 10^3 bp.

49. The method of any one of claims 1 - 48, wherein said target nucleic acid molecule is DNA or RNA.

50. The method of any one of claims 1 - 49, wherein said target nucleic acid molecule comprises a pre-defined sequence.

51. The method of any one of claims 1 - 50, wherein the target nucleic acid molecule comprises at least one single-stranded region.

52. The method of claim 50, wherein said pre-defined sequence is a single-stranded region.
53. The method of any one of claims 19—28 and 38—52, wherein said peroxidase enzyme is a haem peroxidase.

54. The method of claim 53, wherein said haem peroxidase is selected from the group consisting of horseradish peroxidase, cytochrome c peroxidase, glutathione peroxidase, myeloperoxidase, thyroid peroxidase, eosinophil peroxidase, lactoperoxidase, ascorbate peroxidase, peroxidasin, prostaglandin H synthase, E. coli catalase-peroxidase, M. tuberculosis catalase-peroxidase, Bacteroides fragilis catalase-peroxidase, lignin peroxidase, plant ascorbate peroxidase, Haem chloroperoxidase, manganese peroxidase, stigma specific peroxidase, Euphorbia characias latex peroxidase, Arthromyces ramosus peroxidase, sorghum grain peroxidase SPC4, soybean peroxidase, Phanerochaete chrysosporium manganese-dependent peroxidase, and lacrimal gland peroxidase.

55. The method of claim 19 or claim 38, wherein said oxidase enzyme is laccase.

56. The method of any one of claims 1 - 55, wherein said method is carried out in an aqueous solution.

57. The method of any one of claims 1 - 56, wherein the positively chargeable precursor is an aromatic amine.

58. The method of claim 57, wherein said aromatic amine is selected from the group consisting of aniline, pyridineamine, pyrrole and imidazole.

59. The method of claim 57 or claim 58, wherein the target nucleic acid molecule is comprised in a solution of a pH selected in the range of about 1.7 to about 7.0, when said polymerisable positively chargeable precursor is added.

60. The method of claim 57 or claim 58, wherein the pH is brought to a value in the range of about 1.7 to about 7.0 after said polymerisable positively chargeable precursor has been added.

61. The method of claim 59 or 60, wherein the pH is selected in the range of about 2.0 to about 5.5.
62. The method of any one of claims 1 - 61, wherein said electroconductive polymer is contacted with an enhancer reagent before determining the presence of the target nucleic acid molecule.

63. The method of claim 62, wherein said enhancer reagent is selected from the group consisting of an acid, a base, an organic solvent, a pesticide and a cyclodextrine compound.

64. The method of any one of claims 1 - 63, wherein (a) comprises immobilising a blocking agent on the immobilisation unit.

65. The method of any one of claims 1 - 64, wherein the target nucleic acid molecule is comprised in a sample selected from the group consisting of a soil sample, an air sample, an environmental sample, a cell culture sample, a bone marrow sample, a rainfall sample, a fallout sample, a space sample, an extraterrestrial sample, a sewage sample, a ground water sample, an abrasion sample, an archaeological sample, a food sample, a blood sample, a serum sample, a plasma sample, a urine sample, a stool sample, a semen sample, a lymphatic fluid sample, a cerebrospinal fluid sample, a naspharyngeal wash sample, a sputum sample, a mouth swab sample, a throat swab sample, a nasal swab sample, a bronchoalveolar lavage sample, a bronchial secretion sample, a milk sample, an amniotic fluid sample, a biopsy sample, a nail sample, a hair sample, a skin sample, a cancer sample, a tumour sample, a tissue sample, a cell sample, a cell lysate sample, a virus culture sample, a forensic sample, an infection sample, a nosocomial infection sample, a production sample, a drug preparation sample, a biological molecule production sample, a protein preparation sample, a lipid preparation sample, a carbohydrate preparation sample, a solution of a nucleotide, a solution of polynucleotide, a solution of a nucleic acid, a solution of a peptide, a solution of a polypeptide, a solution of an amino acid, a solution of a protein, a solution of a synthetic polymer, a solution of a biochemical composition, a solution of an organic chemical composition, a solution of an inorganic chemical composition, a solution of a lipid, a solution of a carbohydrate, a solution of a combinatory chemistry product, a solution of a drug candidate molecule, a solution of a drug molecule, a solution of a drug metabolite, a suspension of a cell, a suspension of a virus, a suspension of a microorganism, a suspension of a metal, a suspension of metal alloy, a solution of a metal ion, and any combination thereof.
66. A kit for electrically detecting a target nucleic acid molecule, said kit comprising
(a) a pair of electrodes, wherein said electrodes are arranged at a distance from one
another and wherein said pair of electrodes is arranged within a sensing zone,
(b) an immobilisation unit arranged within said sensing zone,
(c) a PNA capture molecule, which has a nucleotide sequence that is at least partially
complementary to at least a portion of the target nucleic acid molecule,
(d) a polymerisable positively chargeable precursor, wherein the electrostatic net
charge of said polymerisable positively chargeable precursor is complementary to
the electrostatic net charge of the target nucleic acid molecule, and
(e) a suitable reactant molecule.

67. The kit of claim 66, wherein the immobilisation unit comprises an electric insulator.

68. The kit of claims 66 or 67, wherein said sensing zone is defined by the zone in which
an electric field of said pair of electrodes is effective.

69. The kit of any one of claims 66 - 68, wherein the reactant molecule is an oxidant.

70. The kit of claim 69, wherein the oxidant is at least one of a ruthenium
tris(bipyridinium) complex, a persulfate, a peroxide, a chromate, a dichromate, a
manganate, a permanganate, oxygen, ozone, a persulfate, a halogene, a chlorite, a
chloride, a perchloride, a chlorate, a iodate, a nitrate, a sulfoxide and osmium tetroxide.

71. The kit of any one of claims 66 - 70, further comprising a suitable initiator.

72. The kit of claim 71, wherein the initiator is one of a halogen molecule, an azo
compound, a persulfate molecule, and a peroxide compound.

73. The kit of any one of claims 66 - 72, further comprising a suitable catalyst.

74. The kit of claim 73, wherein the catalyst is selected from the group consisting of a
metal chloride, a metal bromide, a metal sulphate and an enzyme.

75. The kit of claim 73, wherein the reactant is a substrate molecule for said catalyst.

76. The kit of any one of claims 73 to 75, wherein said catalyst is in solution or is coupled
to a probe nucleic acid molecule, wherein said probe nucleic acid molecule is
complementary to at least a portion of the target nucleic acid molecule.
77. The kit of any one of claims 66 - 76, wherein said PNA capture molecule has a nucleic acid sequence of a length of about 7 to about 30 bp.

78. The kit of claim 77, wherein said nucleic acid sequence is of a length of about 10 to about 20 bp.

79. The kit of any one of claims 66 - 78, wherein said PNA capture molecule is immobilised on said immobilisation unit.

80. The kit of claim 79, comprising means for immobilizing said PNA capture molecule to said immobilisation unit.

81. A kit for electrically detecting a target nucleic acid molecule, said kit comprising
   (a) a pair of electrodes, wherein said electrodes are arranged at a distance from one another and wherein said pair of electrodes is arranged within a sensing zone,
   (b) an immobilisation unit, arranged within said sensing zone,
   (c) a nucleic acid capture molecule, which has a nucleotide sequence that is at least partially complementary to at least a portion of the target nucleic acid molecule,
   (d) a polymerisable positively chargeable precursor, wherein the electrostatic net charge of said polymerisable positively chargeable precursor is complementary to the electrostatic net charge of the target nucleic acid molecule,
   (e) a substrate molecule, and
   (f) an enzyme attached to a probe nucleic acid molecule, wherein said probe nucleic acid molecule is at least partially complementary to at least a portion of the target nucleic acid molecule.

82. The kit of claim 81, wherein the immobilisation unit comprises an electric insulator.

83. The kit of claims 81 or 82, wherein said sensing zone is defined by the zone in which an electric field of said pair of electrodes is effective.

84. The kit of any one of claims 81 - 83, wherein the substrate molecule is an oxidant.

85. The kit of claim 84, wherein the oxidant is at least one of a persulfate, a peroxide, a chromate, a dichromate, a manganate, a permanganate, oxygen, ozone, a halogene, a chlorite, a chloride, a perchloride, a chlorate, a iodate, a nitrate, a sulfoxide and osmium tetroxide.
86. The kit of any one of claims 81 - 85, wherein said nucleic acid capture molecule has a nucleic acid sequence of a length of about 7 to about 30 bp.

87. The kit of claim 86, wherein said nucleic acid sequence is of a length of about 10 to about 20 bp.

88. The kit of any one of claims 81 - 87, wherein said nucleic acid capture molecule is one of DNA, RNA and PNA.

89. The kit of any one of claims 81 - 88, wherein said nucleic acid capture molecule is immobilised on said immobilisation unit.

90. The kit of claim 81 - 88, comprising means for immobilising said nucleic acid capture molecule to said immobilisation unit.

91. The kit of any one of claims 66 - 90, wherein the distance at which the electrodes of said pair of electrodes are arranged from one another is selected in the range between about 0.5 nm to about 10 μm.

92. The kit of claim 91, wherein said distance is selected in the range between about 1 nm to about 800 nm.

93. The kit of any one of claims 74 - 92, wherein said enzyme is a peroxidase enzyme or an oxidase enzyme.

94. The kit of any one of claims 76 - 93, wherein said probe nucleic acid molecule has a nucleic acid sequence of a length of about 5 to about 50 bp.

95. The kit of any one of claims 66 - 94, wherein said target nucleic acid molecule is DNA or RNA.

96. The kit of any one of claims 66 - 95, wherein said target nucleic acid molecule comprises a pre-defined sequence.

97. The kit of any one of claims 66 - 96, wherein the target nucleic acid molecule comprises at least one single-stranded region:

98. The kit of claim 96, wherein said pre-defined sequence is a single-stranded region.
Figure 3
Fig. 5
Fig. 12

Control sample

Conductance (nS)

4.3%

Target DNA 1E-10

Target DNA 1E-11

SBM 1E-10

SBM 1E-11
INTERNATIONAL SEARCH REPORT

PCT/SG2007/000037

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.
C12Q 1/26 (2006.01)  C12Q 1/68 (2006.01)
C12Q 1/28(2006.01)  G01N 27/327 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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[X] Further documents are listed in the continuation of Box C  
[ ] See patent family annex

* Special categories of cited documents
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"D" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
02 April 2007

Date of mailing of the international search report
6 APR 2007

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Form PCT/ISA/210 (second sheet) (April 2007)
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<td>US 601 801 8 A (LYKNE A SAMEULSON et al) 25 January 2000 (See Abstract, figures 1-3, claims)</td>
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<td>A</td>
<td>LE FLOCH et al, Ferrocene-Functionalized cationic polythiophene for the label-free electrochemical detection of ONA. <em>Advanced Materials</em>, 17: 1251-1254, 2005</td>
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</table>
**INTERNATIONAL SEARCH REPORT**

**Box No. II** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. **X** Claims Nos.: 66-98 (PARTIALLY)
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   Claims 66-98 are directed towards kits of parts suitable for electrically detecting a target nucleic acid molecule. The searched was only performed in so far as the kits relate to the method of the invention.

3. Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a)

**Box No. III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.
This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX