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(54) Title: SENSORS FOR DETECTING TARGET NUCLEIC ACIDS AND THEIR USE

(57) Abstract: The present disclosure relates to sensors for detecting a nucleic acid in a sample as well as to methods for their preparation and use. For example, the sensors can comprise a first probe configured to increase photocurrent in the presence of a target nucleic acid and optionally a second probe configured to decrease photocurrent in the presence of a target nucleic acid.

SENSORS FOR DETECTING TARGET NUCLEIC ACIDS AND THEIR USE

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims the benefit of priority from co-pending U.S. provisional application no. 63/495,997 filed on April 13, 2023, the contents of which are incorporated herein by reference in their entirety.

FIELD

[002] The present disclosure relates to photoelectrochemical sensors for detecting a nucleic acid in a sample as well as to methods for their preparation and use. The sensors can advantageously comprise two probes that cause a signal change in opposite directions.

BACKGROUND

[003] Point of care (POC) diagnostics are important to advancements in medicine, for example, because they can eliminate the need for laboratory testing and give a caregiver valuable time to personalize and administer a treatment plan (Wang and Kricka, 2018; Bhalla et al., 2016). The detection and quantification of specific deoxyribonucleic acid (DNA) strands and fragments in biofluids can yield a definitive diagnosis for certain diseases (Rashid and Yusof, 2017). However, the heterogeneous presentation of other diseases renders single-target detection insufficient for complete diagnosis (Crawley et al., 2014). Many proposed multiplexed systems are reliant on external reagents or enzymes, making rapid, simple analysis infeasible in a POC setting (Clifford et al., 2021; Mollarasouli et al., 2019). Commercially available biosensors at present monitor physiological properties like heart rate or sleep quality, blood concentrations of compounds like glucose, or a single biomarker at a point in time (such as for pregnancy or a COVID-19 test). To truly advance the field, reagentless, multiplexed biosensor platforms are desirable.

[004] Photoelectrochemical cells (PEC) have separate excitation and detection mechanisms, yielding a lower signal-to-noise ratio than other electrochemical transducers (Zhao and Ding, 2019; Yu et al., 2022). PECs use the energy given from light to generate a current within a 3-electrode system in electrolyte without changing applied potential (Han et al., 2018). The working electrode is a photoactive material which desirably has good photocatalytic activity, stability and controllable morphology, and good biocompatibility (Li et al., 2019). For this purpose, ZnO nanoparticles have been used in past research. They are n-type semiconductors that are low-cost, environmentally friendly, and non-toxic (Daghrir et

al., 2013). They have many applications in electrochemistry as catalysts (Larina et al., 2019), solar cells (Han et al., 2010; Jiang et al., 2018) and electrochemical biosensors (Han et al., 2017; Feng et al., 2020; Xia et al., 2014; Pradhan et al., 2011). However, their wide bandgap limits light absorption to the UV range, and they are saddled with rapid electron-hole recombination rates. Modifications have been made to the nanoparticle to enhance photocurrent and shift the sensitivity into the visible range of light (Han et al., 2017; Sulciute et al., 2021). Previous signal enhancement strategies for ZnO photoelectrodes have primarily employed costly bench-top synthesis procedures that would be difficult to scale.

[005] Polyethyleneimine (PEI) is a cationic polymer which has one amine group for every two carbon atoms along the polymeric chain (Sanchez-Cortes et al., 2002). It is low-cost, easy to fabricate and environmentally friendly (Zhou et al., 2012). This polymer is versatile due to the ease of attaching organic groups, making it popular for polymeric multifunctional catalysts (Spetnagel and Klotz, 1976). In electrochemistry, PEI reduces the work function of metal oxides, metals and graphene by forming a dipole moment between its amine groups and the electrode (Zhou et al., 2012; Kim and Park, 2014). Additionally, PEI suppresses ZnO suspension agglomeration and reduces the roughness of the drop-cast thin film (Jia et al., 2016; Woo et al., 2014). Jia et al. (2016) reported that the performance of an organic solar cell improved with PEI-ZnO as the cathodic buffer layer, compared to unmodified ZnO. Woo et al. (2014) reported that a PEI-ZnO nanolayer lowers short circuit current density and open circuit voltage, enhancing the average efficiency of an inverted polymer solar cell. Chen et al. (2015) reported that blending PEI within a ZnO film for the electron transport layer improves electron mobility, improving the power conversion efficiency of a polymer solar cell. Linear PEI (LPEI) has been reported to be more effective than branched PEI for improving power conversion efficiency, and simplifies characterization due to its linear chain (Yan et al., 2015; Tanaka et al., 1983). The electrochemical advantages of LPEI may be limited by film size, as a thick layer will act as a resistor (Yan et al., 2015).

SUMMARY

[006] A multiplexed biosensor could enable the identification of multiple targets, which may, for example, improve accuracy in the detection of diseases which may present with more than one distinctive biomarker. The present disclosure includes an example of a platform for photoelectrochemical analysis of single-stranded DNA (ssDNA), using a linear polyethyleneimine (LPEI) modified-zinc oxide photoelectrode. An increase in photocurrent

was observed after LPEI modification. Two different exemplary nucleic acid capture probes were deposited onto the LPEI-ZnO photoelectrode for affinity-based ssDNA analysis via signal-off or signal-on transduction. The signal-off response was based on an increase in steric hinderance upon probe-target hybridization. The unique signal-on mechanism used a partially hybridized double-stranded DNA probe labelled with a gold (Au) nanoparticle that selectively forms a heterojunction with ZnO via toehold displacement reaction in the presence of the target. The specificity of each assay was determined using a mismatched DNA target, which resulted in an insignificant signal change. Thus, this exemplary platform and platforms of the present disclosure may, for example enable reagentless and multiplexed analysis of target DNA.

[007] Accordingly, the present disclosure includes a sensor for detecting a target nucleic acid in a sample, the sensor comprising:

- a first photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and

- a first probe, the first probe comprising:

- a first nucleic acid strand complementary to the target nucleic acid; and

- a second nucleic acid strand that is shorter than the first nucleic acid strand, the second nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the first photoelectrode and the second end coupled to a conductive nanoparticle, wherein the second nucleic acid strand is partially complementary to the first nucleic acid strand and hybridized to the first nucleic acid strand so as to leave an unhybridized region of the first nucleic acid strand distal to the second end of the second nucleic acid strand such that hybridization of the unhybridized region with the target nucleic acid causes separation of the first nucleic acid strand from the second nucleic acid strand, and wherein the second nucleic acid strand is capable of changing conformation subsequent to separation of the first nucleic acid strand so as to bring the conductive nanoparticle in contact with the first photoelectrode, forming a heterojunction, thereby increasing photocurrent.

[008] The present disclosure also includes a method of detecting a target nucleic acid in a sample, the method comprising:

- contacting the sample with a sensor as described herein; and

measuring photocurrent,
wherein an increase in photocurrent indicates the presence of a target nucleic acid complementary to the first nucleic acid strand; and/or
a decrease in photocurrent indicates the presence of a target nucleic acid complementary to the third nucleic acid strand.

[0009] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating embodiments of the disclosure, are given by way of illustration only and the scope of the claims should not be limited by these embodiments, but should rather be given the broadest interpretation consistent with the description as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The embodiments of the disclosure will now be described in greater detail with reference to the attached drawings, in which:

[0011] **FIG. 1** shows an exemplary three-electrode cell setup for photoelectrochemical characterization. The photoelectrode serves as the working electrode (Working) and is coupled with a platinum counter electrode (Counter) and an Ag/AgCl reference electrode (Ref). Also shown is the light source (Light). Once electrolyte is added, a dark box is placed over the setup to impede ambient light from illuminating the working electrode surface.

[0012] **FIG. 2** shows a schematic diagram of exemplary photocurrent output. Reported measurements are (a) the average photocurrent measured at 25-35 seconds.

[0013] **FIG. 3** shows exemplary scanning electron microscopy (SEM) images of ZnO (upper image) and LPEI-ZnO (lower image) coatings. Scale bar in each image shows 400 nm. The modification of ZnO with LPEI did not significantly effect the surface features of the coatings; there was no noticeable change in morphology of the coating.

[0014] **FIG. 4** shows exemplary ^{13}C ssNMR spectrum of as-received LPEI (upper spectrum) and LPEI-ZnO (middle spectrum). LPEI modification of ZnO is confirmed by the shift in the 41.66 ppm peak to 45.11 ppm and the introduction of 161.89 peak. This was verified by testing a 1:1 w/w ratio of as-received LPEI and LPEI-ZnO, confirming the shift in the main peak and the emergence of the 161.89 peak in an exemplary ^{13}C ssNMR spectrum (lower spectrum).

[0015] **FIG. 5** shows exemplary images from ASTM D3359 image and adhesion classification, where the coating is white and the areas of removed coating are dark. The tape test removed significantly more film when ZnO NPs were not modified with LPEI (upper image) in comparison to addition of LPEI (lower image). Scale bars in each image show 200 μm .

[0016] **FIG. 6** shows exemplary IPCE for (i) ZnO and (ii) LPEI-ZnO. The addition of LPEI led to a modest increase in IPCE at wavelengths at or below 450 nm; LPEI-ZnO has a higher photon-to-electron conversion efficiency than ZnO at all wavelengths less than 450 nm.

[0017] **FIG. 7** shows results from exemplary UV-VIS measurement of (i) ZnO and (ii) LPEI-ZnO suspensions in H_2O . There was insignificant difference between the absorption spectra obtained from the two suspensions.

[0018] **FIG. 8** shows the exemplary derived Tauc plots for ZnO (upper) and LPEI-ZnO (lower) suspensions in H_2O , showing that the LPEI modification does not impact band gap. The E_g of both materials is about 3.16 eV.

[0019] **FIG. 9** shows exemplary Mott-Schottky measurements of (i) ZnO and (ii) LPEI-ZnO thin films. Mott-Schottky analysis and V_{FB} measurement in 2 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, showing LPEI-ZnO has a lower V_{FB} than ZnO; the LPEI modification to the ZnO thin film lowered V_{FB} by 49 mV.

[0020] **FIG. 10** is a schematic showing band structure of LPEI-ZnO as determined in Example 1 of the present disclosure. Under 365 nm light irradiation, the electron-hole pair drives a redox reaction between the semiconducting nanocomposite and AA, producing a photocurrent.

[0021] **FIG. 11** is a plot showing exemplary photocurrent output with varying ZnO concentrations. All samples had 1 g/L LPEI and were tested with 365 nm light irradiation and 0.3 V applied potential. The photocurrent increased with increasing ZnO concentration, where 5 g/L ZnO produced the highest photocurrent.

[0022] **FIG. 12** is a plot showing exemplary photocurrent output at different applied potentials upon illumination with a 365 nm light source. Photocurrent increased with increasing potential, where applying 0.3 V produced the highest photocurrent. -300mV and -200mV had no reading.

[0023] **FIG. 13** is a plot showing exemplary photocurrent output at 365 nm light irradiation, 0.3 V applied potential and 5 g/L ZnO. Thin films of 1g/L LPEI did not produce any photocurrent. The addition of 1 g/L LPEI to ZnO increased photocurrent by 50% on average. The addition of 1g/L BPEI to ZnO caused a reduction in photocurrent.

[0024] **FIG. 14** is a plot showing exemplary photocurrent measurements after photoelectrode storage for up to 7 days, showing a relatively stable output; revealing no significant trend in photocurrent change.

[0025] **FIG. 15** is a schematic diagram illustrating the mechanism of detection for an embodiment of the signal-off assay, in which the amine-terminated ssDNA probe hybridizes with complementary target, reducing photocurrent through steric hinderance.

[0026] **FIG. 16** shows exemplary results from signal-off detection of 0.5 μM target DNA in PBS: signal-off photocurrent results for (+) LPEI-ZnO/P1/T1, and (-) LPEI-ZnO/P1/NC (upper); and average photocurrent change from signal-off LPEI-ZnO/P1 (lower). The signal-off mechanism results in a 60% decrease in photocurrent.

[0027] **FIG. 17** is a schematic diagram illustrating the mechanism of detection for an embodiment of signal-on DNA detection of target ssDNA via a toehold strand displacement reaction. The non-bound strand of the AuNP-conjugated dsDNA probe hybridizes the target, leaving the surface immobilized strand to form a hairpin and bring the AuNP in contact with the electrode surface, forming a heterojunction and increasing photocurrent.

[0028] **FIG. 18** shows exemplary scanning transmission electron microscopy (STEM) micrographs of synthesized AuNPs. Scale bar in upper image shows 150 nm. Scale bar in lower image shows 50 nm. 12.5 nm was the average diameter of 32 nanoparticles measured.

[0029] **FIG. 19** shows plots showing exemplary fluorescence calibration curve (upper); and ratio of probe DNA to AuNP after conjugation (lower).

[0030] **FIG. 20** is a plot showing exemplary photocurrent at different signal-on P1:PolyT ratios after incubation with 0.5 μM of target DNA, showing that the photocurrent increases as the P1 relative concentration decreases.

[0031] **FIG. 21** (left) shows exemplary Mott-Schottky plots of detection mechanisms before and after hybridization for signal-on mechanism, where (i) is signal-on LPEI-ZnO/P1/P2 and (ii) is LPEI-ZnO/P1/P2/T2. V_{FB} was reduced by about 40 mV after hybridization. The shallower slope of (ii) is also indicative of an increase in charge carrier density. **FIG. 21** (right) shows exemplary Mott-Schottky plots of detection mechanisms before and after hybridization for signal-on mechanism after incubation with a mismatch, where (i) is signal-on LPEI-ZnO/P1/P2 and (iii) is LPEI-ZnO/P1/P2/NC. V_{FB} was unchanged. While not wishing to be

limited by theory, the shallower slope of (ii) may have been caused by some probe DNA being removed during rinsing cycles, reducing the probe's steric hinderance effect.

[0032] FIG. 22 shows Mott-Schottky measurements in 2 mM $K_3[Fe(CN)_6]$ of signal-on (i) LPEI-ZnO/P1/P2 and (ii) LPEI-ZnO/P1/P2/T2 wherein the signal-on hybridization mechanism causes V_{FB} to decrease by 51 mV (upper); and a schematic showing a band diagram of heterojunction after signal-on hybridization (lower). The AuNP injects hot electrons into the photoelectrode, boosting photocurrent.

[0033] FIG. 23 shows exemplary results from signal-on detection of 0.5 μ M target DNA in PBS: signal-on photocurrent results for (+) LPEI-ZnO/P1/P2/T2, and (-) LPEI-ZnO/P1/P2/NC (upper); and average photocurrent change from signal-on LPEI-ZnO/P1 (lower). The signal-on mechanism results in a 25% increase in photocurrent.

DETAILED DESCRIPTION

I. Definitions

[0034] Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the disclosure herein described for which they would be understood to be suitable by a person skilled in the art.

[0035] As used herein, the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “include” and “includes”) or “containing” (and any form of containing, such as “contain” and “contains”), are inclusive or open-ended and do not exclude additional, unrecited elements or process/method steps. As used herein, the word “consisting” and its derivatives, are intended to be close ended terms that specify the presence of stated features, elements, components, groups, integers, and/or steps, and also exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The term “consisting essentially of” and any form thereof, as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of these features, elements, components, groups, integers, and/or steps.

[0036] Terms of degree such as “substantially”, “about” and “approximately” as used herein mean a reasonable amount of deviation of the modified term such that the end result is not significantly changed. These terms of degree should be construed as including a deviation

of at least $\pm 5\%$ of the modified term if this deviation would not negate the meaning of the word it modifies.

[0037] As used in this disclosure, the singular forms “a”, “an” and “the” include plural references unless the content clearly dictates otherwise.

[0038] The term “and/or” as used herein means that the listed items are present, or used, individually or in combination. In effect, this term means that “at least one of” or “one or more” of the listed items is used or present.

[0039] The term “nucleic acid” as used herein refers to a polynucleotide, for example, a ribonucleotide (RNA) or a deoxyribonucleotide (DNA) and includes polynucleotides including suitable modified nucleotides and/or derivatives capable of forming base-pair relationships.

II. Sensors and Methods

[0040] Biosensors are an important aspect of diagnostic medicine since they may, for example, enable biomarker detection at point-of-care and/or at-home settings, which may, for example, make diagnosis faster and less resource-intensive than laboratory methods. As some diseases present heterogeneously, they may require detection of multiple biomarkers. To this end, reagentless, multiplexed biosensors that are simple to produce and use are needed. The present disclosure includes an example of a photoelectrochemical biosensor platform capable of multiplexed, reagentless DNA detection, using a zinc oxide/linear polyethyleneimine photoelectrode and fabricated with a simple drop-casting technique. Due to a lowering of flat-band potential, this modification improved the photocurrent response to 365 nm light. This platform was then modified to detect multiple biomarkers via DNA hybridization, with either signal-off or signal-on transduction. The signal-off response was from steric hinderance caused by probe-target hybridization. The unique signal-on mechanism was based on toehold-strand displacement, using a dual-strand DNA probe labelled with a gold nanoparticle that selectively forms a heterojunction with the nanocomposite. With UV excitement, the gold nanoparticle injects hot electrons into the conduction band of ZnO, which would increase photocurrent. In the presence of each respective target, the signal-off mechanism decreased photocurrent by 60% and the signal-on mechanism increased photocurrent by 25%. An insignificant photocurrent change was observed in the presence of a mismatched DNA, showing that the sensor has excellent selectivity. Such platforms may be useful, for example, in the detection of disease DNA in biological samples.

[0041] Accordingly, the present disclosure includes a sensor for detecting a target nucleic acid in a sample, the sensor comprising:

- a first photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and

- a first probe, the first probe comprising:

- a first nucleic acid strand complementary to the target nucleic acid; and

- a second nucleic acid strand that is shorter than the first nucleic acid strand, the second nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the first photoelectrode and the second end coupled to a conductive nanoparticle, wherein the second nucleic acid strand is partially complementary to the first nucleic acid strand and hybridized to the first nucleic acid strand so as to leave an unhybridized region of the first nucleic acid strand distal to the second end of the second nucleic acid strand such that hybridization of the unhybridized region with the target nucleic acid causes separation of the first nucleic acid strand from the second nucleic acid strand, and wherein the second nucleic acid strand is capable of changing conformation subsequent to separation of the first nucleic acid strand so as to bring the conductive nanoparticle in contact with the first photoelectrode, forming a heterojunction, thereby increasing photocurrent.

[0042] In an embodiment, the sensor further comprises a second photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and a second probe, the second probe comprising a third nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the second photoelectrode, the third nucleic acid strand complementary to a target nucleic acid, and wherein hybridization of the target nucleic acid with the third nucleic acid strand causes an increase in steric hindrance, thereby decreasing photocurrent.

[0043] Accordingly, the present disclosure also includes a sensor for detecting a target nucleic acid in a sample, the sensor comprising:

- a first photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and

- a first probe, the first probe comprising:

- a first nucleic acid strand complementary to the target nucleic acid; and

- a second nucleic acid strand that is shorter than the first nucleic acid strand, the second nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the first photoelectrode and the second end coupled to a conductive nanoparticle, wherein the second nucleic acid strand is partially complementary to the first nucleic acid strand and hybridized to the first nucleic acid strand so as to leave an unhybridized region of the first nucleic acid strand distal to the second end of the second nucleic acid strand such that hybridization of the unhybridized region with the target nucleic acid causes separation of the first nucleic acid strand from the second nucleic acid strand, and wherein the second nucleic acid strand is capable of changing conformation subsequent to separation of the first nucleic acid strand so as to bring the conductive nanoparticle in contact with the first photoelectrode, forming a heterojunction, thereby increasing photocurrent; and

- a second photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and

- a second probe, the second probe comprising a third nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the second photoelectrode, the third nucleic acid strand complementary to a target nucleic acid, and wherein hybridization of the target nucleic acid with the third nucleic acid strand causes an increase in steric hindrance, thereby decreasing photocurrent.

[0044] The first end of the third nucleic acid strand is immobilized via any suitable means, the selection of which can be made by a person skilled in the art. In an embodiment, the first end of the third nucleic acid strand is immobilized via a covalent bond. In another embodiment, the first end of the third nucleic acid strand is immobilized via a covalent bond

formed via reaction of an NH_2 group at the 5' end of the third nucleic acid strand with a complementary reactive group on the surface.

[0045] In an embodiment, the first nucleic acid strand and third nucleic acid strand are complementary to different target nucleic acids.

[0046] The first end of the second nucleic acid strand is immobilized via any suitable means, the selection of which can be made by a person skilled in the art. In an embodiment, the first end of the second nucleic acid strand is immobilized via a covalent bond. In another embodiment, the first end of the second nucleic acid strand is immobilized via a covalent bond formed via reaction of an NH_2 group at the 5' end of the second nucleic acid strand with a complementary reactive group on the surface.

[0047] In an embodiment, the first nucleic acid strand, the second nucleic acid strand, the third nucleic acid strand, if present, and the target nucleic acid(s) comprise single strand DNA. It will be appreciated by a person skilled in the art that the sequences of the first nucleic acid strand, the second nucleic acid strand and the third nucleic acid strand, if present, will vary depending on the nucleic acid sequence of the target nucleic acid.

[0048] In an embodiment, the material comprising the conductive substrate, the photoactive material and/or the polymer coated on the photoactive material of the first photoelectrode and the material comprising the conductive substrate, the photoactive material and/or the polymer coated on the photoactive material of the second photoelectrode, respectively, are at least substantially the same.

[0049] The conductive substrate can comprise any suitable material or combinations thereof, the selection of which can be made by a person skilled in the art. In an embodiment, the conductive substrate comprises a conductive metal (e.g., a gold electrode), a conductive oxide (e.g., a transparent conducting oxide such as indium tin oxide), screen-printed conductive electrodes (printed from any suitable material or combinations thereof) or combinations thereof. In an embodiment, the conductive substrate comprises indium tin oxide (ITO). In an embodiment, the conductive substrate comprises a screen-printed electrode.

[0050] The photoactive material can comprise any suitable material or combinations thereof, the selection of which can be made by a person skilled in the art. In an embodiment, the photoactive material comprises a metal oxide. In another embodiment, the metal oxide comprises, consists essentially of or consists of zinc oxide (ZnO) nanoparticles.

[0051] In an embodiment, the first photoelectrode comprises the polymer. In another embodiment, the second photoelectrode comprises the polymer. In another embodiment, the polymer comprises polyethyleneimine. The polyethyleneimine can be any suitable polyethyleneimine or combination thereof, the selection of which can be made by the person skilled in the art. In an embodiment, the polyethyleneimine is linear.

[0052] In an embodiment, the conductive nanoparticle comprises a metal. The metal can comprise any suitable metal or combination thereof, the selection of which can be made by a person skilled in the art. In an embodiment, the conductive nanoparticle comprises, consists essentially of or consists of a gold nanoparticle. The second end of the second nucleic acid strand can be coupled to the conductive nanoparticle by any suitable means, the selection of which can be made by a person skilled in the art and may depend, for example, on the identity of the conductive nanoparticle. In an embodiment, the second end of the second nucleic acid strand is coupled to the conductive nanoparticle via an Au-S bond formed via reaction of a SH group at the 3' end of the second nucleic acid strand with the gold nanoparticle.

[0053] In an embodiment, the change in confirmation comprises formation of a hairpin.

[0054] In an embodiment, the sample is a biofluid.

[0055] The present disclosure also includes a photoelectrode as described herein, for example, in respect to the sensors and relevant embodiments with respect to such sensors.

[0056] The present disclosure also includes a method of detecting a target nucleic acid in a sample, the method comprising:

- contacting the sample with a sensor as described herein; and
- measuring photocurrent,
- wherein an increase in photocurrent indicates the presence of a target nucleic acid complementary to the first nucleic acid strand; and/or
- a decrease in photocurrent indicates the presence of a target nucleic acid complementary to the third nucleic acid strand.

[0057] The contacting of the sample with the sensor can comprise any suitable methods, means and/or conditions. For example, it would be appreciated by a person skilled in the art that the time for contacting is suitable for the target nucleic acid to hybridize with the first nucleic acid strand and/or the third nucleic acid strand, as the case may be. The measuring of

the photocurrent can be carried out by any suitable methods and/or means, the selection of which can be made by a person skilled in the art having regard to the present disclosure.

[0058] In an embodiment, the method of detecting the target nucleic acid in the sample is for use in diagnosing a disease, disorder or condition associated with the presence of the target nucleic acid complementary to the first nucleic acid strand and/or the target nucleic acid complementary to the third nucleic acid strand. Accordingly, the present disclosure also includes a use of a sensor as described herein in the diagnosis of a disease, disorder or condition associated with the presence of the target nucleic acid complementary to the first nucleic acid strand and/or the target nucleic acid complementary to the third nucleic acid strand.

[0059] The present disclosure also includes a method for preparing a sensor for detecting a target nucleic acid in a sample as described herein. In an embodiment, the method comprises depositing the photoactive material onto a surface of the conductive substrate. In another embodiment, the sensor comprises the polymer, and the method comprises depositing the polymer onto a top surface of the photoactive material which has been deposited onto the surface of the conductive substrate. In another embodiment, the sensor comprises the polymer, and the method comprises depositing the first probe onto a top surface of the polymer which has been deposited onto the top surface of the photoactive material of the first photoelectrode. In another embodiment, the method comprises depositing the first probe onto a top surface of the photoactive material which has been deposited onto the surface of the conductive substrate of the first photoelectrode. In another embodiment, the sensor comprises the second photoelectrode and the polymer, and the method comprises depositing the second probe onto a top surface of the polymer which has been deposited onto the photoactive material of the second photoelectrode. In another embodiment, the sensor comprises the second photoelectrode, and the method comprises depositing the second probe onto a top surface of the photoactive material which has been deposited onto the surface of the conductive substrate of the second photoelectrode. In an embodiment, the method comprises deposition of the photoactive material, polymer, first probe and/or second probe via a method comprising drop-casting. In an embodiment, the conductive substrate comprises a screen-printed electrode. It will be appreciated by a person skilled in the art that in embodiments wherein the sensor comprises the first photoelectrode and the second photoelectrode, the sensor may comprise a dual screen-printed electrode and could readily adapt the method for preparation accordingly. For example, the skilled person would readily appreciate that the

first probe can be deposited in a region coupled to a first working electrode and the second probe can be deposited in a region coupled to a second working electrode.

[0060] The following are non-limiting examples of the present disclosure:

EXAMPLES

Example 1

[0061] An objective was to use facile synthesis methods to produce a PEC biosensor capable of detecting two different ssDNA targets without a target-labeling step. Other objectives included: the development of a synthesis procedure to produce an LPEI-ZnO photoelectrode and characterize its surface morphology, chemical structure, and adhesion properties; to characterize the photoelectrochemical properties of the LPEI-ZnO photoelectrode (as the light absorbance, photon-to-electron conversion efficiency and shifts in VFB potential would provide useful information in understanding the mechanism for photocurrent production); to optimize material chemistry and photoelectrochemical parameters, such as applied potential bias, to maximize base photocurrent and increase the signal-to-noise ratio; and to design, optimize, and validate the signal-off and signal-on mechanisms of nucleic acid analysis.

[0062] An exemplary LPEI-ZnO nanocomposite photoelectrode was prepared that may, for example, be suited for a reagentless, multiplexed DNA biosensing platform via a facile layer-by-layer drop-casting technique at room temperature. The structure and dispersion of the nanocomposite was evaluated using solid-state nuclear magnetic resonance (ssNMR) spectroscopy and scanning electron microscopy (SEM), and the adhesion using ASTM D3359 (2012). The electrochemical and photoelectrochemical properties of the nanocomposite were assessed using incident photon-to-electron conversion efficiency (IPCE), Mott-Schottky analysis, ultraviolet-visible spectroscopy (UV-VIS), and photoelectrochemical measurements. After construction and characterization of the photoelectrode, a multiplexed biosensor was constructed using DNA hybridization as the bioreceptor, with both signal-off and signal-on transduction for different targets. The signal-off mechanism used a short single strand DNA (ssDNA) probe which hybridizes with the target DNA to form a dual-strand DNA (dsDNA), increasing steric hinderance and lowering photocurrent. The signal-on mechanism, based on toehold strand displacement, used a gold nanoparticle (AuNP)-conjugated probe DNA strand partially hybridized into rigid dsDNA by a target-recognition piece. In the presence of the target, the target-recognition piece detaches

from the probe. The AuNP-labelled probe then reforms its natural hairpin structure and brings the AuNP in contact with the photoactive surface. The underlying mechanism behind the resulting increase in photocurrent was characterized by measuring flat-band potential via Mott-Schottky analysis after the formation of the heterojunction. With multiple specific and sensitive detection methods and simple, low temperature, non-toxic manufacturing, this biosensor platform may for example, be tailored for sensitive and selective detection of multiple DNA biomarkers in biological samples.

I. Experimental Methods

[0063] (a) Materials: Indium Tin Oxide (ITO) on glass (30-60 ohm/sq) square substrates, ZnO nanoparticles (<100 nm), linear PEI (MW 10,000) hydrochloride, L-Ascorbic acid (AA), potassium ferrocyanide, gold(III) chloride (49% metal basis), trisodium citrate dihydride, and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Sigma-Aldrich. Potassium chloride, sodium chloride, denatured ethanol, and acetone were obtained from Fisher Scientific. Branched PEI (MW 10,000) was obtained from Polysciences. Phosphate buffered saline (PBS) 1x concentrate (0.01 M, pH 7.4) was obtained from Gibco, and PBS 10x concentrate (0.1 M, pH 7.4) was obtained from Fisher Scientific. The Pt counter electrode was obtained from Sigma-Aldrich, and the Ag/AgCl reference electrode was obtained from Gamble Technologies Ltd. The DNA strands used to test signal-off and signal-on detection were obtained dry in tubes from Integrated DNA Technologies. The strands used to test the signal-off assay are listed in Table 1, and the strands used to test the signal-on assay are listed in Table 2. The 365 nm UV light source was obtained from the Darkbeam company.

Table 1: ssDNA for signal-off detection.

DNA	Sequence
Probe DNA (P1)	NH ₂ -AGGGAGATCGTAAGCAAAAA (SEQ ID NO:1)
Target DNA (T1)	TTTTTGCTTACGATCTCCC (SEQ ID NO:2)
Non-Complementary Target DNA/Mismatched DNA (NC)	TTTTTTTTTTTTTTTTTTTTT (SEQ ID NO:3)

Table 2: ssDNA for signal-on detection

DNA	Sequence
Thiol-Terminated Probe DNA (P1)	NH₂ -TCTAGAGGCCCTATAGTGAG TCGTATTAGCCTCTAGATTTTTT- SH (SEQ ID NO:4)
Hybridizing Probe DNA/ Target-Recognition Piece (P2)	TTGTTCTATCATTTCATCTAGAGGCTAA TACGACTCACTATAGG (SEQ ID NO:5)
Target DNA (T2)	CCTATAGTGAGTCGTATTAGCCTCTAGA TGAATGATAGAACAA (SEQ ID NO:6)
Non-Complementary Target DNA/Mismatched DNA (NC)	TTTTTTTTTTTTTTTTTTTTT (SEQ ID NO:3)
Fluorescent Probe DNA (Fluorescent P1)	FL -TCTAGAGGCCCTATAGTGAG TCGTATTAGCCTCTAGATTTTTT- SH (SEQ ID NO:7)
Poly-T	TTTTTT-SH

[0064] (b) Photoelectrode Fabrication: ITO on glass substrates were washed with deionized (DI) water, ethanol, and then acetone. They were then air-dried and plasma-cleaned using a Henneker Plasma HPT-200 plasma cleaner for 3 minutes using maximum power (200 W) and low pressure in an air environment. Following this, the ITO surface was masked with Scotch masking tape, leaving an area of 1 cm × 1 cm exposed. ZnO nanoparticles were suspended in de-ionized (DI) H₂O at a concentration of 5 g/L by mixing for 5 minutes, then sonicating for 25 minutes using a Branson 2800 Sonicator. Once complete, 50 µL of suspension was drop-cast onto the exposed ITO surface and air-dried at room temperature. Once dry, LPEI hydrochloride was dissolved into DI water at a concentration of 1 g/L by stirring for 5 minutes. 50 µL of LPEI suspension was drop-cast on top of the ZnO film and allowed to air-dry at room temperature overnight.

[0065] (c) Surface Characterization: The surface morphology of the photoactive layer was analyzed using scanning electron microscopy (SEM) with a Helios Nanolab 650 FIB-SEM after sputter-coating a 5 nm conductive iridium thin film (Leica EM MED 020). The structure of the LPEI-ZnO photoelectrode was identified using carbon-13 solid-state nuclear magnetic resonance spectroscopy (¹³C ssNMR), using a Bruker Wide Bore Solid State AV400 spectrometer. To produce the LPEI-ZnO powdered sample, a 5:1 w/w ratio of ZnO and LPEI hydrochloride were mixed in water and allowed to air dry over several days. The resulting thick, brittle film was crushed into a fine powder to be used for analysis. The spectrum of as-received

LPEI hydrochloride was tested as the control. Ultraviolet-visible (UV-VIS) spectroscopy was performed on a LPEI-ZnO suspension in DI water using an Agilent Cary 60 UV-VIS Spectrophotometer from 250 nm to 700 nm. Three replicates were tested where applicable.

[0066] Tauc plots were produced from these spectra by converting the light wavelength into $h\nu$ by the following:

$$h\nu \text{ (eV)} = \frac{1239.8}{\lambda} \quad ,$$

where λ is the wavelength of light.

[0067] The absorbance measurement can be converted to α by the following:

$$\alpha = 2.303 * \frac{A}{l} \quad ,$$

where A is the absorbance measurement and l is the path length (1 cm).

[0068] Adhesion tests were conducted using ASTM D3359: Standard Test Methods for Rating Adhesion by Tape Test. The cross-cut method was used to test the samples, and the classifications were determined according to the ASTM document.

[0069] **(d) Photoelectrochemical Characterization:** Photoelectrochemical measurements were conducted by chronoamperometry using a three-electrode cell setup under a dark box, shown in FIG. 1. An as-prepared LPEI-ZnO photoelectrode was connected to the cell as the working electrode using copper tape. It was accompanied by an Ag/AgCl reference electrode and a Pt foil counter electrode. The supporting electrolyte was 0.1 M AA in 10x PBS. A Darkbeam 5W 365 nm flashlight was used to illuminate the electrode surface. The setup was covered with an aluminum-foil lined cardboard box to isolate the cell from ambient light. Chronoamperometric measurements were taken by a Palmsens EmStat Pico potentiostat. Current data was collected for 60 seconds, where the surface was illuminated between 20 and 40 seconds. To facilitate comparisons, a sample's photocurrent was considered the average of current measurements between 25-35 seconds (shown in FIG. 2). Three replicates per sample type were measured and compared using the average of current measurements between 25-35 seconds.

[0070] LPEI-ZnO photoelectrode stability during storage was evaluated by measuring the photocurrent over 7 days. Prior to testing, samples were stored in the dark. Each sample was conditioned for 10 seconds at 0.3 V under UV light prior to measurement. The

chronoamperometric measurements were taken with an applied voltage of 0.3 V. The average photocurrent between 25-35 seconds for three repeats was reported.

[0071] Incident photon to current efficiency (IPCE) is a measure of the electrode's ability to convert light photons into electrons, and is denoted by the following equation:

$$IPCE(\%) = \frac{1240 * I}{\lambda * P_{inc}} * 100 \quad ,$$

where I is the photocurrent (A/cm^2), λ is the incident wavelength, and P_{inc} is the incident optical power of the light source. The LPEI-ZnO working electrode was tested in a two-electrode cell configuration with an Ag/AgCl reference electrode in a solution containing 0.1 M PBS and 0.1 M AA as the electrolyte. Spectra were measured using a Newport Oriel IQE 200 system. The system was calibrated with a silicon detector. The light output was modulated from 300 nm to 600 nm at 10 nm intervals.

[0072] Mott-Schottky analysis was performed using a Gamry Reference 600 potentiostat. A 3-electrode cell system was used with an Ag/AgCl reference electrode and a Pt foil counter-electrode. Measurements were taken in the dark. The electrolyte was 2 mM potassium ferrocyanide, 0.1 M potassium chloride, and 10 mM PBS. The experiments were performed at a frequency of 1 kHz and an alternating current excitation of 10 mV. The linear region of the obtained curve can be approximated by the following formula:

$$\frac{1}{C^2} = \frac{2}{\epsilon \epsilon_0 A^2 e N_D} (V - V_{fb}) \quad ,$$

where C is the interfacial capacitance (F), ϵ is the dielectric constant of the semiconductor, ϵ_0 is vacuum permittivity, A is the area (cm^2), e is the charge of an electron, N_D is the charge carrier density, and V_{fb} is the flat-band potential of the semiconductor. From this formula, the slope increases with decreasing charge carrier density, and V_{fb} can be determined by extrapolating the linear region to the point of intersection with the X-axis.

[0073] (e) Gold Nanoparticle Synthesis: Gold nanoparticles (AuNP) were synthesized using the citrate reduction method, in line with a literature procedure (Grabar et al., 1995). 250 mL of 1 mM gold (III) chloride solution was brought to a boil and stirred vigorously. The starting colour of the solution was pale yellow. 25 mL of 38 mM sodium citrate dihydride solution was quickly added to the vortex, and the colour of the solution quickly shifted to dark red. The solution was allowed to boil for 10 minutes, after which the flask was removed

from the heat and stirred vigorously for 15 minutes. Then, the suspension was removed from the stir plate and was allowed to reach room temperature. Particle size was characterized using a Helios Nanolab 650 FIB-SEM using the scanning transmission electron microscopy (STEM) detector and analyzed using ImageJ software, where the diameter of 32 nanoparticles was measured. The suspension was stored at 4°C.

[0074] (f) Gold Nanoparticle-DNA Conjugation: The AuNP-P1 conjugation procedure was adapted from literature (Wang et al., 2017; Zhang et al., 2007). First, signal-on P1 and P2 were hybridized by mixing 100 μ M suspensions of each together, heating at 85°C for 10 minutes and slowly cooling to room temperature over two hours. Meanwhile, 1 mL of AuNP suspension was centrifuged at 4°C at 16,200g for 15 minutes. The supernatant was discarded, and the AuNPs were resuspended in DI water. 99 μ L of hybridized (dsDNA) probe DNA suspension was reduced by adding 1 μ L of 10 mM TCEP and incubating for 30 minutes. 35 μ L of the reduced probe suspension was added to 1 mL of AuNP suspension and incubated at room temperature for 16 hours. To remove unconjugated AuNPs, 9 μ L of buffer containing 10 mM PBS and 2 M NaCl were slowly added, and the suspension was incubated for 30 minutes. This was repeated 6 times, for a total of 54 μ L added, then incubated for 40 hours at room temperature. The resulting suspension was then centrifuged at 18,600g at 4°C for 30 minutes and resuspended in 10 mM PBS. This was repeated a total of 3 times, then stored at 4°C.

[0075] The ratio of signal-on P1 to AuNP after conjugation was determined by fluorescence measurements. The concentration of AuNPs in the synthesized suspension were determined by measuring the light absorbance at 450 nm. Beer-Lambert law, shown in the equation below, was used to calculate the concentration.

$$Absorbance = \alpha cl$$

wherein in this equation, α is the molar absorption coefficient, c represents the molar concentration, and l represents the light path length. Fluorescent signal-on P1 were used in place of amine-terminated P1 for the conjugation procedure in 1:0, 1:1, 1:5, 1:10, 1:20, 1:40 and 0:1 ratios to PolyT. A calibration curve was produced by suspending the fluorescent P1 strands in PBS at 0.1 nM, 1 nM, 10 nM, 100 nM, and 1000 nM concentrations. Then, in a 96-well plate, the AuNP-conjugated fluorescent P1 suspension was incubated with 20 mM dithiothreitol overnight, then fluorescence was measured at an excitation wavelength of 495 nm and an absorbance wavelength of 520 nm using a Filtermax F5 Multi-Mode microplate

reader. The fluorescence measurements for three repeats of the suspension were taken, and the P1:PolyT ratio was determined using the calibration curve.

[0076] (g) Multiplexed DNA Analysis: Prior to adding the bioreceptor layer, the LPEI-ZnO photoelectrode was carefully rinsed with 10 mM PBS. Each subsequent step was conducted by drop-casting 50 μ L of each DNA suspension for signal-off, and 35 μ L of each DNA suspension for signal-on. First, a 1 μ M suspension of a probe DNA was drop-cast and incubated for one hour at room temperature. Afterwards, excess solution was removed, and the surface was thoroughly rinsed with 10 mM PBS. Following this, the electrodes were incubated for one hour at room temperature with either 0.5 μ M of corresponding complementary (T1 for signal-off, T2 for signal-on) or NC DNA. After a final 10 mM PBS rinse, the photocurrent of these photoelectrodes was tested as described above. The results for relative photocurrent were quantified by relative photocurrent change by using the equation below:

$$\% \Delta I = \left| \frac{I_{Target} - I_{Probe}}{I_{Probe}} \right| * 100 \quad ,$$

where I_{Target} represents the photocurrent after photoelectrode is incubated with probe suspension, then with buffer containing complementary or non-complementary strands, and I_{Probe} corresponds to the photocurrent when the photoelectrode was incubated with only the probe DNA. The photocurrent for a sample is the average of measured currents between 25-35 seconds of the chronoamperometric program, and three repeats were taken for each sample type.

II. Results and Discussion

(a) Photoelectrode Characterization and Photocurrent Optimization

[0077] (i) Surface Characterization: SEM was used to compare the morphology of the ZnO nanoparticle thin film with the LPEI-ZnO thin film, as shown in FIG. 3. The as-received nanoparticles had non-uniform size and morphology but had good surface coverage on the ITO substrate after drop-casting. Surface modification of the drop-casted ZnO layer with LPEI did not change the morphology or surface coverage of the particles.

[0078] ^{13}C ssNMR is a method used to determine the chemical structure of polymers in a powdered state. It produces a characteristic spectrum that is used to identify the bonds to each carbon atom in the polymer chain. To characterize the mechanism of binding between LPEI and ZnO NPs, ^{13}C ssNMR was performed on as-received LPEI hydrochloride and on LPEI-ZnO. The relevant peak shifts from literature for analysis of LPEI and LPEI-ZnO are found in Table 3.

Table 3: Relevant ^{13}C ssNMR peak shifts (Clark, 2020).

Bonding	Chemical Shift (ppm)
$\text{R}_3\text{-CH}$	25-35
$\text{R-CH}_2\text{-NH}_2$	37-45
C=O	170-185

[0079] The formation of a nanocomposite was confirmed using ^{13}C ssNMR, where the spectra of the nanocomposite and of as-received LPEI were compared (FIG. 4) The ssNMR spectrum of the as-received LPEI had just one characteristic peak at $\delta 41.66$ ppm, shown in FIG. 4 (upper spectrum). Each bond in the chain is either C-N or C-C, so this peak is characteristic of the whole chain, and is in agreement with past literature on LPEI structure. In the LPEI-ZnO spectrum, shown in FIG. 4 (middle spectrum), the characteristic peak shifted slightly to $\delta 45.11$ ppm. This is indicative of C-C and C-N deshielding as the introduced ZnO nanoparticles bond to the LPEI, and indicates formation of the modified material. Additionally, a new signal emerged at $\delta 161.89$ ppm. While not wishing to be limited by theory, this new peak may correspond to new C=O bonds in the material, or from amine group quaternization. To verify that these new peaks were not the result of experimental error, a mixed sample containing as-received LPEI and LPEI-ZnO powder were tested at a 1:1 weight ratio, with the spectrum shown in FIG. 4 (lower spectrum). Here, a division of the original and shifted characteristic signal are seen, as well as the additional peak at $\delta 161.55$ ppm. This confirms the successful modification of ZnO with LPEI.

[0080] To evaluate the surface adhesion of the coatings before and after modification with LPEI, a tape test was carried out according to ASTM D3359. As shown in FIG. 5 (upper image), the majority of the unmodified ZnO thin film was removed, and what remained was noticeably more translucent, indicating it was much thinner. This indicated a 1B classification (over 65% of film removed). As shown in FIG. 5 (lower image), much less of the film surface area was removed. This indicated a 4B classification (less than 15% removed). Thus, the addition of LPEI substantially improved the adhesion of the photoactive thin film.

[0081] (ii) *Photoelectrochemical Characterization:* IPCE, or quantum efficiency (QE), is a measure of the number of charge carriers generated per photon injected. This metric is widely

used in solar cell development. For the purposes of this work, IPCE showed at which wavelengths the photocurrent was strongest, and whether LPEI-ZnO had a higher QE than unmodified ZnO. This was measured using a two-electrode cell in 0.1 M PBS electrolyte containing 0.1 M AA. As shown in FIG. 6, when compared to ZnO, LPEI-ZnO showed a modest increase in IPCE for all wavelengths less than 450 nm. At 365 nm, the IPCE of the LPEI-ZnO is greater than that of ZnO by about 20%. To determine any changes in light absorption at relevant wavelengths, UV-VIS spectra were collected from both ZnO and LPEI-ZnO suspensions in H₂O. As shown in FIG. 7, there was no significant change in light absorption of ZnO after modification with LPEI. Although LPEI does not improve absorption in the visible spectrum, it does not significantly impede the passage of light to the ZnO nanoparticles. However, this means that the light absorption properties do not explain the IPCE increase.

[0082] UV-VIS spectra can be used to estimate the E_g of a semiconductor after conversion to a Tauc plot, which is based on the following equation:

$$(\alpha * hv)^{\frac{1}{\gamma}} = B(hv - E_g) \quad ,$$

where α is the energy-dependent absorption coefficient, h is the Planck constant, v is the photon's frequency and B is a constant. As shown in FIG. 8, Tauc plots were produced for both ZnO and LPEI-ZnO. The linear region of the plots can be extrapolated to the x-axis to estimate the E_g . In practice, both ZnO and LPEI-ZnO have E_g of approximately 3.16 eV, which is in agreement with the specification for the as-received ZnO material by the manufacturer. In contrast, Chakraborti et al. (2012) reported that their BPEI-ZnO material, which was synthesized with trisodium-citrate-capped ZnO, had an increased E_g over native ZnO. The use of LPEI in the synthesis reported in this disclosure is therefore advantageous, for example, because the nanoparticles do not lose any light sensitivity due to widening E_g .

[0083] It is known that BPEI reduces the work function of ZnO through dipole moment formation, but the effect of LPEI on ZnO is unconfirmed. V_{FB} is the applied voltage where there is no band bending or charge depletion. Mott-Schottky analysis is an electrochemical impedance test at constant frequency, and is a powerful tool used to determine semiconductor properties. It is conducted in a 3-electrode cell which is isolated from light, with electrolyte containing 2 mM potassium ferrocyanide.

[0084] The Mott-Schottky equation, shown below, was used to calculate the V_{FB} and other electrochemical properties of a semiconductor.

$$\frac{1}{C^2} = \frac{2}{\epsilon\epsilon_0 A^2 e N_D} \left(V_{app} - V_{FB} - \frac{k_B T}{e} \right) ,$$

wherein, C is the interfacial capacitance, V_{app} is the applied potential, N_D is the charge carrier density, ϵ is the relative permittivity of the semiconductor, ϵ_0 is vacuum permittivity, A is the area (cm^2), k_B is the Boltzmann constant, T is the temperature, and e is the charge of an electron. Upon analyzing the Mott-Schottky equation, all values apart from V and C are constant, meaning the plot of $1/C^2$ versus V_{app} is linear, and the value of V_{FB} can be estimated by where the plot intersects the V_{app} axis. Different photoactive semiconductor materials can be easily compared by their V_{FB} and N_D . The slope is calculated by the equation below:

$$\text{slope} = \frac{2}{\epsilon\epsilon_0 A^2 e N_D} .$$

[0085] Notably, with everything held constant, an increase in charge carrier density would result in a decrease in slope. Using the Mott-Schottky equation, the impedance results were converted into capacitance measurements, and plotted according to applied potential, as shown in FIG. 9. The plots of ZnO and LPEI-ZnO both had positive slopes, confirming that the n-type behaviour of ZnO is unaffected by the modification of LPEI. Then, the linear region of Mott-Schottky plots were extrapolated to determine V_{FB} . As shown on the inset of FIG. 9, V_{FB} decreased by 49 mV after modification with LPEI.

[0086] All the factors that impact the photocurrent (i_P) can be summarized by the simplified version Gärtner-Butler equation, shown below:

$$i_P = I_\lambda \alpha_\lambda \left(\frac{2e\epsilon_0\epsilon}{N_D} \right)^{\frac{1}{2}} (V_{App} - V_{FB})^{\frac{1}{2}} ,$$

where i_P increases with an increasing difference between applied potential (V_{App}) and V_{FB} , given the photon flux at a given light wavelength (I_λ), the absorption coefficient at a given light wavelength (α_λ), the charge of an electron (e), the relative permittivity (ϵ), the vacuum permittivity (ϵ_0), and the majority charge carrier density (N_D). By holding all other parameters constant, lowering the V_{FB} using photosensitizing materials is a useful method to increase output photocurrent. According to the simplified version Gärtner-Butler equation, the photocurrent varies inversely with the square root of V_{FB} at constant applied potential, so the

modification of ZnO with LPEI would result in an increase in photocurrent. The similar slopes of the two lines indicate that there was no significant change in charge carrier density in this system. Thus, the decrease in V_{FB} is one of the factors driving an increase in IPCE of ZnO after modification with LPEI.

[0087] With the electrochemical parameters determined experimentally, the underlying mechanism of the LPEI-ZnO material becomes clear. As shown in the band diagram in FIG. 10, when activated by light with energy above its 3.16 eV E_g , electrons begin moving from the V_B to the C_B , leaving holes in the V_B . This catalyzes a redox reaction with the surrounding electrolyte, oxidizing the AA and pushing the electrons from the C_B . These electrons are now free to move to the ITO substrate and through the circuit. With the reduction in V_{FB} , the energy at which this occurs is reduced, creating a larger potential difference with the circuit applied potential. The result is a boosted photocurrent when compared to the unmodified ZnO NP photoelectrode.

[0088] (iii) *Photoelectrode Optimization*: To obtain a sensitive PEC biosensor, the photoelectrode desirably has a maximized photocurrent density so that the relatively small changes in signal upon probe-target hybridization are easily measurable. To do so, the concentration of ZnO and V_{app} in the cell were both varied to find the combination that generated the largest photocurrent. The LPEI concentration was not tested since increasing film thickness increases overall resistance, which would outweigh the work function benefits. As shown in FIG. 11, when all other parameters were held constant, the photocurrent output increased with increasing ZnO concentration. While not wishing to be limited by theory this was most likely because a higher density of nanoparticles increased the photoactive surface area of the electrode, yielding more light absorption and generation of electron-hole pairs. Colloidal suspensions with a ZnO concentration greater than 5 g/L were observed to be unstable, which resulted in inconsistent surface coverage and photocurrent generation. Thus, a desirable concentration of ZnO was found to be 5 g/L.

[0089] Similarly, the applied potential in the cell was optimized for maximum, consistent photocurrent. The inventors expected that the photocurrent would follow the simplified version Gärtner-Butler equation. As shown in FIG. 12, this theory was validated, since the photocurrent increased with increasing applied potential. This experiment was limited to 300 mV since surface degradation was observed after applying higher potentials. Therefore, the applied potential for subsequent experiments was 300 mV.

[0090] With the optimal parameters determined, a head-to-head photocurrent comparison between the ZnO and LPEI-ZnO thin films could be conducted. Both thin films were fabricated by drop casting a layer of a suspension containing 5 g/L ZnO, but a second layer of 1 g/L LPEI was drop casted onto the initial ZnO film to create LPEI-ZnO. The test was conducted with an applied potential of 300 mV and irradiation with an ultraviolet light source (365 nm). As shown in FIG. 13, a 1 g/L LPEI thin film does not independently produce photocurrent. However, the addition of 1 g/L LPEI to a 5 g/L ZnO thin film increased the photocurrent by up to 50% over an unmodified ZnO thin film, indicating that modification of ZnO with LPEI provides a significant improvement in photocurrent. In contrast, the addition of 1 g/L BPEI to the ZnO thin film reduced the photocurrent, indicating that BPEI acts more as an insulator rather than a photosensitizer.

[0091] As shown in FIG. 14, no significant trends are seen by testing the photoelectrodes after storage for up to 7 days after synthesis, indicating that the photoelectrodes remain stable after seven days in storage. Thus, there is prospective for long-term shelf life for LPEI-ZnO photoelectrodes.

(b) Multiplexed DNA Analysis

[0092] (i) *Signal-Off Nucleic Acid Detection*: A schematic of the signal-off nucleic acid assay used is shown in FIG. 15, where the probe DNA was a single strand (ssDNA) that was immobilized on the surface of the sensor via amine bonding. It was then incubated with the sample fluid. If the target is present in the fluid, it will hybridize with the probe spontaneously forming a DNA duplex and remain immobilized on the electrode surface. The hybridized double-stranded DNA (dsDNA) reduces the photocurrent due to an increase in steric hinderance. This is indicative of a “positive” test result. If there is no hybridization event, then the photocurrent should not be reduced. This is indicative of a “negative” test result.

[0093] The probe attachment to the photoelectrode is important for the functionality of the bioreceptor layer. Simple physical adsorption is insufficient, as the orientation of the ssDNA strand may block part of the strand, impeding its ability to hybridize. In this application, terminating 5' with an amine group allowed covalent bonding with the LPEI-ZnO photoelectrode surface. For each sample, the LPEI-ZnO photoelectrode was produced and then incubated with 1 μ M of P1 suspension for 1 hour. After rinsing, it was incubated with a buffer suspension that contained either 0.5 μ M of the target (results denoted by (+)), or 0.5 μ M of mismatched target (results denoted by (-)). Finally, the samples were rinsed thoroughly with buffer and the resulting photocurrent was measured. FIG. 16 (upper) shows the photocurrent

of the (+) sample was significantly lower than the (-) sample, indicating the hybridization event occurred. This can be quantified using the equation above, which calculates the change relative to a photoelectrode that was only incubated with the probe. FIG. 16 (lower) shows a significant and consistent decrease when the sample was exposed to (+), where the photocurrent decreased by about 60% on average. This result indicates that the hybridization event was the most likely root cause of the signal decrease. Any nonspecific physical adsorption of nucleic acid would have impacted the (-) sample as well, but since this photocurrent change was insignificant, it is evident that nonspecific absorption had a minimal effect.

[0094] (ii) *Signal-On Nucleic Acid Detection*: The signal-on assay is based on toehold strand displacement (TSD), which is a nucleic acid hybridization mechanism where one ssDNA in a duplex is exchanged with another to form a new duplex. These exchanged strands cannot be identical, or else the reaction will be very slow. Therefore, the mechanism must rely on dsDNA probes where it is favourable for one strand to break this duplex and bond with the target. To do so, Srinivas et al. (2013) designed a partially-hybridized complex where one of the strands (P2) was much longer than the other (P1), leaving an overhanging region called a toehold. The target, which is a perfect complement to P2, invades the duplex and hybridizes P2 using the toehold region. P2 then separates from P1 to complete its duplex with the target.

[0095] To enable signal-on analysis of ssDNA without the need for a separate target-labelling step, a modified TSD assay was used (FIG. 17). Referring to the exemplary schematic diagram shown as an embodiment in FIG. 17, P1 is immobilized to the sensor surface via the amine-terminated 5'. P2 is the much longer "target recognition" strand that is partially hybridized, leaving the overhanging toehold. Two steps take place in this assay:

[0096] P2 hybridizes with the target via the overhanging toehold, and eventually separates from P1. P1 forms a hairpin. The newly hybridized dsDNA containing the target and P2 are released into the bulk of the incubating solution. After rinsing, it would be removed, leaving only the P1 hairpin on the sensor surface. If the target is not present, then P1 will remain attached to P2. This method is achievable because the reaction is thermodynamically favourable at room temperature, and does not require the use of enzymes or external reagents, nor does it require any spacers.

[0097] To convert this change into a measurable signal, an AuNP was employed to amplify the photocurrent post-hairpin formation. In the design, the AuNP is conjugated to P1 via Au-S bonding to the thiol 3' terminus of the P1 nucleic acid, holding it far from the

photoactive surface. After the hybridization event, P1 is designed to form a hairpin, which would bring AuNP into contact with the sensor surface. The formed heterojunction causes an increase in photocurrent. This differs from previous work, where a conjugated hairpin was hybridized into straight dsDNA by the target for signal-off fluorescent or electrochemical sensing, and did not involve TSD (Wang et al., 2017; Fan et al., 2003; Marras et al., 1999).

[0098] (iii) *AuNP Synthesis and Conjugation Characterization*: AuNPs were synthesized using the citrate reduction method. STEM images of the synthesized particles are shown in FIG. 18. Particle sizes were measured in ImageJ and were approximately 12.4 nm +/- 1 nm based on 32 nanoparticle measurements. Based on past literature, this is an adequate average particle size for conjugation on DNA (Saha et al., 2020; Zhang et al., 2007). The suspension was stored at 4°C.

[0099] Next, to determine the concentration of AuNPs in the synthesized suspension, the simplified Beer-Lambert law was rewritten to solve for concentration, as shown below:

$$c = \frac{\text{Absorbance}}{al}$$

[00100] As shown in Table 4, the average AuNP concentration was 18.9 nM.

Table 4: Using Beer-Lambert law to determine AuNP concentration in suspension.

Parameter	Quantity	Reference (if applicable)
Absorbance	1.28	—
AuNP size	12.4 nm	—
Extinction coefficient for AuNP size	$1.21 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$	Haiss et al. (2007)
Light pathway	0.56 cm	Promega (2009)
Concentration	18.9 nM	—

[00101] If conjugated incorrectly, it would be possible for multiple DNA strands to conjugate to each AuNP. This would decrease the number of AuNPs in the bioreceptor layer, reducing the signal-on effect. To characterize the conjugation procedure and verify the DNA:AuNP ratio, a fluorescence experiment was performed. As shown in FIG. 19 (upper), the calibration curve was produced by diluting the fluorescent P1 strands in PBS. With a coefficient of determination of 0.9999, this calibration curve was used to quantify the concentration of P1. Next, fluorescent P1 strands were conjugated to AuNPs as per the described procedure, so that all unconjugated fluorescent P1 strands and unconjugated AuNPs were removed from the

suspension. Following this, the conjugation bonds were broken by incubation with dithiothreitol to free the fluorescent P1, and the fluorescence measurement was taken. These readings were inputted into the calibration curve regression equation to determine the ratio of P1:AuNP. It was assumed that the thiol terminated PolyT strands would act as a blocker for the binding sites of the AuNP, so as the PolyT concentration increases, the amount of AuNP-conjugated fluorescent P1 should decrease. This is reflected in FIG. 19 (lower), where the ratio of P1 to AuNP decreased with relative increasing PolyT concentration. Interestingly, the ratio of P1 to AuNP with no PolyT presence is about 1:1.7. This means that there is a high likelihood of only one P1 strand being bound to each AuNP. The PolyT did act as a blocker at higher concentrations, meaning they were effective at acting as conjugation blockers for P1.

[00102] This trend was then evaluated in photocurrent output using the amine-terminated P1. Since the PolyT strands do not have an amine terminus, there is no way for them to be immobilized with the photoelectrode aside from non-specific adsorption as they would be removed by the rinsing steps. Since the previous experiment showed that the amount of suspended AuNP-conjugated P1 decreased with increasing relative PolyT concentration, the probe density on the photoelectrode should also decrease. With less steric hinderance caused by their presence, the photocurrent should increase. To test this hypothesis, AuNPs were conjugated to amine-terminated P1 at different ratios with PolyT. Once the conjugation was complete, the suspension was drop-cast onto LPEI-ZnO photoelectrodes. They were hybridized with T2, and their photocurrent was tested. As shown in FIG. 20, the photocurrent increased as the relative PolyT concentration increased. As mentioned above, the effect of steric hinderance would decrease as the probe density decreased. Interestingly, the photocurrent at 0:1 was still low compared to the results for LPEI-ZnO in FIG. 13. As indicated by the error bars, the photocurrent was somewhat constant with three repeats, indicating that the amount of non-specific physical adsorption was relatively consistent.

[00103] (iv) *LPEI-ZnO/AuNP Heterojunction Characterization:* With confirmation that the AuNP conjugation was successful, the LPEI-ZnO/AuNP heterojunction was then characterized to determine the mechanism for photocurrent increase. Experimentation was required to confirm whether a potential mechanism would be relevant with the AuNP particle size and injected light wavelength. In terms of LSPR mechanisms, light scattering occurs when incoming light gets trapped in the nanoparticle complex, but only occurs when the nanoparticle is relatively large. Since the AuNPs in this system were 12.4 nm in diameter on average, it was

unlikely that light scattering would be responsible for generating any signal in this system (Warren and Thimsen, 2012). PIRET is a mechanism that is possible for smaller nanoparticles, but the LSPR peak and light absorption band edge of the semiconductor must overlap (Wu, 2018). Saha et al. (2020) tested the plasmon excitation band for AuNPs with a diameter of 12 nm on average, and found it was about 510-520 nm. Since the excitation wavelength for the LPEI-ZnO material in this work is 365 nm, it is unlikely that any significant plasmonic excitation would occur, so PIRET is unlikely to be the underlying mechanism of the signal generation. Plasmonic hot electron generation occurs when charge carriers form on the surface of the AuNP, becoming redox-active with the electrolyte. If the energy of the light-induced charge-carrier surpasses the heterojunction interfacial barrier, as it would for small AuNPs with lower E_f , these hot charge carriers can transfer into the semiconductor (Govorov et al., 2013). Thus, in the case of the AuNP/LPEI-ZnO heterojunction in this work, where the light was high-energy and the average diameter of AuNPs were small, while not wishing to be limited by theory, plasmonic hot electron injection would be the most likely plasmonic mechanism.

[00104] To evaluate any non-LSPR mechanisms and to produce an accurate band diagram for the generated heterojunction, electrochemical characterization was performed on the signal-on photoelectrode before and after hybridization to evaluate the differences. Mott-Schottky analysis was conducted on the signal-on assay before and after exposure to the target. As shown in FIG. 21 (left), V_{FB} was shifted by 40 mV after hybridization. This is indicative of an improvement in photocurrent according to the simplified version Gärtner-Butler equation. Since E_f of AuNPs would usually lie below that of LPEI-ZnO, the reduction in V_{FB} is indicative of Fermi-level equilibration, meaning the heterojunction has formed a direct charge transfer complex. This change must be due to the hybridization, as there was no change in V_{FB} after incubation with the mismatch DNA FIG. 21 (right).

[00105] Another interesting feature of the Mott-Schottky analysis is the change in slopes of the regression lines before and after hybridization. The slope of the Mott-Schottky plot has an inverse relationship to the charge-carrier density. As shown in FIG. 21, the slope of the Mott-Schottky curve decreases after hybridization in the signal-on system. An increase in charge carrier density would be driven by hot electron injection, as the AuNPs provide the additional charge carriers. The slope flattened slightly after incubation with the mismatch, but while not wishing to be limited by theory, this may be due to some probe DNA being removed during the rinsing cycles prior to testing. This would mean that the steric hinderance effect of the probe

DNA would be reduced, allowing for more LPEI-ZnO surface area to interface with the electrolyte. It cannot be due to the formation of the heterojunction since V_{FB} remained the same.

[00106] Based on the electrochemical characterization, FIG. 22 shows a comprehensive diagram that incorporates the photocharging and hot electron injection mechanisms. The semiconductor bands bend close to the AuNP surface, causing electron flow towards the ITO. With Fermi-level equilibration, the hot electrons can be injected directly into the semiconductor and improving charge separation in the semiconductor photogenerated electron-hole pairs. The resulting hot holes could then oxidize the electrolyte, in addition to the redox reaction occurring between the semiconductor and the electrolyte. The resulting photocurrent is increased.

[00107] (v) *PEC Detection of Target DNA*: To evaluate the signal-on assay for PEC nucleic acid analysis, the LPEI-ZnO photoelectrode was incubated for 1 hour with a suspension containing the AuNP-conjugated rigid dsDNA duplex that forms the TSD probe. It was then incubated with a buffer suspension that contained either 0.5 μM of the target (results denoted by (+)), or 0.5 μM of mismatched target (results denoted by (-)). Finally, the samples were rinsed thoroughly with buffer and their photocurrent was tested. As shown in FIG. 23 (upper), the photocurrent of the (+) sample increased in comparison to the (-) sample. This difference was consistent and significant when compared to probe-only photoelectrodes. In this case, the photocurrent increased by 25% on average, compared to an insignificant change after incubation with (-). The consistency of these changes indicates that the rinsing steps effectively reduced non-specific physical adsorption.

[00108] Thus, by combining two assays that cause a signal change in opposite directions, multiplexed recognition can be conducted using the same base photoelectrode, circumventing the need for a separate target labeling step and using minimal external reagents. Since the production of this device requires simple drop-casting, this may, for example, provide a useful pathway towards scalable manufacturing and use.

III. Conclusions/Overview

[00109] Biosensors represent an avenue towards rapid and portable diagnosis of a medical condition, as they give clinicians crucial information about a patient's condition without the need of laboratory assistance. The rapid sample-to-result time could be the difference between a positive and negative outcome for the patient. However, most commercially available biosensors have not been focused on disease biomarker detection. The commercial applications

and research efforts in the disease detection field have focused on single biomarker detection, even though some diseases present heterogeneously. Methods used in research have also used bench-scale synthesis procedures, or may rely upon numerous external reagents, impacting manufacturing scalability and complicating ease-of-use for the user. Therefore, a goal of this work was to develop a photoelectrochemical biosensor for multiplexed DNA detection that uses inexpensive, non-toxic materials and low-temperature, facile processing methods, without relying on a target-labeling step. The findings of the work included the following:

[00110] First, the LPEI-ZnO thin film was characterized. Modification of ZnO with LPEI did not alter coating surface morphology compared to pure ZnO thin films. ^{13}C ssNMR indicated that there was a change in the chemistry of LPEI after it was added to ZnO, indicating that a bond had formed between LPEI and ZnO. Surface adhesion of ZnO thin films to ITO increased significantly post-modification with LPEI compared to unmodified ZnO films. Modification with LPEI also slightly increased the IPCE of ZnO with light irradiation of $\lambda < 450$ nm. Although the LPEI modification did not change the light absorbance properties of the material or the E_g , a decrease in V_{FB} was observed by Mott-Schottky analysis. According to the Gärtner-Butler equation, photocurrent has an inverted linear dependence on V_{FB} , so, while not wishing to be limited by theory, the Mott-Schottky plot explained the likely reason for an increase in IPCE in the relevant wavelengths for this device. Next, the chemistry of the LPEI-ZnO photoelectrode and experimental parameters were varied to produce maximum photocurrent in a three-electrode cell. To do so, a ZnO NP suspension and an LPEI solution were drop-cast onto an ITO substrate in a layer-by-layer fashion. Photocurrent measurements at different concentrations under 365 nm light exposure showed that a 5 g/L ZnO suspension covered with a 1 g/L LPEI solution produced the highest photocurrent. The impact of applied potential was evaluated, and it was found that 0.3 V generated the highest photocurrent. Ultimately, the photocurrent after LPEI modification showed a 50% increase over the unmodified ZnO thin film with 365 nm light irradiation. This result was consistent over 7 days in storage. A photoelectrode with only LPEI produced no photocurrent. To study the influence of the molecular structure of PEI, a BPEI-ZnO thin film was evaluated and found to generate a much lower photocurrent than LPEI-ZnO at the same concentration. The photoelectrode was then modified to detect ssDNA using two different assays. The signal-on and signal-off assays were designed to cause an increase or decrease in photocurrent upon probe-target recognition, respectively. An objective was to characterize this mechanism to determine the reason for the

photocurrent increase. After the conjugation procedure, the ratio of DNA to conjugated AuNPs was about 1:1.7, meaning it was likely that most DNA strands were only conjugated to one AuNP. With an effective conjugation procedure established, the next step was to characterize the heterojunction that would form between LPEI-ZnO and AuNPs in the presence of the target. UV-VIS measurements confirmed an increase in light absorption and a decrease in E_g , and Mott-Schottky measurements confirmed a decrease in V_{FB} . This indicated that the combination of plasmonic hot electron injection and photocharging would cause an increase in photocurrent after formation of the heterojunction. The signal-on and signal-off mechanisms were also evaluated. Probe-target hybridization caused a decrease in photocurrent of 60%, for the signal-off assay, while no significant photocurrent change was observed in the presence of non-complementary ssDNA. A 25% boost in photocurrent was observed when exposed to target ssDNA for the signal-on assay, while exposure to mismatched ssDNA did not result in any significant changes in photocurrent. This indicates that both assays were useful at identifying their respective targets. Thus, the biosensor presented herein may, for example, be useful in portable analysis of multiple nucleic acids such as for applications in point-of-care diagnostics.

[00111] While the present disclosure has been described with reference to examples, it is to be understood that the scope of the claims should not be limited by the embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

[00112] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE DESCRIPTION

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CLAIMS:

1. A sensor for detecting a target nucleic acid in a sample, the sensor comprising:
 - a first photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and
 - a first probe, the first probe comprising:
 - a first nucleic acid strand complementary to the target nucleic acid; and
 - a second nucleic acid strand that is shorter than the first nucleic acid strand, the second nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the first photoelectrode and the second end coupled to a conductive nanoparticle, wherein the second nucleic acid strand is partially complementary to the first nucleic acid strand and hybridized to the first nucleic acid strand so as to leave an unhybridized region of the first nucleic acid strand distal to the second end of the second nucleic acid strand such that hybridization of the unhybridized region with the target nucleic acid causes separation of the first nucleic acid strand from the second nucleic acid strand, and wherein the second nucleic acid strand is capable of changing conformation subsequent to separation of the first nucleic acid strand so as to bring the conductive nanoparticle in contact with the first photoelectrode, forming a heterojunction, thereby increasing photocurrent.
2. The sensor of claim 1, further comprising a second photoelectrode comprising a conductive substrate, a photoactive material and optionally a polymer coated on the photoactive material, the polymer capable of reducing the work function of the photoactive material; and a second probe, the second probe comprising a third nucleic acid strand having a first end and a second end, the first end immobilized on a surface of the second photoelectrode, the third nucleic acid strand complementary to a target nucleic acid, and wherein hybridization of the target nucleic acid with the third nucleic acid strand causes an increase in steric hindrance, thereby decreasing photocurrent.
3. The sensor of claim 2, wherein the first end of the third nucleic acid strand is immobilized via a covalent bond formed via reaction of an NH_2 group at the 5' end of the third nucleic acid strand with a complementary reactive group on the surface.

4. The sensor of claim 2 or 3, wherein the first nucleic acid strand and third nucleic acid strand are complementary to different target nucleic acids.
5. The sensor of any one of claims 1 to 4, wherein the first end of the second nucleic acid strand is immobilized via a covalent bond formed via reaction of an NH_2 group at the 5' end of the second nucleic acid strand with a complementary reactive group on the surface.
6. The sensor of any one of claims 1 to 5, wherein the first nucleic acid strand, the second nucleic acid strand, the third nucleic acid strand, if present, and the target nucleic acid(s) comprise single strand DNA.
7. The sensor of any one of claims 1 to 6, wherein the conductive substrate comprises indium tin oxide (ITO).
8. The sensor of any one of claims 1 to 7, wherein the conductive substrate comprises a screen-printed electrode.
9. The sensor of any one of claims 1 to 8, wherein the photoactive material comprises a metal oxide.
10. The sensor of claim 9, wherein the metal oxide comprises zinc oxide (ZnO) nanoparticles.
11. The sensor of any one of claims 1 to 10, wherein the first photoelectrode comprises the polymer and the polymer comprises polyethyleneimine.
12. The sensor of any one of claims 2 to 4 or any one of claims 5 to 11 as dependent on any one of claims 2 to 4, wherein the second photoelectrode comprises the polymer and the polymer comprises polyethyleneimine.
13. The sensor of claim 11 or 12, wherein the polyethyleneimine is linear.

14. The sensor of any one of claims 1 to 13, wherein the conductive nanoparticle comprises a metal.
15. The sensor of claim 14, wherein the conductive nanoparticle comprises a gold nanoparticle.
16. The sensor of claim 15, wherein the second end of the second nucleic acid strand is coupled to the conductive nanoparticle via an Au-S bond formed via reaction of a SH group at the 3' end of the second nucleic acid strand with the gold nanoparticle.
17. The sensor of any one of claims 1 to 16, wherein the change in confirmation comprises formation of a hairpin.
18. The sensor of any one of claims 1 to 17, wherein the sample is a biofluid.
19. A method of detecting a target nucleic acid in a sample, the method comprising:
 - contacting the sample with a sensor as defined in any one of claims 1 to 18; and
 - measuring photocurrent,
 - wherein an increase in photocurrent indicates the presence of a target nucleic acid complementary to the first nucleic acid strand; and/or
 - a decrease in photocurrent indicates the presence of a target nucleic acid complementary to the third nucleic acid strand.
20. A use of a sensor as defined in any one of claims 1 to 18 in the diagnosis of a disease, disorder or condition associated with the presence of the target nucleic acid complementary to the first nucleic acid strand and/or the target nucleic acid complementary to the third nucleic acid strand.

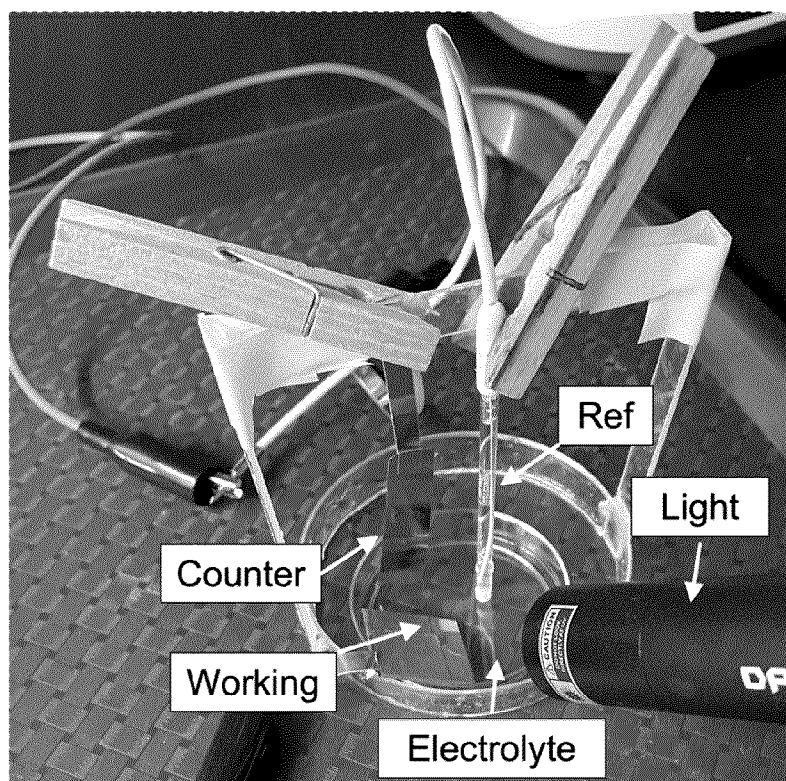
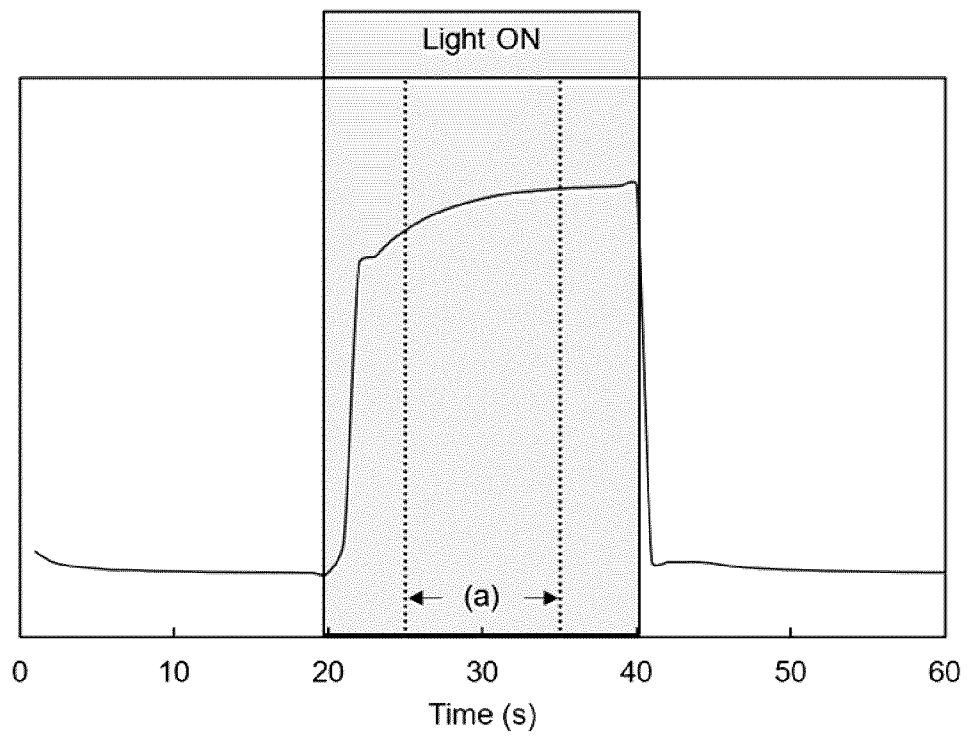
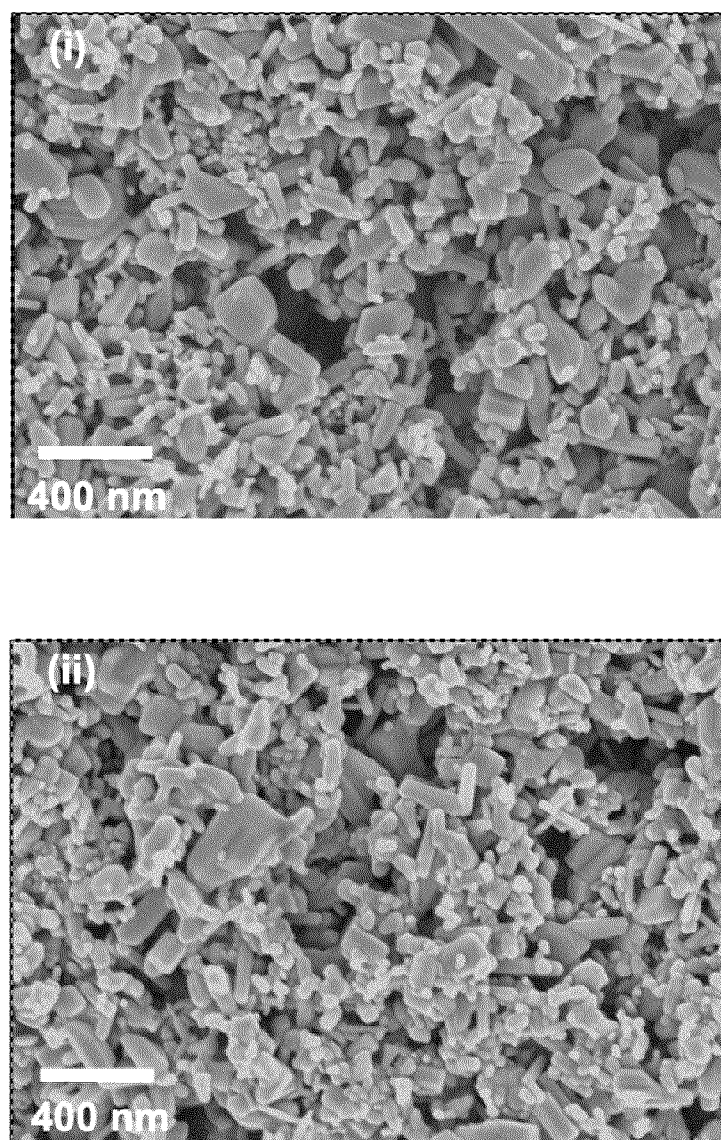


FIG. 1

**FIG. 2**

**FIG. 3**

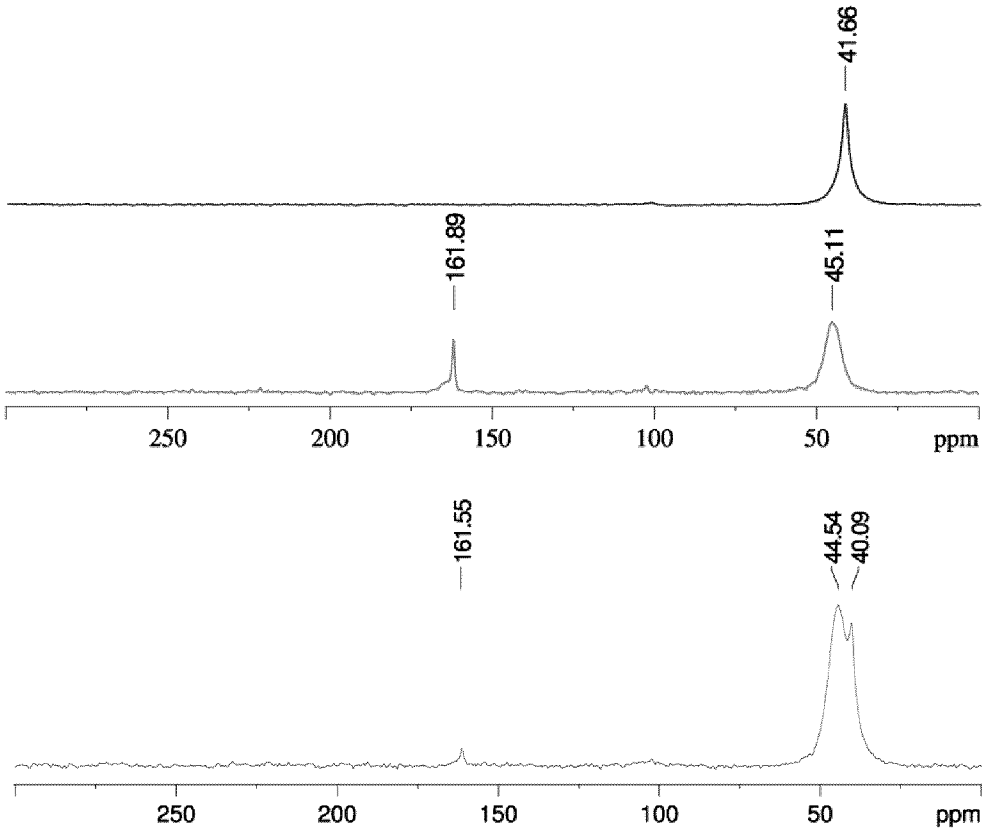


FIG. 4

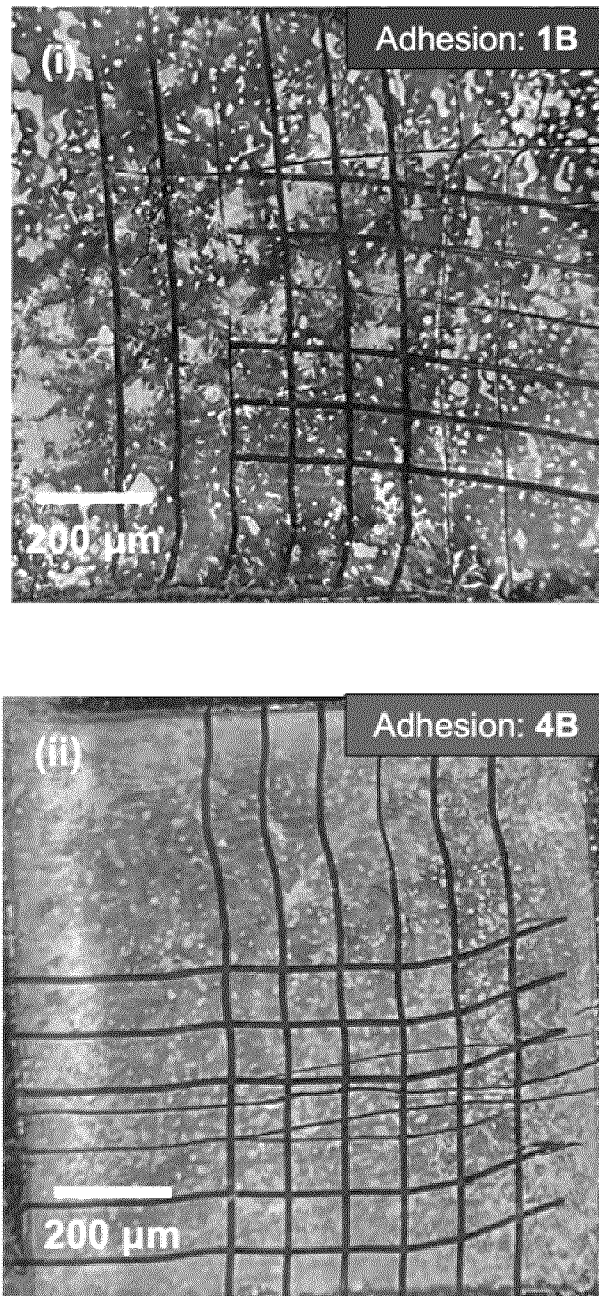


FIG. 5

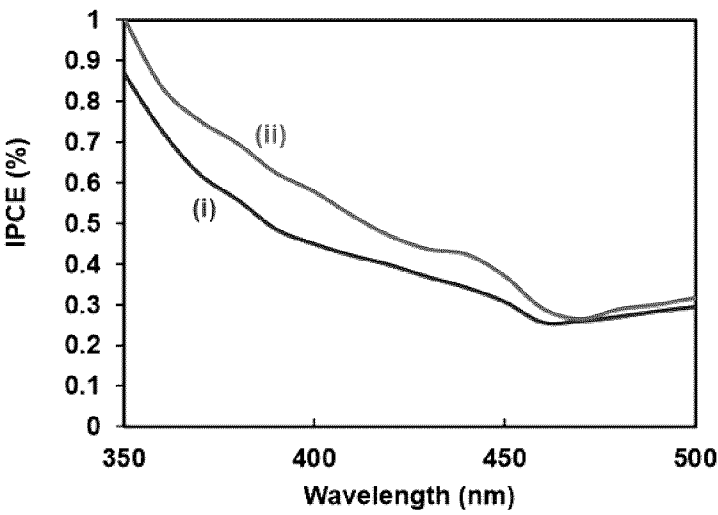


FIG. 6

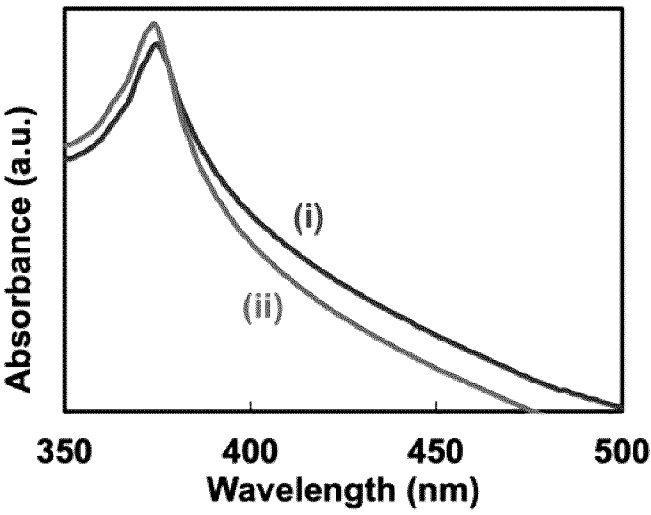


FIG. 7

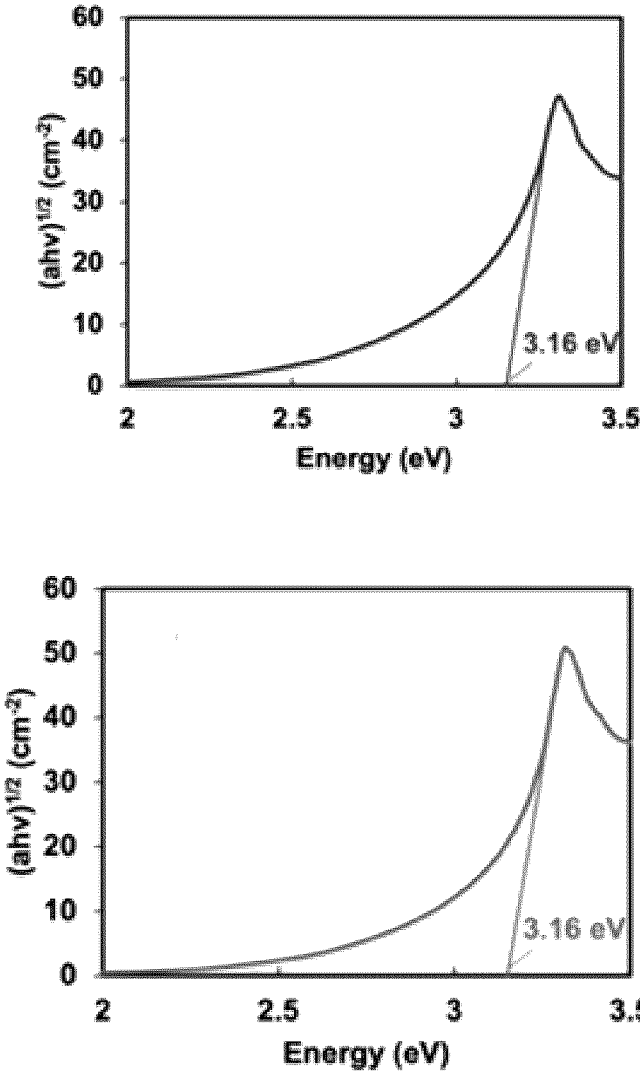


FIG. 8

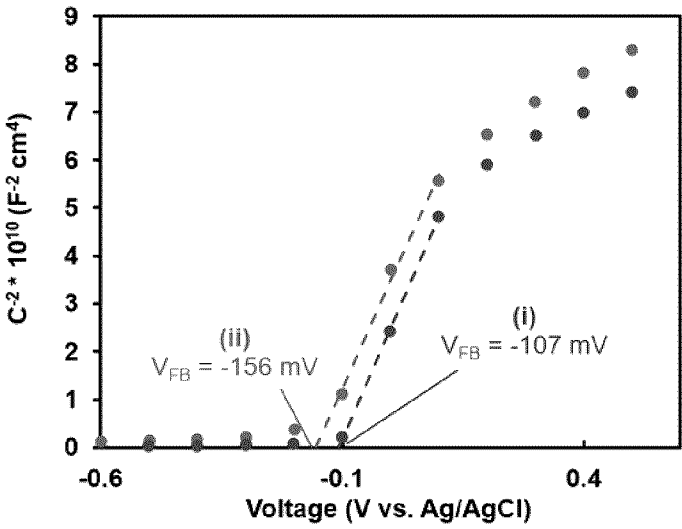


FIG. 9

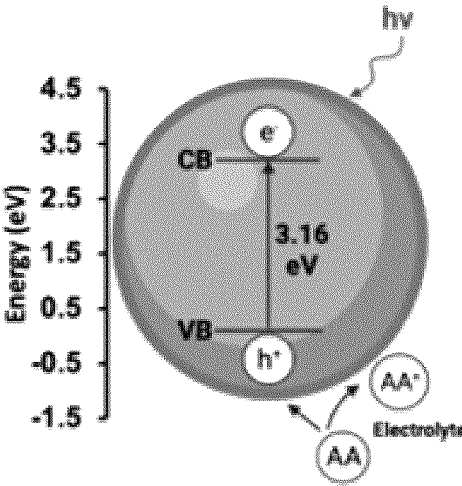


FIG. 10

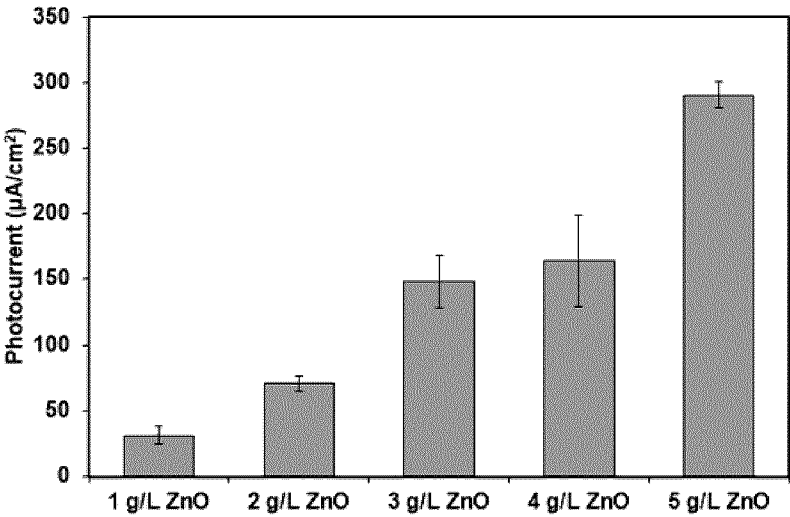


FIG. 11

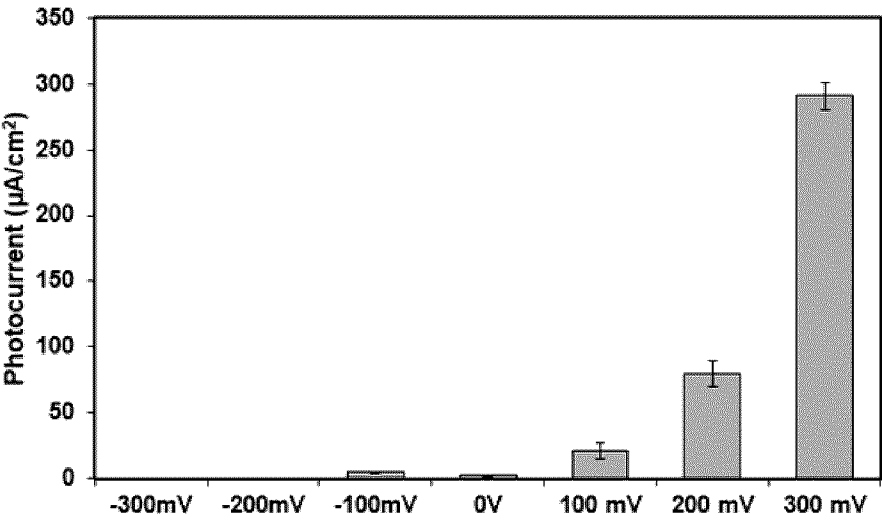


FIG. 12

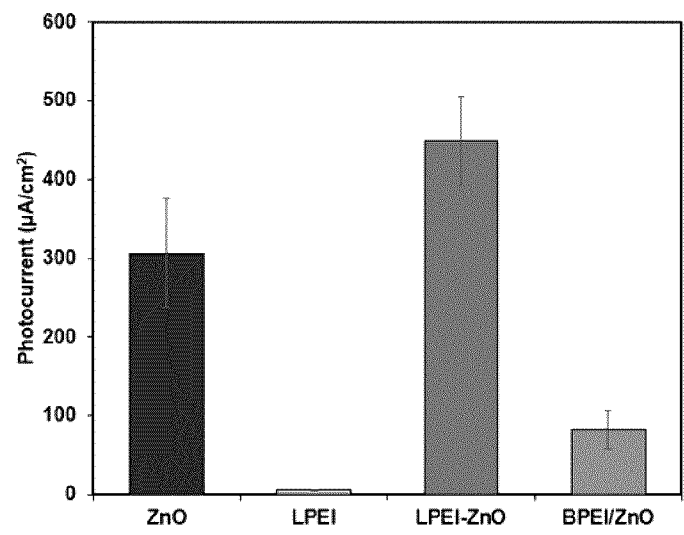


FIG. 13

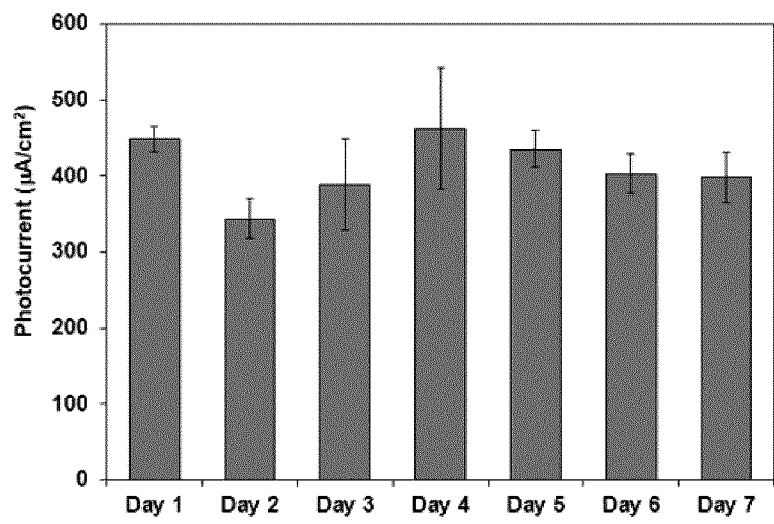


FIG. 14

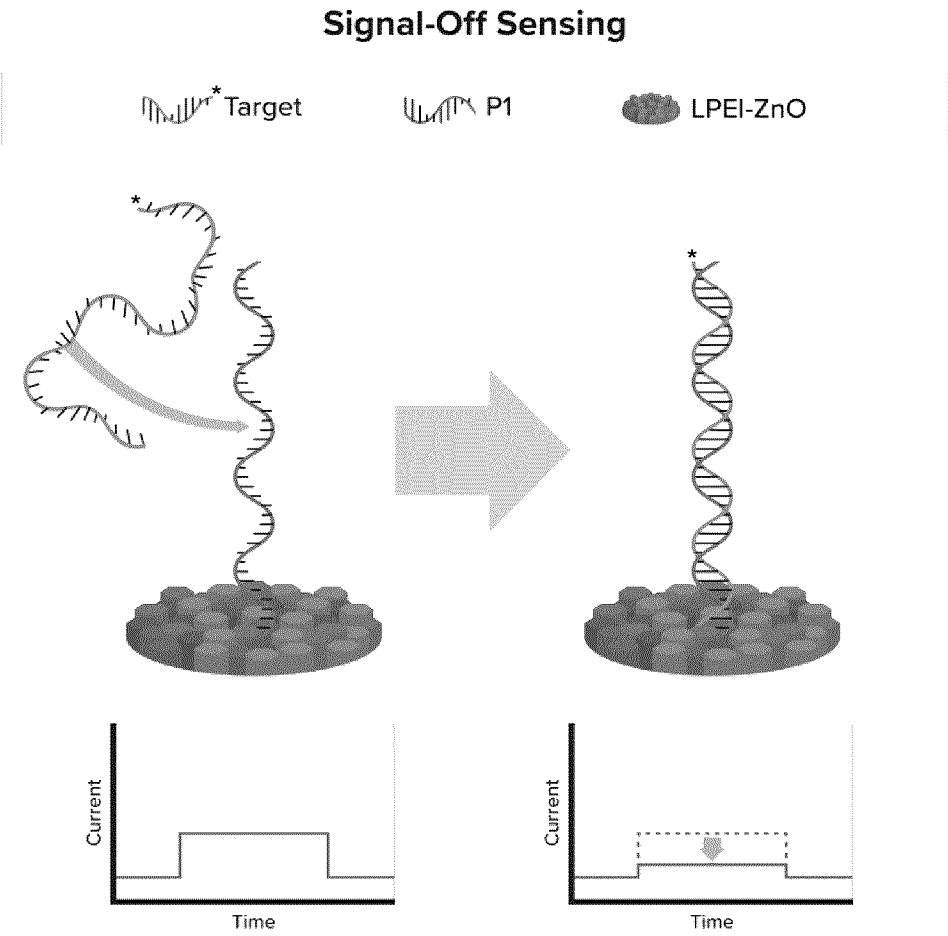


FIG. 15

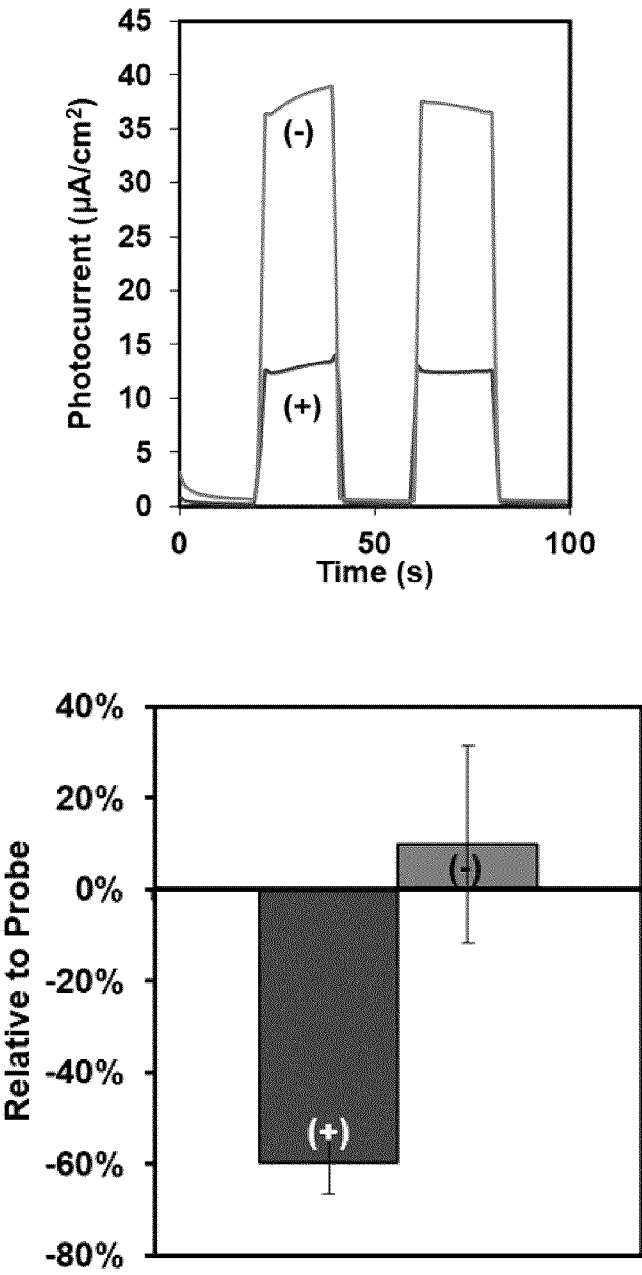


FIG. 16

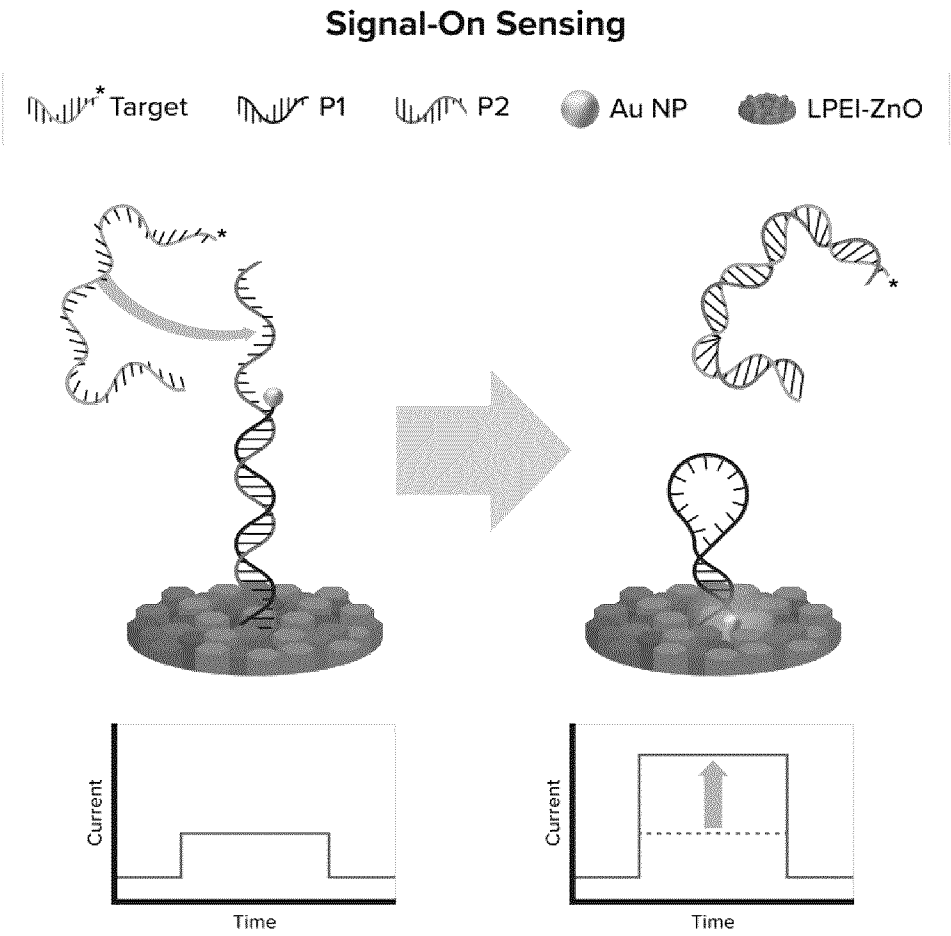


FIG. 17

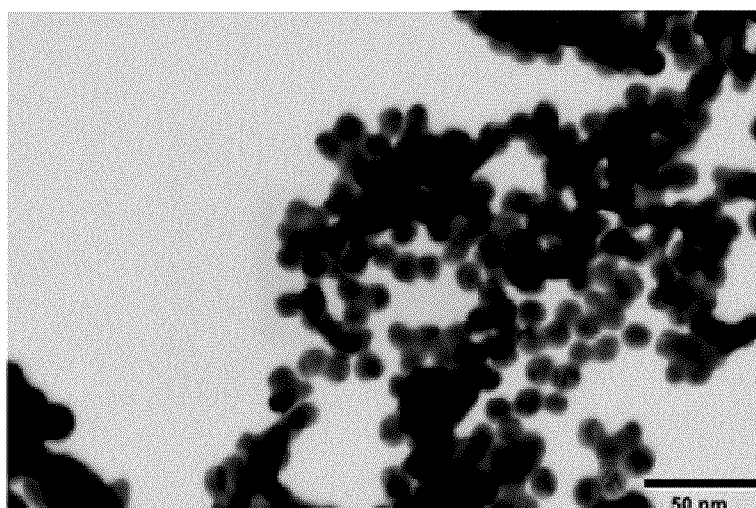
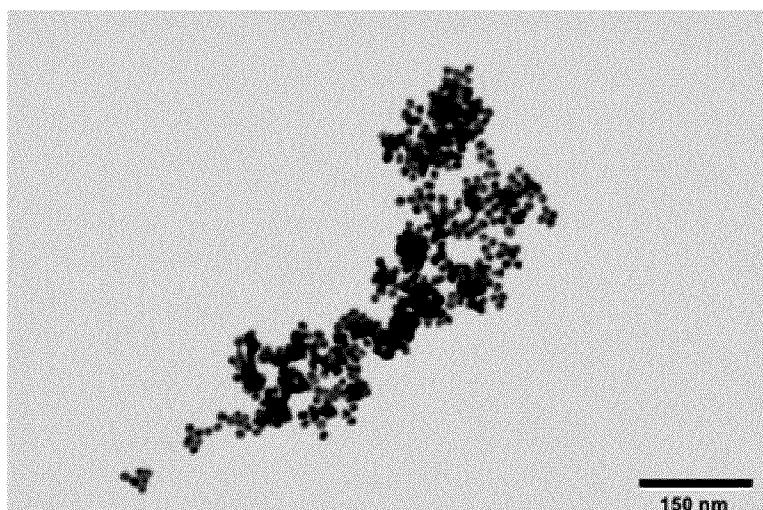


FIG. 18

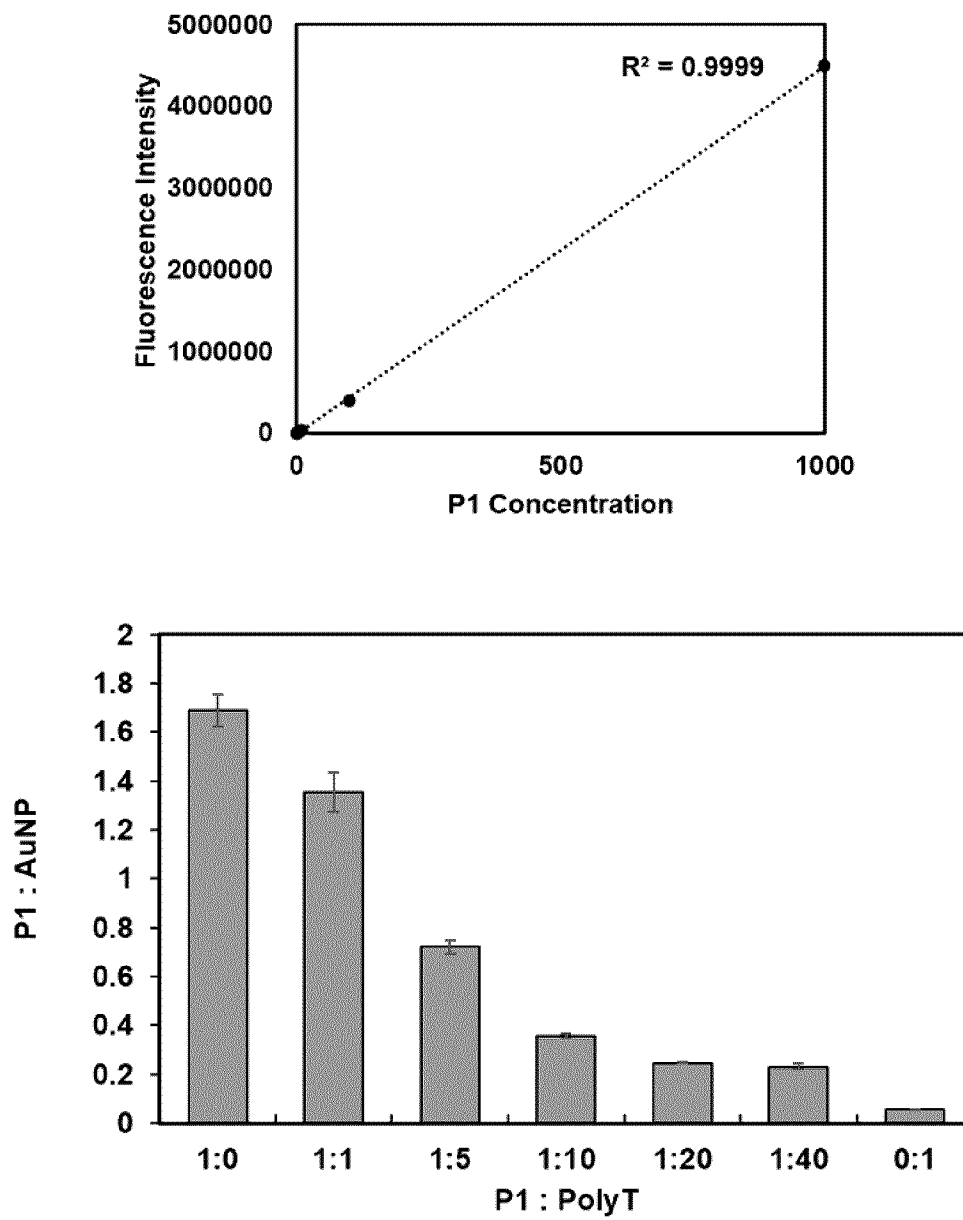


FIG. 19

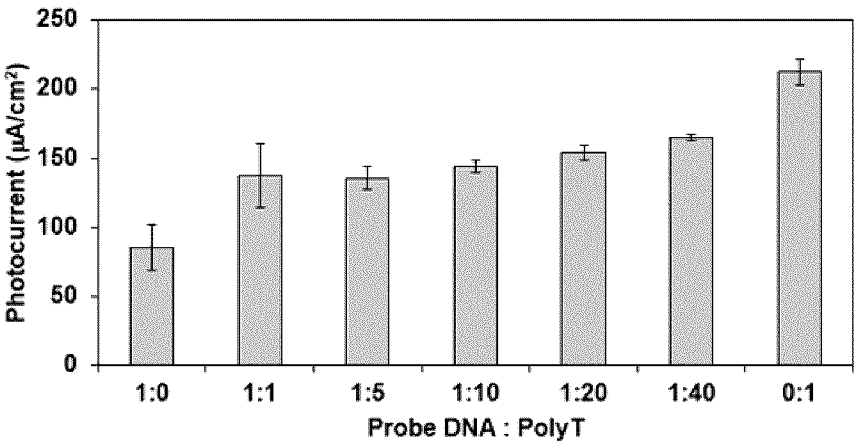


FIG. 20

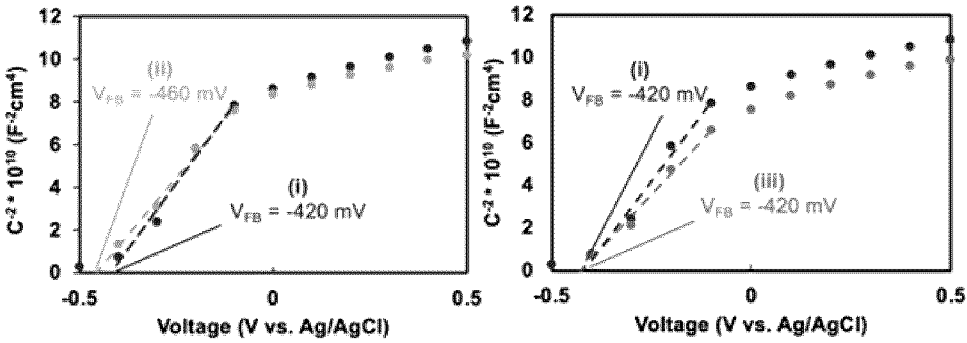


FIG. 21

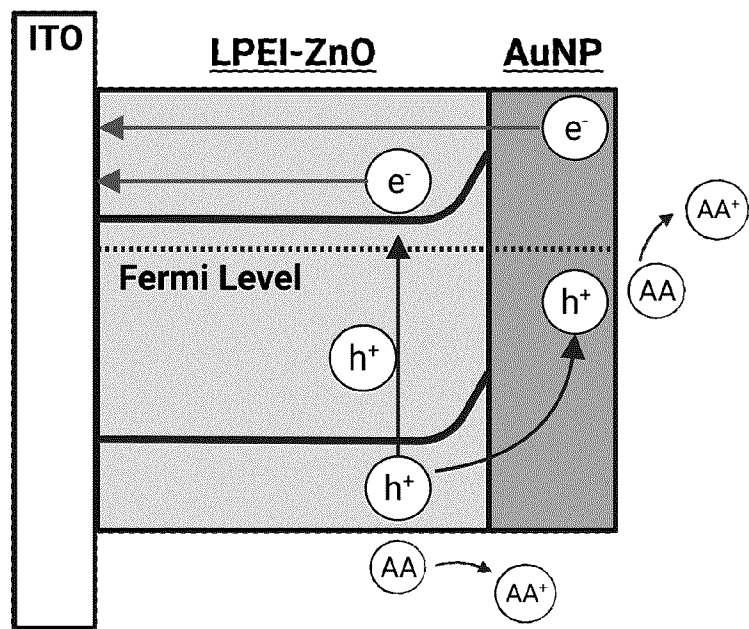
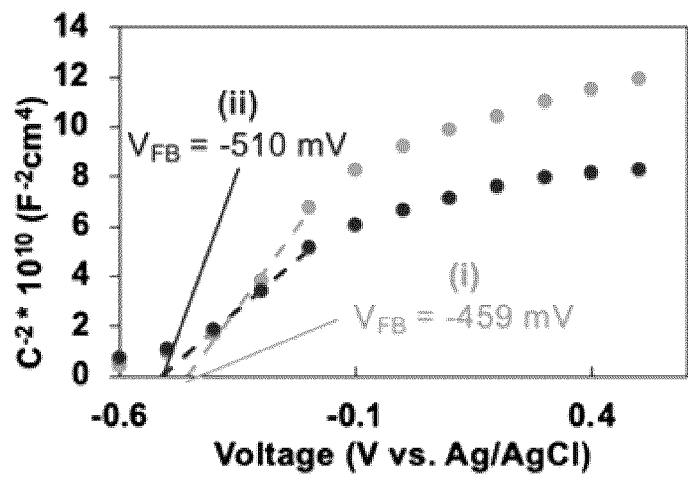


FIG. 22

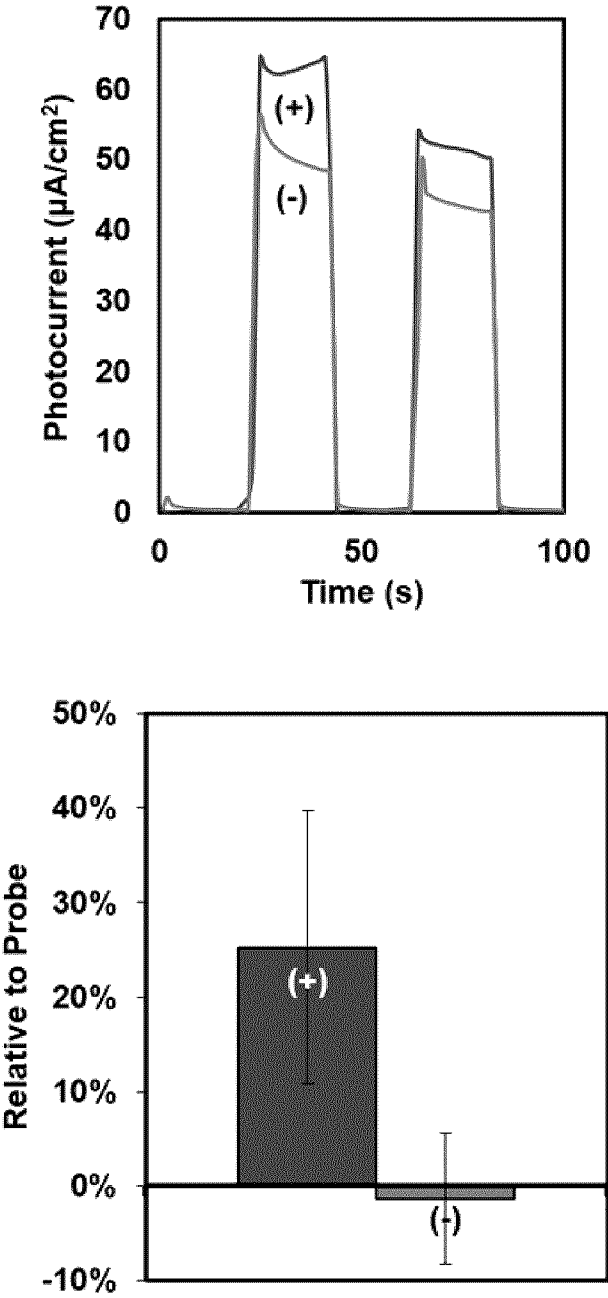


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2024/050478

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **G01N 27/327** (2006.01), **B82Y 5/00** (2011.01), **B82Y 15/00** (2011.01)CPC: **G01N 27/3276** (2020.01), **B82Y 5/00** (2020.01), **B82Y 15/00** (2020.01), **G01N 27/3278** (2020.01)

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)

IPC: **G01N 27/327** (2006.01), **B82Y 5/00** (2011.01), **B82Y 15/00** (2011.01) CPC: **G01N 27/3276** (2020.01), **B82Y 5/00** (2020.01), **B82Y 15/00** (2020.01), **G01N 27/3278** (2020.01)

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

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

Questel Orbit. Ebscohost. Canadian Patent Database. Search terms: photoelectrode, nucleic acid, strand, complementary, DNA, polynucleotide, work function, heterojunction, hairpin, displace

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN112444545 A (TANG LIN, ZHU XU, PENG BO, OUYANG XILIAN, TAN JISUI, YU JIANGFANG) 05 March 2021 (05-03-2021) Whole document.	1-20
A	US20220065806A1 (SOLEYMANI LEYLA, SAHA SUDIP, VICTORIOUS AMANDA) 03 March 2022 (03-03-2022) Whole document.	1-20
A	US20220221418A1 (SOLEYMANI LEYLA, SAHA SUDIP, VICTORIOUS AMANDA) 14 July 2022 (14-07-2022) Whole document.	1-20
A, P	CN117517424 A (LÜ SHUZHEN, WANG HUIJIE, BI SAI) 06 February 2024 (06-02-2024) Whole document.	1-20

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* "A" "D" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance document cited by the applicant in the international application earlier application or patent but published on or after the international filing date 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) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 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 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
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Date of the actual completion of the international search
13 June 2024 (13-06-2024)Date of mailing of the international search report
19 June 2024 (19-06-2024)Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Adam MacKenzie (819) 639-8506

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2024/050478

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed.
- b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13^{ter}.1(a)),
 - ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CA2024/050478

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CN112444545 A	05 March 2021 (05-03-2021)	CN112444545 B	11 March 2022 (11-03-2022)
US20220065806 A1	03 March 2022 (03-03-2022)	US11874247 B2	16 January 2025 (16-01-2024)
US20220221418A1	14 July 2022 (14-07-2022)		
CN117517424 A	06 February 2024 (06-02-2024)		