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(54) Title: USE OF LONGITUDINALLY DISPLACED NANOSCALE ELECTRODES FOR VOLTAGE SENSING OF BIOMOLECULES AND OTHER ANALYTES IN FLUIDIC CHANNELS

(57) Abstract: Devices and methods for detecting an analyte are provided. Devices for voltage sensing of analytes may comprise a fluidic channel defined in a substrate, a pair of sensing electrodes disposed in a fluidic channel for sensing voltage therein, and a pair of electromotive electrodes for applying potential along the fluidic channel. The pair of sensing electrodes may include a first and second sensing electrode disposed at two discrete locations along the length of the fluidic channel and the pair of electromotive electrodes may be disposed at a first end and a second end of the fluidic channel. The fluidic channel may include a nanochannel or a microchannel. Methods for detecting an analyte may include the steps of disposing the analyte in a fluidic channel; applying a potential along the fluidic channel to generate an electrophoretic force therein such that the analyte is translocated from a first end of the fluidic channel to a second end of the fluidic channel; and measuring a voltage signal between a pair of sensing electrodes disposed in the fluidic channel as the analyte moves past the sensing electrodes.

USE OF LONGITUDINALLY DISPLACED NANOSCALE ELECTRODES FOR
VOLTAGE SENSING OF BIOMOLECULES AND OTHER ANALYTES IN
FLUIDIC CHANNELS

Related Application

[0001] This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Serial Number 61/093,885, filed September 3, 2008; the entirety of which is incorporated herein by reference.

Field of Invention

5 [0002] The present invention relates generally to biopolymer sequencing. More particularly, in certain embodiments, the invention relates to determining the length of biopolymers and the distances of probes bound to the biopolymer.

Background

[0003] The technique known as Coulter counting was first proposed by Wallace H. Coulter 10 in the late 1940s as a technique for the high speed counting of red blood cells. Also referred to as resistive pulse sensing, Coulter counting may be used to measure physical parameters of analytes in electrolyte solution including size (volume), charge, electrophoretic mobility and concentration. In this technique, two reservoirs of solution are separated by a fluidic constriction of known dimensions. The application of a constant DC voltage between the two 15 reservoirs results in a baseline ionic current that is measured. The magnitude of the baseline current is related to the conductivity of the electrolyte, the applied potential, the length of the channel, and the cross-sectional area of the channel. If an analyte is introduced into a reservoir, it may pass through the fluidic channel and reduce the observed current due to a difference in 20 conductivity between the electrolyte solution and analyte. The magnitude of the reduction in current depends on the volume of electrolyte displaced by the analyte while it is in the fluidic channel.

[0004] A benefit of the resistive pulse sensing technique is that it may be scaled down to 25 enable the detection of nanoscale analytes through the use of nanoscale fluidic constrictions. This capability led to the development of solid-state nanopores for detecting nanoscale molecules such as DNA.

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[0005] In the case of DNA translocation through a nanopore, the physical translocation is driven by the electrophoretic force generated by the applied DC voltage. This driving force and the detected signal are, therefore, typically inseparably coupled. The decoupling of these two effects may be desirable because the optimal potential for physical translocation is different from that of optimal measurement.

5 **[0006]** Transverse electrodes have been proposed to provide a transverse electric field and electric current to sense biomolecules confined in a nanofluidic channel. *See* Liang and Chou 2008 Liang, X; Chou, S.Y., *Nanogap Detector Inside Nanofluidic Channel for Fast Real-Time Label-Free DNA Analysis*. *Nano Lett.* **2008**, 8, 1472-1476, which is incorporated herein by reference in its entirety. The analytes are moved through the channel with an electrophoretic force generated by current-carrying electrodes at the ends of the nanochannel, therefore decoupling the measurement from the translocation speed.

Summary

10 **[0007]** Embodiments of the present invention provide devices and methods that use electrodes to sense voltage changes, rather than to generate transverse electric currents, thereby reducing degradation of the electrodes. In particular, a device described herein utilizes longitudinally displaced electrodes for electronic sensing of biomolecules and other nanoscale analytes in fluidic channels. Embodiments enable characterization of nanoscale analytes, including, e.g., analysis of DNA strands having probes attached thereto.

15 **[0008]** More particularly, embodiments of the present invention may utilize sensing electrodes for electronic sensing of analytes, e.g., DNA in fluidic channels. The sensing electrodes in the fluidic channel may be used to determine the length of the analyte or they may be used to determine the distance between probes hybridized to a target strand of DNA. The device design is similar to nanochannel devices used for optical detection. Two micro-scale liquid reservoirs may be fabricated at a distance of 100 to 200 μm . One or more fluidic channels may connect the two reservoirs. A cap may be fabricated by drilling holes that will allow fluid introduction to each reservoir and to provide access for macroscopic electrodes. In use, a voltmeter may be used to monitor the potential difference between two sensing electrodes.

[0009] The DNA to be analyzed may be introduced to one of the microfluidic reservoirs.

Macroscopic electrodes may be connected to a power supply and used to apply a potential between the two reservoirs. DNA fragments may be electrophoretically driven from the microscopic reservoir into the nanochannels. As each DNA fragment moves down the fluidic channel, it may enter and exit the pair of sensing electrodes disposed in the fluidic channel.

[0010] In the absence of DNA, the fluidic channel contains only the ionic solution and typically have a baseline potential difference measured between the two sensing electrodes. As DNA enters the fluidic channel, the potential measured between the two sensing electrodes may change because the DNA has a conductivity different from that of the ionic solution. When

DNA enters the fluidic channel, the conductivity of the channel between the two sensing electrodes will typically be reduced as DNA is less conductive than the buffer solution (See de Pablo, P.J.; Moreno-Herrero, F; Colchero, J.; Gomez-Herrero, J.; Herrero, P.; Baro, A.M.; Ordejon, P.; Soler, J.M.; Artacho, E. Absence of dc-Conductivity in *Phys. Rev. Lett.* **2000**, 85, 4992-4995, which is incorporated by reference in its entirety). When a portion of the DNA that has a probe hybridized to the DNA enters the fluidic channel the potential may change further. The measured signal may be analyzed to determine the length of the DNA and/or distances between probes.

[0011] In an aspect, an embodiment of the invention includes a device for voltage sensing of analytes. The device may include a fluidic channel defined in a substrate, and a pair of sensing electrodes disposed in the fluidic channel for sensing voltage therein. The pair of sensing electrodes may include a first and a second sensing electrode disposed at two discrete locations along a length of the fluidic channel. A pair of electromotive electrodes may be disposed at a first end and a second end of the fluidic channel for applying a potential along the fluidic channel. The fluidic channel may include or consist essentially of a nanochannel or a microchannel.

[0012] One or more of the following features may be included. The substrate may include or consist essentially of silicon, silicon dioxide, fused silica, and/or gallium arsenide. Each of the sensing and electromotive pairs of electrodes may include or consist essentially of platinum, gold, chrome, titanium, silver chloride, silver, and graphene.

[0013] The first sensing electrode may be disposed on a first side of the fluidic channel and the second sensing electrode may be disposed on an opposing side of the fluidic channel. Each of the first and second sensing electrodes may be disposed on a first side of the fluidic channel,

or each of the first and second sensing electrodes may transverse the fluidic channel. The first sensing electrode may transverse the fluidic channel and the second sensing electrode may be disposed on a side of the fluidic channel.

[0014] The pair of electromotive electrodes may include macroscopic electrodes for

5 generating a constant, changing, or oscillating electrophoretic force in the fluidic channel for translocation of an analyte disposed therein.

[0015] A measurement tool, such as a voltmeter, may be provided for measuring a voltage sensed by the pair of sensing electrodes. The device may include a plurality of fluidic channels. A voltage amplifier may be disposed on the substrate.

10 [0016] The fluidic channel may have a width selected from a range of 1 nm to 5 μ m, a depth selected from a range of 1 nm to 5 μ m, and/or a length selected from a range of 1 μ m to 10 cm.

[0017] The description of elements of the embodiments of other aspects of the invention may be applied to this aspect of the invention as well.

15 [0018] In another aspect, the invention features a method for detecting an analyte, the method including disposing the analyte in a fluidic channel. A potential is applied along the fluidic channel and the analyte is translocated from a first end of the fluidic channel to a second end of the fluidic channel. A voltage signal is measured between a pair of sensing electrodes disposed in the fluidic channel as the analyte moves past the pair of sensing electrodes, with the 20 pair of sensing electrodes including a first and a second electrode disposed at two discrete locations along a length of the fluidic channel. The fluidic channel may include or consist essentially of a nanochannel or a microchannel.

[0019] The potential applied along the fluidic channel may include generating an electrophoretic force therein. Translocating the analyte may include using a pressure differential and/or a chemical gradient.

25 [0020] One or more of the following features may be included. The analyte may include a biopolymer, such as a deoxyribonucleic acid, a ribonucleic acid, and/or a polypeptide. The biopolymer may include or consist essentially of a single-stranded molecule. The analyte may include or consist essentially of a biopolymer having at least one probe attached thereto.

30 [0021] The voltage signal may change when the biopolymer moves through a volume between the sensing electrodes and further change when the portion of the biopolymer containing the probe moves through the volume between the sensing electrodes. A time

between voltage signal changes may be recorded. A duration of a change in the voltage signal may indicate a presence of a probe, and the voltage signal may be used to determine a distance between two probes on the biopolymer.

[0022] A duration of a change in the voltage signal may be used to determine a length of the analyte. Multiple pairs of sensing electrodes may be used to measure a single analyte molecule as it passes through the fluidic channel.

[0023] The description of elements of the embodiments of other aspects of the invention may be applied to this aspect of the invention as well.

[0024] In yet another aspect, a method for determining a sequence of a biopolymer may include preparing an analyte including the biopolymer. The analyte may be disposed in a fluidic channel. A potential may be applied along the fluidic channel and the analyte is translocated from a first end of the fluidic channel to a second end of the fluidic channel. A voltage signal may be measured between a pair of sensing electrodes disposed in the fluidic channel as the analyte moves past the pair of sensing electrodes, the voltage signal corresponding to locations along the biopolymer, the pair of sensing electrodes including a first and a second electrode disposed at two discrete locations along a length of the fluidic channel. The fluidic channel may include or consist essentially of a nanochannel or a microchannel.

[0025] The potential applied along the fluidic channel may include generating an electrophoretic force therein. The analyte may be translocated by using a pressure differential and/or a chemical gradient.

[0026] One or more of the following features may be included. Preparing the analyte may include hybridizing the biopolymer with a probe. A change in the voltage signal may correspond to a location along the hybridized biopolymer containing the probe. The voltage signal may be processed using a computer algorithm to reconstruct the sequence of the biopolymer. The biopolymer may include or consist essentially of a double-stranded biopolymer target molecule. Preparing the analyte may include contacting the target molecule with a first probe having a first probe specificity for recognition sites of the target molecule to form a first plurality of local ternary complexes, the first probe having a first predicted recognition site sequence. The voltage signal may be used to determine positional information of the first plurality of local ternary complexes. The positional information may include a parameter to a spatial distance between two local ternary complexes.

[0027] Preparing the analyte may further include contacting the target molecule with a second probe having a second probe specificity for recognition sites of the target molecule to form a second plurality of local ternary complexes, the second probe having a second predicted recognition site sequence.

5 **[0028]** The voltage signal may be used to determine positional information of the second plurality of local ternary complexes. Positional information of at least the first and second plurality of local ternary complexes may be aligned to determine a DNA sequence of the target.

[0029] The biopolymer may include or consist essentially of a double-stranded nucleic acid target molecule having a plurality of binding sites disposed along the sequence thereof.

10 Preparing the analyte may include adding a plurality of probe molecules having a first sequence specificity to the double stranded nucleic acid target molecule.

[0030] The probe molecules having the first sequence specificity and the target molecule may be incubated so as to effectuate preferential binding of the first probe molecules to both a first binding site and a second binding site of the target molecule. The voltage signal may be 15 used to measure a parameter related to a distance between the first binding site and the second binding site.

20 **[0031]** Preparing the analyte may include contacting the biopolymer with a first probe to create at least one probe-target complex at a recognition site of the biopolymer for which the first probe has a known specificity, while leaving uncomplexed, regions of the biopolymer for which the first probe is not specific. Preparing the analyte may include contacting the biopolymer with a second probe to create at least one probe-target complex at a recognition site of the biopolymer for which the second probe has a known specificity, while leaving uncomplexed, regions of the target for which the second probe is not specific.

25 **[0032]** The voltage signal may be used to detect and record complexed and uncomplexed regions of the biopolymer to create a first probe map of the first probe and a second probe map of the second probe, the first probe map and the second probe map incorporating information on the relative position of the hybridization of the probes. A candidate sequence may be determined by aligning at least two probe sequences using positional information or a combination of overlapping sequences of the probe molecules and positional information. The 30 first and second probe maps may incorporate information on an error of the positional information for each probe. A candidate sequence may be determined by ordering at least two probe sequences using positional information and parameters relating to the error in positional

information or a combination of overlapping sequences of the probe molecules and positional information and error in positional information.

[0033] The description of elements of the embodiments of other aspects of the invention may be applied to this aspect of the invention as well.

5 Brief Description of Figures

[0034] Figure 1 is a schematic diagram illustrating a longitudinally displaced transverse electrode device configuration in accordance with an embodiment of the invention;

Figure 2 is a schematic diagram illustrating a longitudinally displaced transverse electrode device configuration in accordance with another embodiment of the invention;

10 Figure 3 is a schematic diagram illustrating a longitudinally displaced continuous transverse nanoscale electrode device configuration in accordance with another embodiment of the invention;

15 Figure 4 is a schematic diagram illustrating a longitudinally displaced nanoscale electrode device configuration with electrodes disposed on the same side of a nanochannel, in accordance with an embodiment of the invention;

Figure 5 is a schematic depiction of a DNA molecule;

Figure 6 is a schematic depiction of an RNA molecule;

Figure 7 is a schematic depiction of a hybridizing oligonucleotide (or probe);

20 Figure 8 is a schematic depiction of a single strand DNA molecule hybridized with a probe; and

Figure 9 is a graph of an exemplary voltage signal determined in accordance with an embodiment of the invention.

Detailed Description

Fabrication of fluidic channel and sensing electrodes

25 **[0035]** Embodiments of the invention include devices and methods for performing sequence analysis by hybridization (“SBH”). Referring to Figure 1, in an embodiment a device 100 includes a fluidic channel 105, e.g., a micro- or nanochannel. A pair of electromotive electrodes 110, 110' are disposed at a first and a second end of the fluidic channel 105 for applying a potential along the fluidic channel. A pair of sensing electrodes 115A, 115B are disposed at two discrete locations along a length of the fluidic channel. The sensing electrodes

may be in electrical communication with a measurement tool 120 that measures a voltage sensed by the sensing electrodes. The fluidic channel 105 may be defined in a substrate comprising either silicon, silicon dioxide, fused silica, or gallium arsenide, and may contain an electrolytic solution. The electromotive electrode pair 110, 110' may include at least one anode

5 110' and cathode 110' in contact with the electrolytic solution to provide a constant or changing current to drive the analyte 125 through the fluidic channel 105. In an alternate embodiment, a pressure differential, such as a positive pressure, may be used to drive the analyte 125 through the fluidic channel 105. Pressure may be supplied with a fluid pump or with a pressurized gas line. Other methods of applying pressure may be envisioned by one of
10 skill in the art. In some embodiments, a chemical potential gradient may be used to move molecules through the fluidic channel 105. The analyte may also be translocated by using a chemical potential gradient. Chemical potential gradients may be created with concentration gradients. For instance, a fluidic channel may have one end immersed in a fluid that has a higher salt concentration than the fluid at the other end of the channel. The differential in salt
15 concentration at the ends of the fluidic channel causes an osmotic pressure that can drive analytes through the channel.

[0036] As the analyte 125, which may be any biopolymer including, but not limited to, polypeptides, DNA or RNA, passes through the fluidic channel 105, it will pass between the pair of sensing electrodes 115A, 115B (each individually referred to herein as "A" and "B").
20 The sensing electrodes 115A, 115B contacting the fluidic channel 105 may be used to measure the changes in conductance of the electrolytic volume between them. The changes in conductance between the sensing electrodes 115A, 115B may be measured using a measurement tool 120, e.g., a voltmeter.

[0037] By making the longitudinal distance, e.g., a length along the fluidic channel 105, 25 between sensing electrodes 115A, 115B small, the device 100 retains high sensitivity for an analyte 125 passing therethrough. Each sensing electrode 115A, 115B in the pair may be disposed on opposite sides of the fluidic channel 105 as in Figure 1, where tips of the sensing electrode 115A, 115B are in contact with the electrolytic solution are entirely or partially across from one another, or as in Figure 2, in which the tips of the sensing electrodes 115A, 115B are 30 not across from each other, but are rather longitudinally displaced with respect to one another by a selected distance. Alternatively, each sensing electrode 115A, 115B in a pair may cross the fluidic channel 105, as shown in Figure 3. Referring to Figure 4, in a third arrangement,

two sensing electrodes 115A, 115B in a pair may be on the same side of the fluidic channel 105.

[0038] The devices 100 described herein may be formed by the fabrication of a trench to define a fluidic channel 105 having nanoscale dimensions, and the fabrication of nanoscale electrodes. A typical device 100 may also have a microscale fluidic structure for introduction of buffers and samples. Thus, the techniques described herein employing nanochannels are also applicable to devices including microchannels. Some or all of the structures may also be sealed with a cap in order to provide closed channels.

[0039] Fluidic channels may be formed in the substrate by, e.g., lithographic and etch steps. The substrate may be, e.g., a silicon-on-insulator wafer, with, for example, an (100) Si surface, a Si wafer, or a fused silica substrate. Lithography in the sub-100 nanometer (nm) regime may be performed by various techniques, including the following: electron beam lithography (EBL), nanoimprint lithography (NIL) or deep ultraviolet optical lithography (DUV OL). *See* Liang, X.; Morton, K.J.; Austin, R.H.; Chou, S.Y., Single sub-20 nm wide, centimeter-long nanofluidic channel fabricated by novel nanoimprint mold fabrication and direct imprinting, *Nano Lett.* **2007**, 7, 3774-3780; Austin, M.D.; Ge, H.; Wu, W.; Li, M.; Yu, Z.; Wasserman, D.; Lyon, S.A.; Chou, S.Y., Fabrication of 5 nm line width and 14 nm pitch features by nanoimprint lithography, *App. Phys. Lett.* **2004**, 84, 5299-5301; and Guo, J., Recent progress in nanoimprint technology and its applications, *J. Phys. D: Appl. Phys.* **2004**, 37, R123-R141. Each of these references is incorporated herein by reference in its entirety. The current industry standard in micro and nanofabrication is optical lithography due to its low cost and high throughput. At present, optical lithography has been successfully used in the mass production of devices with critical dimensions as small as 32 nm. EBL and NIL are presently used extensively in academic research environments due to their versatility and capability of producing sub-10 nm features reproducibly. Any of these methods may be used to pattern the fluidic trenches described herein.

[0040] The removal of material for the formation of the fluidic trenches may be performed by, e.g., etching. Wet etching includes the immersion of the material in a solution capable of selective removal. Dry etching, i.e., reactive ion etching (RIE), involves the exposure of the sample to a charged plasma. For the resolution and control required of nanoscale fabrication, RIE is preferable due to its consistency, controllability, and efficiency. Microfluidic channels or reservoirs leading to the nanoscale channels may be etched using either wet or dry methods.

[0041] The resulting channels have preferred dimensions of width and depth ranging from 1 nm to 5 μ m, more preferably 1 nm to 1 μ m, and more preferably 10 nm to 100 nm. The channels may have a length selected from a range of, e.g., 1 micrometer (μ m) to 10 centimeters (cm).

5 **[0042]** The size of the channel may be chosen with regard to the persistence length of the analyte. For example, a randomly coiled polymer (e.g., DNA) may be elongated when introduced into a confined space, such that when the confinement space becomes smaller the extent of elongation becomes greater. In some embodiments, it may be preferable to elongate the analyte to measure length or distance between probes. Depending on the cross-sectional 10 size and the persistence length it may be useful to have the geometric mean of the width and depth of the channel be between 5% and 500% of the persistence length of the analyte. For example, for double-stranded DNA, under conditions where the persistence length is 50 nm, it may be preferable to have, e.g., a fluidic channel with a width and depth between 2.5 nm and 250 nm. In other embodiments, for more rigid polymers such as RecA coated DNA, under 15 conditions where the persistence length is 950 nm, it may be preferable to have, e.g., a fluidic channel with a width and depth between 45 nm to 4.75 μ m.

[0043] After the channels are formed, sensing electrodes are fabricated. Similar to etching and lithography, numerous metal deposition techniques suitable for fabrication of sensing electrodes exist in conventional microfabrication process flows. Each technique has positive 20 and negative attributes and a list of the materials that may be deposited using that technique. The three primary techniques are: electron beam evaporation, thermal evaporation, and sputtering. The sensing electrodes have thicknesses ranging from 5 nm to 100 nm at the point where the sensing electrodes intersect the fluidic channels. The sensing electrodes may be wider and/or thicker in regions distal to the fluidic channels and approaching contact pads 25 disposed at the perimeter of the device.

[0044] To complete the device, a cap layer may be introduced to prevent evaporation of liquid from the fluidic channel. The cap may be formed over just the nanoscale fluidic paths or over all of the fluidic channels. In the latter case, the cap structure preferably has holes or ports to allow for the introduction of fluid and samples into the fluidic paths. In another 30 embodiment, the entire substrate, i.e., wafer, may be capped. The cap may be made of a glass plate such as borosilicate glass, phosphosilicate glass, quartz, fused silica, fused quartz, a silicon wafer or other suitable substrates. Various techniques are suitable for accomplishing

this step including anodic bonding. In anodic bonding, an underlying silicon wafer and a glass substrate are pressed together and heated while a large electric field is applied across the joint. Anodic bonding has been demonstrated to form a strong bond between a silicon wafer and the capping substrate. Direct silicon bonding has been used to join two silicon wafers. The latter 5 method involves pressing the two wafers together under water. Other methods use an adhesive layer, such as a photoresist, to bond the cap to the substrate.

[0045] An exemplary fabrication process for defining the proposed sensing element is as follows. A suitable substrate, such as a conventional (100) p-type silicon wafer, is thermally 10 oxidized in a hydrated atmosphere to grow a thick (e.g., >1 μm) silicon-dioxide (SiO_2) layer.

This SiO_2 layer may serve as insulation between subsequently formed adjacent metal sensing 15 electrodes, and may also reduce overall device capacitance.

[0046] Using conventional high resolution optical lithography, the pattern of the fluidic channel may be transferred to a first photoresist masking layer. RIE with an anisotropic etch species, such as Cl_2 , may be used to transfer the pattern into the SiO_2 layer. The preferred 20 width and depth of the channel may be determined by the requirements for the device sensitivity. The smaller the volume of the channel between two sensing electrodes, the more sensitive the device is. Channel size, width, and depth, may also be determined by the size or behavior of the analyte. In one embodiment, the device described herein is used to detect 25 strands of DNA. It may be desirable to fabricate the channel with dimensions that extend the DNA strand within the channel. For instance for double-stranded DNA, it has been found that the use of channels with dimensions of 100 nm or less are able to extend the biopolymer. *See* Tegenfeldt, J.O et al. *The dynamics of genomic-length DNA molecules in 100-nm channels*.

Proc. Nat. Acad. Sci. USA, 2004, 101, 10979-10983, which is incorporated herein by reference in its entirety. Upon completion of the dry etch procedure, residual resist is removed and the 25 substrate vigorously cleaned.

[0047] Following the etching of the fluidic channel, embedded metal sensing electrodes are fabricated. Conventional high resolution optical lithography may be used to transfer the metal electrode pattern to a second photoresist masking layer. RIE with an anisotropic etch species, such as Cl_2 , will be used to transfer the pattern into the SiO_2 layer. Preferably the depth of 30 these trenches exceeds or equals the depth of the fluidic channel. Upon completion of pattern transfer to the SiO_2 layer, a thin metal adhesion promotion layer may be deposited. A suitable layer is tantalum with a thickness of 30 - 50 \AA , deposited via electron beam evaporation. Next,

the sensing electrode material is deposited without exposing the substrate to atmosphere. A preferred metal for the bulk of the sensing electrodes is platinum, also deposited via electron beam evaporation. Other examples of suitable metals include gold, chrome, titanium, silver chloride, silver, and graphene. The thickness of the metal is dictated by the depth of the etched 5 trenches, such that the resultant metal trace is approximately planar with a top surface of the SiO₂ layer. Upon completion of the metal deposition, the substrate is immersed in a photoresist solvent that will lift-off excess metal from the surface and the substrate is vigorously cleaned. Chemical-mechanical polishing (CMP) may be performed to remove excess metal extending over the SiO₂ top surface, thereby planarizing a top surface of the metal to be level with the 10 SiO₂ top surface.

[0048] To complete the fabrication of the sensor, a cap layer is preferably adhered to the sensor surface to provide a leak-free seal, enabling fluidic conduction. Preferred cap materials include borosilicate glass, fused silica, fused quartz, quartz, or phosphosilicate glass. Holes may be created in the cap layer to provide access to fluidic inlet, fluidic outlet and metal 15 sensing electrodes. A typical method for making holes in glass wafers is ultrasonic etching, which allows for highly controllable pattern transfer to glass substrates. Anodic bonding may then be used to bond the glass cap layer to the underlying substrate, e.g., silicon wafer. The anodic bonding of two layers provides a strong and leak-free seal.

[0049] An exemplary device with a pair of such nanoscale sensing electrodes 115A, 115B 20 is illustrated in Figure 1, i.e., electrodes A and B. Electric current is transferred in the form of ionic flow in an electrolyte solution confined in the fluidic channel 105, e.g., a nanochannel. The role of the electrolyte is to maintain a uniformly distributed electric field in the fluidic channel. Typical electrolyte solutions have been described in applications of electrophoresis to 25 separations of DNA molecules. The most common electrolytes for electrophoretic separation of DNA are Tris boric acid EDTA (TBE) and tris acetate EDTA (TAE). *See, e.g., Sambrook, J.; Russell, D.W. *Molecular Cloning: A Laboratory Manual 3rd ed.* Cold Spring Harbor Press, 2001.* However, any conductive medium may be used.

Operation of Device

[0050] During operation, a constant current is supplied by applying a potential to a pair of 30 macroscopic electrodes, e.g., electromotive electrodes 110, 110' disposed at opposing ends of the fluidic channel 105 and in contact with the electrolytic solution. The electromotive

electrodes are preferably in electrical communication with the wires leading to the ends of the fluidic channel 105 illustrated in Figures 1-4. A potential may be applied along the fluidic channel 105 to generate an electrophoretic force therein, such that the analyte 125 is translocated from a first end of the fluidic channel 105 to a second end of the fluidic channel.

5 The electromotive electrodes may generate a constant or oscillating electrophoretic force in the fluidic channel 105 for translocation of an analyte 125 disposed therein. The voltage between the electromotive electrodes may be constant or it may be changed over the course of a measurement. For instance, it may be desirable to reduce the voltage once a DNA molecule has entered the fluidic channel 105 and before the DNA molecule has entered the volume

10 between the sensing electrodes, in order to slow the passage of the DNA molecule through the volume between the sensing electrodes. Controlling the rate of passage of the DNA molecule through the volume between sensing electrodes 115A, 115B allows for more versatile detection of the DNA.

[0051] As an example of the placement of sensing electrodes, a width of 20 nm may be
15 assumed for each of sensing electrodes A and B in Figure 1. Electrode A may be shifted along the fluidic channel 105 relative to electrode B, by, e.g., 10 nm or 30 nm. Distances between sensing electrodes from 30 nm to 100 nm or from 30 nm to 500 nm, or from 30 nm to 5 μ m can be incorporated into a single device. For analytes of sufficient length, distances up to e.g., 500 μ m may be used, e.g., up to 300 μ m, 200 μ m, or 100 μ m may be used. Although electrodes
20 with any distance therebetween may be fabricated, since DNA is difficult to obtain at a length greater than 500 μ m, any electrode distance that is greater than 500 μ m would be superfluous, as long as the length of the DNA does not exceed 500 μ m. The smaller displacement between electrodes A and B is an example of an embodiment in which there is overlap of the electrodes, even though they are displaced with respect to one another. In some embodiments, as shown in
25 Figure 2, there may be no overlap between sensing electrodes 115A, 115B.

[0052] The voltage across sensing electrodes 115A, 115B is proportional to the local impedance in the fluidic channel 105 between sensing electrodes 115A, 115B. The spacing of the electrodes is determined by several factors. The smaller the distance between electrodes in a sensing pair, all other factors being constant, the smaller the particle that can be detected by
30 the sensing pair. However, fabrication limits may make it difficult to consistently place the electrodes in a pair at small distances. Thus, the selected distance is a trade-off between fabrication reproducibility and sensitivity of the device 100. The choice of separation distance

and thus whether the electrodes are overlapping or non-overlapping depends on these constraints.

[0053] The resulting sensing electrode 115A, 115B arrangement provides a means to separate the current and voltage probes and can be used to employ 4-point sensing in a fluidic channel. In an embodiment, the macroscopic, electromotive electrodes 110, 110' at the ends of the fluidic channel 105 provide a current while the nanoscale sensing electrodes 115A, 115B disposed across the fluidic channel 105 are used to measure voltage. The voltage electrodes preferably have an output impedance higher than the impedance of the volume being measured.

[0054] The following calculations demonstrate the feasibility of this device concept. The fluidic channel may be subject to a constant electric field equal to the potential difference along the length of the channel divided by the length of the channel, i.e., 100 mV applied longitudinally to a 10 μ m long fluidic channel results in a field of $100 \text{ mV}/10 \mu\text{m} = 10 \text{ mV}/\mu\text{m}$ or 0.01 mV/nm. The potential difference between electrodes A and B separated by 10 nm is then the product of the distance between electrodes and the electric field or:

$$10 \text{ nm} \times 0.01 \text{ mV/nm} = 0.1 \text{ mV}.$$

[0055] Similarly, a potential difference of 0.3 mV exists between electrodes A and B when the spacing is 30 nm. Each of these potentials is readily detectable with conventional electronic measurement tools. When a DNA molecule or any other analyte passes between a pair of sensing electrodes, the impedance between the sensing electrodes changes due to a resistivity difference between the electrolyte and the molecule. The resulting transient change in the potential is measured, while maintaining a constant current.

[0056] For the example shown in Figure 1, assuming a substantially constant velocity, the duration of each voltage pulse detected by the sensing electrodes 115A, 115B is proportional to the length of the DNA or other analyte 125 that passes between the two sensing electrodes.

After determining the speed of the analyte 125 in the fluidic channel, the measured duration of the pulse may be used to calculate the distance the analyte 125 moved (velocity \times time = distance) while passing through the volume between sensing electrodes, which would be equal to the analyte's 125 length.

[0057] It is important to note that by shifting one of the transverse electrodes along the fluidic channel 105 by a distance of 10-50 nm, and using a fluidic channel 105 with a diameter of about 10 nm, the volume separating the two sensing electrodes 115A, 115B may be viewed as having a sensitivity equivalent to that of a conventional solid-state nanopore.

[0058] In use, the voltage between a pair of sensing electrodes 115A, 115B, e.g., V_{AB} , may be sensed by a measurement tool 120, e.g., a voltmeter, configured to measure the potential difference between the sensing electrode 115A, 115B pair. In a preferred embodiment, the voltmeter 120 may be in electrical communication with each of the sensing electrodes 115A,

5 115B in the pair via metal contact pads connected to nanowires leading to the sensing electrodes.

[0059] Generally, an analyte 125 may be detected in the fluidic channel 105 as follows.

The analyte, e.g., the biopolymer strand and probes, is transferred from a chamber into the fluidic channel in an electrolytic solution. Typically, an electrolyte may be added to the fluidic

10 channel by a pipette, a syringe, or a pump. An analyte sample size may be as small as

practically possible, as the device allows the detection of single molecules. The fluid may wet the fluidic channels by capillary action. Analyte may be introduced into the microscale areas either with the original electrolyte or after by pumping in a new solution. An analyte, such as DNA, which may be hybridized to one or more probes, may be drawn into the fluidics channel

15 by the potential. For small analytes, one could use diffusion, fluid flow, or a potential.

[0060] The fluidic channel may have a width that is no smaller than approximately the same width as the analyte, and may be sufficiently large such that large molecules bound to the analyte may pass through the fluidic channel. For example, the width of the fluidic channel may be selected from a range of 1 nm to 200 nm. The fluidic channel may be sufficiently deep 20 to allow large molecules bound to the analyte to pass through and yet shallow enough to be approximately the same size as the analyte. The fluidic channel depth may be, e.g., selected from a range of 1 nm to 200 nm. The length of the fluidic channel may be selected such that the entire analyte is contained in the fluidic channel.

[0061] In an embodiment, the sensing electrodes and fluidic channel may be preferably

25 arranged such that the entire analyte enters the fluidic channel before it enters the volume between sensing electrodes. This configuration provides an advantage of reducing the effect of the analyte on the conductance of the fluidic channel. For instance, if one is beginning to measure the change in potential of a volume between sensing electrodes while the conductance of the whole fluidic channel is changing due to more analyte entering the fluidic channel, the 30 analysis becomes more complex. In a preferred embodiment, the analyte may be contained completely in the channel when it exits the volume between sensing electrodes. Thus, the length of the fluidic channel preferably has a minimum length that is approximately three times

the length of the analyte (assuming that the volume between sensing electrodes is only as long as the analyte, which is a minimal requirement but not optimal). The length of a 1 kb piece of DNA is about 330 nm, so a length of the fluidic channel is preferably at least 1 μ m in length. The longest piece of DNA suitable for analysis with the described methods may be 10

5 megabases (Mb), which corresponds to a preferred fluidic channel of at least 10 mm. More preferably, the length of a fluidic channel is ten times the length of the analyte, and thus a more preferred upper limit for a channel length is 100 mm (10 cm). Thus, the fluidic channel length is preferably selected from a range from 1 μ m to 10 cm. Longer and shorter fluidic channel lengths are also possible.

10 [0062] In some embodiments, the structure of the fluidic channel may facilitate entry of the analyte into the channel, e.g., the fluidic channel may comprise a series of posts (e.g., U.S. Patent No. 7,217,562, which is incorporated by reference in its entirety) and/or a funnel shape.

[0063] The analyte is translocated through the fluidic channel by a current that is supplied by applying a potential to the two electromotive electrodes disposed at opposing ends of the 15 fluidic channel and in contact with the electrolytic solution. The electromotive electrodes may generate a constant or oscillating electrophoretic force in the fluidic channel for translocation of an analyte disposed therein. The voltage between the macroscopic electrodes may be constant or it may be changed over the course of a measurement. For example, the voltage may be reduced once a DNA molecule has entered the fluidic channel and before the DNA molecule 20 has entered the volume between the sensing electrodes, to slow the passage of the DNA molecule through the volume between sensing electrodes.

[0064] A voltage signal reflecting a change in potential between the pair of sensing electrodes may be monitored. As the analyte moves through a volume between the sensing electrodes, the voltage signal changes. The signal may be elevated or depressed for a period of 25 time that reflects the length of the analyte, e.g., a probe-target complex, or the length of the intervening regions without probes. A typical analyte is non-conductive and will impede the flow of ions in the electrolyte. Therefore, the potential – and voltage signal – typically increase as the analyte flows through the volume between sensing electrodes. In some embodiments, e.g., a low salt electrolyte and a charge-carrying analyte, the potential and voltage signal may 30 decrease as the analyte flows through the volume between sensing electrodes. The voltage signal further changes when the portion of the analyte containing the hybridized probe moves through the volume between the sensing electrodes.

Determination of Analyte Length and Probe Location

[0065] In an embodiment, a method for detecting the relative position of probes hybridized to a biopolymer and/or the length of the biopolymer. Nanopores may be used as detectors to determine the distance between hybridization sites as described in U.S. Patent Publication No. 5 2007/0190542 A1, which is incorporated herein by reference in its entirety. The construction of a nanochannel device incorporating voltage detectors is described herein. In both the nanopore and the fluidic channel (e.g., a nanochannel), the distance between hybridization sites on the target biopolymer may be inferred from the time between the detection of a first hybridization position and a subsequent hybridization position as the biopolymer moves 10 through the nanopore or fluidic channel. The technology disclosed herein allows the determination of biopolymer length and distances between hybridization positions.

[0066] In particular, as used herein, a “probe” means any molecule or assembly of molecules capable of sequence –specific covalent or non-covalent binding to a target molecule. A probe may be, but is not limited to, a DNA sequence, an RNA sequence, antibodies or 15 antibody fragments. The terms “nucleotide” and “base” are used interchangeably and mean a molecule consisting of a phosphate group, a sugar and one of five nitrogen-containing bases that can make up DNA or RNA polynucleotide chains or strands. For DNA, the nitrogen-containing bases include cytosine (C), adenine (A), guanine (G) and thymine (T) and the sugar is a 2-deoxyribose. For RNA, a the deoxyribose sugar is replaced by a ribose sugar instead of 20 deoxyribose and uracil bases (U) instead of thymine bases (T).

[0067] A DNA probe “library” is a collection of DNA probes of a fixed length which includes a large number of, or possibly all, possible sequence permutations. A plurality of probes may be made up of multiple copies of the same probe with the same sequence 25 selectivity or be made up of two or more probes with different sequence selectivity. A “probe map” means a data set containing information related to the sites along a target sequence at which a probe preferentially binds. A partially hybridized biomolecule is created when the entire length of a sequence selective probe binds to a portion of the length of the target biomolecule. The data set may include absolute positional information referenced to a known sequence, relative information related to distances between binding sites, or both. The data set 30 may be stored in computer media. Further details of the characteristics of probe and spectrum maps may be found in U.S Patent Publication No. 2009-0099786 A1, which is incorporated herein by reference in its entirety.

[0068] A “target,” i.e., the analyte, is a biopolymer, of which length, identity or sequence information is to be determined using embodiments of the present invention. The analyte may be a biopolymer, such as a deoxyribonucleic acid, a ribonucleic acid, proteins, or a polypeptide. The target DNA may be single- or double-stranded. In some embodiments, the analyte is a 5 biopolymer to which probes have been hybridized.

[0069] DNA is the fundamental molecule containing all of the genomic information required in living processes. RNA molecules are formed as complementary copies of DNA strands in a process called transcription. Proteins are then formed from amino acids based on the RNA patterns in a process called translation. The common relation that can be found in 10 each of these molecules is that they are all constructed using a small group of building blocks, such as bases or amino acids, that are strung together in various sequences based on the end purpose that the resulting biopolymer will ultimately serve.

[0070] Analytes may be prepared for analysis, e.g., as disclosed in U.S. Patent Publication No. 2007/0190542, which is incorporated herein by reference in its entirety. Referring to 15 Figure 5, a DNA molecule 500 is schematically depicted and can be seen to be structured in two strands 505, 510 positioned in anti-parallel relation to one another. Each of the two opposing strands 505, 510 is sequentially formed from repeating groups of nucleotides 515 where each nucleotide 515 consists of a phosphate group, 2-deoxyribose sugar and one of four nitrogen-containing bases. The nitrogen-containing bases include cytosine (C), adenine (A), 20 guanine (G) and thymine (T). DNA strands 505 are read in a particular direction, from the so called the 5' or "five prime" end to the so called the 3' or "three prime" end. Similarly, RNA molecules 600, as schematically depicted in Figure 6 are polynucleotide chains, which differ from those of DNA 500 by having ribose sugar instead of deoxyribose and uracil bases (U) instead of thymine bases (T).

[0071] Traditionally, in determining the particular arrangement of the bases 515 in these organic molecules and thereby the sequence of the molecule, a process called hybridization is utilized. The hybridization process is the coming together, or binding, of two genetic sequences with one another. This process is a predictable process because the bases 515 in the molecules do not share an equal affinity for one another. T (or U) bases favor binding with A 25 bases while C bases favor binding with G bases. This binding is mediated by the hydrogen bonds that exist between the opposing base pairs. For example, between an A base and a T (or

U) base, there are two hydrogen bonds, while between a C base and a G base, there are three hydrogen bonds.

[0072] The principal tool that is used then to determine and identify the sequence of these bases 515 in the molecule of interest is a hybridizing oligonucleotide commonly called a probe

5 700. As Figure 7 illustrates, a DNA probe 700 is a DNA sequence having a known length and sequence composition. Probes 700 may be of any length dependent on the number of bases 515 that they include. For example a probe 700 that includes six bases 515 is referred to as a six-mer wherein each of the six bases 515 in the probe 700 may be any one of the known four natural base types A, T(U), C or G and alternately may include non-natural bases. In this
10 regard the total number of probes 700 in a library is dependent on the number of bases 515 contained within each probe 700 and is determined by the formula 4^n (four raised to the n power) where n is equal to the total number of bases 515 in each probe 700. Accordingly, the general expression for the size of the probe library is expressed as 4^n n-mer probes 700. For the purpose of illustration, in the context of a six-mer probe the total number of possible unique,
15 identifiable probe combinations includes 4^6 (four raised to the sixth power) or 4096 unique six-mer probes 700. It should be further noted that the inclusion of non-natural bases allows the creation of probes that have spaces or wildcards therein in a manner that expands the versatility of the library's range of probe recognition. Probes that include universal bases organized into patterns with natural bases may also be used, for example those described in U.S. Patent Nos.
20 7,071,324, 7,034,143, and 6,689,563, each of which are incorporated herein by reference in their entireties.

[0073] When a target biomolecule, such as single-stranded DNA, is incubated with a sequence selective probe under appropriate conditions, the probe hybridizes or binds to the biomolecule at specific sites. The determination of the relative location of the hybridization sites is useful for constructing maps of the target biomolecule, and for identifying the target molecule.

[0074] When the biopolymer to be analyzed is a double-stranded DNA 500, the process of hybridization using probes 700 as depicted in Figure 8 first requires that the biopolymer strand be prepared in a process referred to as denaturing. Denaturing is a process that is usually
25 accomplished through the application of heat or chemicals, wherein the hydrogen bonds between the two strands of the original double-stranded DNA are broken, leaving a single strand of DNA whose bases are available for hydrogen bonding. After the biopolymer 800 has

been denatured, a single-stranded probe 700 is introduced to the biopolymer 800 to locate portions of the biopolymer 800 that have a base sequence that is complimentary to the sequence that is found in the probe 700. In order to hybridize the biopolymer 800 with the probe 700, the denatured biopolymer 800 and a plurality of the probes 700 having a known sequence are both 5 introduced to a solution. The solution is preferably an ionic solution and more preferably is a salt containing solution. The mixture is agitated to encourage the probes 700 to bind to the biopolymer 800 strand along portions thereof that have a matched complementary sequence. Once the biopolymer strand 800 and probes 700 have been hybridized, the strand 800 is introduced to one of the chambers of a sequencing arrangement. It should also be appreciated 10 to one skilled in the art that while the hybridization may be accomplished before placing the biopolymer strand 800 into the chamber, it is also possible that the hybridization may be carried out in one of these chambers as well. In this case, after the denatured biopolymer has been added to the cis chamber, a drop of buffer solution containing probes 700 with a known sequence are also added to the cis chamber and allowed to hybridize with the biopolymer 800 15 before the hybridized biopolymer is translocated.

[0075] As a specific example of analyte preparation, a nucleotide sample, such as DNA or RNA, may be heated to a denaturing temperature, typically greater than 90° C for DNA and typically between 60-70° C for RNA, in the presence of a selection of probes and in a buffer of 50 mM potassium chloride and 10mM Tris-HCl (pH 8.3). The hybridization may be 20 accomplished in the cis chamber or before placing the analyte in the chamber. The mixture of nucleotide strand and probes are then cooled to allow for primer binding, with the temperature dependent upon the length and composition of the probe. For example, a 6-nucleotide probe would be cooled to room temperature or lower. The nucleotide strand and probe may be allowed to anneal at the low temperature for up to 5 minutes before being passed through a 25 fluidic channel for analysis. The exact temperatures may be easily determined by one of skill in the art without undue experimentation.

[0076] Referring to Figure 1, in some embodiments, electrical signals corresponding to the volume between sensing electrodes 115A, 115B are detected by the sensing electrodes 115A, 115B as an analyte 125, e.g., a biopolymer, is disposed in the fluidic channel. As the 30 biopolymer enters the volume between sensing electrodes, a change in the electrical signal is recorded. Referring to Figure 10, the electrical signal increases linearly at time point T₁ as the analyte enters the volume between sensing electrodes. When the volume between sensing

electrodes is completely filled, the signal stays essentially constant. When the analyte leaves the volume between sensing electrodes, the signal returns to baseline at time point T₄. The sensing electrodes are configured for connection to a measurement tool for capturing the electrical signals corresponding to the volume between sensing electrodes, e.g., a voltmeter.

5 Electrical signals captured by the measurement tool may be recorded as a function of time by a data collection device, e.g., a Stanford Research Instruments SIM970 voltmeter. A time interval between voltage signal changes may be recorded. The duration of the change in the voltage signal over baseline may indicate a presence of an analyte. Another increase over that, as shown in Figure 9 between time points T₂ and T₃, may indicate the presence of a probe

10 hybridized with the analyte. The electrical signal may have fluctuations from noise and from the fact that a long analyte, such as DNA, typically has small bends in it. When a section having a bend enters the volume between sensing electrodes, the electrical signal may increase slightly, and then may decrease slightly when the bend exits the volume between sensing electrodes. Calibration of the electrical signals, therefore, may be needed to determine length.

15 To determine the length of the analyte, one may calibrate the system with one or more standards, e.g., biopolymers of known, varying lengths, to determine the pulse duration and speed of each biopolymer length. Under consistent voltage and electrolyte conditions, this data may be used to create a standard curve for correlating the pulse duration of the analyte with its length.

20 [0077] Similarly, the duration of a change in the voltage signal may be used to determine the location of hybridization of a first plurality of probes and a distance between two probes on the biopolymer. The detected electrical signal corresponding to volume between sensing electrodes of the fluidic channel may be detected by using the sensing electrodes. As shown in Figure 9 at time point T₁, the electrical signal may initially change when the biopolymer moves

25 through a volume between sensing electrodes and further change, at T₂, when a portion of the biopolymer including a hybridized probe moves through the volume between sensing electrodes. The detected electrical signals may indicate the locations of the hybridized probes along the biopolymer. Changes and duration of changes in electrical signals may be analyzed to determine the location of probes with known sequence specificity along the length of the

30 biopolymer. Distances between probes may be based upon the duration between voltage spikes representing hybridized probes just as the duration of spike is used to determine biopolymer length. For example, one may consider the average of the voltage signals when determining

the distance between hybridized probes. Outliers may be excluded if they deviate greatly from other measurements taken of the same molecule. This analysis may be done either visually or with the assistance of a computer program that executes the analysis described herein.

[0078] The calculation of distances between probes may be used to determine the sequence 5 of a biopolymer as follows. An analyte may be prepared by hybridizing a first plurality of probes with a known sequence with the biopolymer such that the first plurality of probes attaches to portions of the biomolecule to produce a partially hybridized biomolecule. The analyte may be disposed in a fluidic channel. A potential may be applied along the fluidic channel to generate an electrophoretic force therein such that the analyte is translocated from a 10 one end of the fluidic channel to another end of the fluidic channel. Changes in the voltage are used to detect the hybridized probe as described above.

[0079] At least a portion of the sequence of the biopolymer may be determined by 15 detecting the hybridization of the first plurality of probes. Its location on the biopolymer may be determined by using a distance from the end of the biopolymer to a probe's site of hybridization or the distance from a probe site of hybridization to another probe site of hybridization. A computer algorithm may be used to process the electrical signals to help determine the sequence of the biopolymer.

[0080] In some embodiments, a second plurality of probes having specificity for 20 recognition sites on the target molecule may be hybridized with the biopolymer, either subsequently or in parallel to the first probe, to form individual pluralities of hybridization and the detecting, analyzing, and determining steps may be repeated with the subsequent plurality of probes.

[0081] The biopolymer may include a double-stranded biopolymer target molecule. The 25 analyte may be prepared by contacting the biopolymer, i.e., the target molecule, with a first probe having a first probe specificity for recognition sites of the target molecule to form a first plurality of local ternary complexes.

[0082] The electrical signals may be used to detect and record complexed and 30 uncomplexed regions of the biopolymer to create a first probe map of the first plurality of probes and subsequent probe maps for each subsequent plurality of probes, the first probe map and subsequent probe maps each including information about the relative positions of the hybridized first and each of the subsequent plurality of probes. Each probe map may include a series of numbers that indicate the distances between probes. The numbers may indicate

distance in terms of base pairs or distance in terms of nanometers. A candidate sequence for at least a portion of the biopolymer may be determined by ordering at least two probe sequences using positional information and/or a combination of overlapping probe binding sequences and positional information.

5 [0083] The first and second probe maps may include information about an error of the positional information for each probe. For example, each indicated distance may have an associated standard deviation, e.g., 100 nm \pm 10 nm. Further, a candidate sequence may be determined by ordering at least two probe sequences using at least one of (i) positional information and parameters relating to the error in positional information or (ii) a combination 10 of overlapping sequences of the probe molecules and positional information and error in positional information.

15 [0084] The sequencing of biopolymers by hybridization of probes to form ternary complexes is further discussed in patent application U.S. Serial No. 12/243,451 which is incorporated herein by reference in its entirety. Additional background information about detection/sequencing may be found in Gracheva, M.E.; Xiong, A.; Aksimentiev, A.; Schulten, K.; Timp, G, Leburton, J.-P. Simulation of the electric response of DNA translocation through a semiconductor nanopore-capacitor, *Nanotechnology* **2006**, *17*, 622-633; and Zwolak, M.; Di Ventra, M. Physical approaches to DNA sequencing and detection, *Rev. Mod. Phys.* **2008**, *80*, 141-165, each of which is incorporated herein by reference in its entirety.

20 *Example of Length Determination*

[0085] A sensing device composed of two microfluidic chambers, one or more fluidic channels connecting the two microfluidic chambers, and a pair of sensing electrodes disposed along the length of each fluidic channel, is filled with an ionic fluid. Typically, the fluid may be water that contains salt.

25 [0086] Multiple copies of a fragment of DNA of unknown length may be introduced into one of the microfluidic chambers that is connected to the fluidic channel that contains a pair of sensing electrodes. Macroscopic electrodes are used to electrophorese the DNA strands from the microfluidic chamber into one or more fluidic channels. As the DNA enters the fluidic channel, it assumes a linear conformation. The degree to which it is linearized depends on a 30 number of factors. Some of those factors are, e.g., the persistence length of the DNA strand, the temperature, the ionic conditions, and the width and depth of the fluidic channel.

[0087] The potential applied by the electromotive electrodes causes the DNA strand to move down the length of the fluidic channel. As the fragment moves down the fluidic channel it passes through the volume between sensing electrodes. When the leading edge of the DNA enters a volume between sensing electrodes, a change in some electrical characteristic such as 5 cross channel current or potential between two sensing electrodes may be recorded. The recorded signal is composed of a time stamp and an indication of change in potential or other electrical property. The value of the electrical property may also be recorded. The value may be subtracted from the background signal or may be an absolute value. A table may be generated by a computer that lists all responses occurring in the volume between sensing 10 electrodes and the time stamp for each response. A computer program may subsequently determine the duration of the signal. As the trailing edge of the DNA strand exits the volume between sensing electrodes, the electrical response typically returns to the value which was observed before the DNA entered the volume. The magnitude of the electrical response depends on the experimental set-up; preferably, the electrical response is equal to at least 3 15 times the magnitude of the root mean square noise for the system.

[0088] A calibrated standard curve may be applied to the measured length in order to calculate the true length of the analyte. For example, the device may be calibrated with a series of DNA fragments of known length that are electrophoresed through the fluidic channel under the same conditions, e.g., ionic strength, temperature, pH as the analyte. The fragments 20 preferably span enough different lengths to cover the range that may be used in the experiment to measure the length of the unknown fragment.

Example of DNA Sequencing

[0089] A target DNA strand of known or unknown sequence may be denatured. Denaturation of the duplex DNA is typically accomplished through the application of heat or 25 chemicals, such that the hydrogen bonds between paired strands are broken. The denatured DNA sample is incubated with a probe of known sequence and base length or divided for incubation with multiple probes, each with their own specific recognitions sequences on the target DNA. In order to hybridize the probe or probes to their recognition sequence or sequences, the conditions for the incubation are chosen such that the probe or probes bind to 30 the known specific recognition site in preference to other sites or mismatch sites. The conditions are also chosen so that more of the probe binding sites on the denatured DNA

strands are bound to a probe than unbound. The solution may be a buffered ionic solution. The solution may be agitated to facilitate binding of the probes. The temperature of the solution may be varied during the course of the incubation. For instance, the temperature of the incubation may be slowly cooled over the course of the hybridization.

5 [0090] Once the denatured target DNA has been hybridized with a probe or probes, the sample is introduced into a microfluidic chamber at one end of the fluidic channel device. The fluidic channel device is filled with an ionic solution, e.g., a salt solution. The solution may also be buffered. The excess probe or probes may be removed prior to the introduction of the sample into the microfluidic chamber. Gel filtration is one method for removing short probes
10 from a longer strand of DNA. Alternatively, other commercially available purification methods are available. Once the target DNA strand with hybridized probes has been introduced into a microfluidic chamber, a potential is applied via electromotive electrodes to drive the DNA from the microfluidic chamber into one or more fluidic channels.

15 [0091] The target DNA, upon entering the fluidic channel, typically assumes a linearized conformation. The narrower the fluidic channel, the more linearized the DNA is forced to become. The voltage applied to the macroscopic electromotive electrodes electrophoretically drives the DNA down the fluidic channel. As the DNA and hybridized probes move down the fluidic channel they enter the volume between sensing electrodes in the fluidic channel.

20 [0092] In the absence of DNA, the volume between sensing electrodes may contain only the ionic solution and have a baseline potential difference measured between the two sensing electrodes. As DNA enters the volume between sensing electrodes, the potential measured between the two sensing electrodes changes because the DNA has a conductivity different from that of the ionic solution. When DNA enters the volume between sensing electrodes, the conductivity of the channel between the two sensing electrodes is typically reduced with
25 respect to the conductivity when only ionic fluid is present between the sensing electrodes. When a portion of the DNA that also has a probe hybridized thereto enters the volume between sensing electrodes, the potential changes further.

30 [0093] As the molecule passes between sensing electrodes, the monitored voltage varies by a detectable and measurable amount. The electrodes detect and record this variation in voltage as a function of time. These variations in voltage are the result of the relative diameter of the molecule that is passing between sensing electrodes at any given time. For example, the portions of the biomolecule that have probes bound thereto are twice the diameter of the

portions of the biomolecule that have not been hybridized and therefore lack probes.

[0094] This relative increase in volume of the biomolecule passing between sensing electrodes causes a temporary increase in resistance between sensing electrodes resulting in a measurable voltage variation. As the portions of the biomolecule that include probes pass

5 between sensing electrodes, the current is further impeded, forming a relative spike in the recorded voltage during passage of the bound portion, which decreases again after the hybridized portion has passed. The sensing electrodes detect and reflect these variations in the monitored current. Further, the measurements of the voltage variations are measured and recorded as a function of time. As a result, the periodic interruptions or variations in resistance
10 indicate where, as a function of relative or absolute position, the known probe sequence has attached to the biomolecule.

[0095] When the DNA or a probe on the target DNA enters a fluidic channel, an electrical signal is recorded. The electrical signal is composed of a time stamp and the value of the changed electrical property. The electrical property value may be subtracted from the

15 background signal or may be an absolute value. A table may be generated by a computer that lists all responses occurring between sensing electrodes and the time stamp for each response. A computer program may subsequently determine the length of the biopolymer and the location of the hybridized probes on the biopolymer. The location of the probe on the biopolymer may be determined in terms of nanometers, base pairs or percentage of total biopolymer length.

20 **[0096]** The probe's location on the biopolymer can be determined according to its distance from the end of the biopolymer. This may be done through a determination of a total length of the biopolymer using a calibrated standard. The duration of the biopolymer signal may be compared to a calibrated standard curve in order to calculate the true length of the analyte. For example, the device may be calibrated with a series of DNA fragments of known length that are
25 electrophoresed through the fluidic channel under the same conditions as the analyte, e.g., ionic strength, temperature, pH. The fragments preferably span enough different lengths to calibrate the sensing electrodes to measure the length of the unknown fragment.

[0097] More of the sequence can be determined through subsequent or parallel hybridization with a second plurality of probes and the detecting, analyzing, and determining
30 steps may be repeated with the subsequent plurality of probes. The designs described herein merge nanopore and fluidic channel technologies and decouple the driving electrophoretic force from the detected signal. By using voltage sensing and by fabricating voltage amplifiers

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directly on the substrate where the sensing electrodes are placed, the device may operate at higher frequencies than has been possible with previous geometries.

[0098] The described embodiments of the invention are intended to be merely exemplary and numerous variations and modifications will be apparent to those skilled in the art. All such variations and modifications are intended to be within the scope of the present invention as defined in the appended claims.

[0099] What is claimed is:

Claims

- 1 1. A device for voltage sensing of analytes, the device comprising:
 - 2 a fluidic channel defined in a substrate;
 - 3 a pair of sensing electrodes disposed in the channel for sensing voltage therein, the pair
 - 4 of sensing electrodes comprising a first and a second sensing electrode disposed at two discrete
 - 5 locations along a length of the fluidic channel; and
 - 6 a pair of electromotive electrodes disposed at a first end and a second end of the fluidic
 - 7 channel for applying a potential along the fluidic channel,
 - 8 wherein the fluidic channel comprises a nanochannel or a microchannel.
- 1 2. The device of claim 1, wherein the substrate comprises a material selected from the
- 2 group consisting of silicon, silicon dioxide, fused silica, and gallium arsenide.
- 1 3. The device of claim 1, wherein each of the sensing and electromotive pairs of electrodes
- 2 comprises a material selected from the group consisting of platinum, gold, chrome, titanium,
- 3 silver chloride, silver, and graphene.
- 1 4. The device of claim 1, wherein the first sensing electrode is disposed on a first side of
- 2 the fluidic channel and the second sensing electrode is disposed on an opposing side of the
- 3 fluidic channel.
- 1 5. The device of claim 1, wherein each of the first and second sensing electrodes is
- 2 disposed on a first side of the fluidic channel.
- 1 6. The device of claim 1, wherein each of the first and second sensing electrodes
- 2 transverses the fluidic channel.
- 1 7. The device of claim 1, wherein the first sensing electrodes transverses the fluidic
- 2 channel and the second sensing electrode is disposed on a side of the fluidic channel.
- 1 8. The device of claim 1, wherein the electromotive pair of electrodes comprises
- 2 macroscopic electrodes for generating a constant, changing, or oscillating electrophoretic force
- 3 in the fluidic channel for translocation of an analyte disposed therein.
- 1 9. The device of claim 1, further comprising a measurement tool for measuring a voltage
- 2 sensed by the pair of sensing electrodes.
- 1 10. The device of claim 9 where the measurement tool comprises a voltmeter.

- 1 11. The device of claim 1, further comprising a plurality of fluidic channels.
- 1 12. The device of claim 1, further comprising a voltage amplifier disposed on the substrate.
- 1 13. The device of claim 1, wherein the fluidic channel has a width selected from a range of
2 1 nm to 5 μ m.
- 1 14. The device of claim 1, wherein the fluidic channel has a depth selected from a range of
2 1 nm to 5 μ m.
- 1 15. The device of claim 1, wherein the fluidic channel has a length selected from a range of 1 μ m
2 to 10 cm.
- 1 16. A method for detecting an analyte, the method comprising the steps of:
2 disposing the analyte in a fluidic channel;
3 applying a potential along the fluidic channel;
4 translocating the analyte from a first end of the fluidic channel to a second end of the
5 fluidic channel; and
6 measuring a voltage signal between a pair of sensing electrodes disposed in the fluidic
7 channel as the analyte moves past the pair of sensing electrodes, the pair of sensing electrodes
8 comprising a first and a second electrode disposed at two discrete locations along a length of
9 the fluidic channel,
10 wherein the fluidic channel comprises a nanochannel or a microchannel.
- 1 17. The method of claim 16, wherein applying a potential along the fluidic channel
2 comprises generating an electrophoretic force therein.
- 1 18. The method of claim 16, wherein translocating the analyte comprises using a pressure
2 differential.
- 1 19. The method of claim 16, wherein translocating the analyte comprises using a chemical
2 gradient.
- 1 20. The method of claim 16, wherein the analyte comprises a biopolymer selected from the
2 group consisting of deoxyribonucleic acids, ribonucleic acids, and polypeptides.
- 1 21. The method of claim 20, wherein the biopolymer comprises a single-stranded molecule.
- 1 22. The method of claim 16, wherein the analyte comprises a biopolymer having at least

2 one probe attached thereto.

1 23. The method of claim 22, wherein the voltage signal changes when the biopolymer
2 moves through a volume between the sensing electrodes and further changes when the portion
3 of the biopolymer containing the probe moves through the volume between the sensing
4 electrodes.

1 24. The method of claim 23, further comprising recording a time between voltage signal
2 changes.

1 25. The method of claim 24, wherein a duration of a change in the voltage signal indicates a
2 presence of a probe, further comprising using the voltage signal to determine a distance
3 between two probes on the biopolymer.

1 26. The method of claim 16, further comprising using a duration of a change in the voltage
2 signal to determine a length of the analyte.

1 27. A method for determining a sequence of a biopolymer, the method comprising:
2 preparing an analyte comprising the biopolymer;
3 disposing the analyte in a fluidic channel;
4 applying a potential along the fluidic channel;
5 translocating the analyte from a first end of the fluidic channel to a second end of the
6 fluidic channel; and

7 measuring a voltage signal between a pair of sensing electrodes disposed in the fluidic
8 channel as the analyte moves past the pair of sensing electrodes, the voltage signal
9 corresponding to locations along the biopolymer, the pair of sensing electrodes comprising a
10 first and a second electrode disposed at two discrete locations along a length of the fluidic
11 channel,

12 wherein the fluidic channel comprises a nanochannel or a microchannel.

1 28. The method of claim 27, wherein applying a potential along the fluidic channel
2 comprises generating an electrophoretic force therein.

1 29. The method of claim 27, wherein translocating the analyte comprises using a pressure
2 differential.

1 30. The method of claim 27, wherein translocating the analyte comprises using a chemical
2 gradient.

1 31. The method of claim 27, wherein preparing the analyte comprises hybridizing the
2 biopolymer with a probe.

1 32. The method of claim 31, wherein a change in the voltage signal corresponds to a
2 location along the hybridized biopolymer containing the probe.

1 33. The method of claim 27, further comprising processing the voltage signal using a
2 computer algorithm to reconstruct the sequence of the biopolymer.

1 34. The method of claim 27, wherein the biopolymer comprises a double-stranded
2 biopolymer target molecule.

1 35. The method of claim 34, wherein preparing the analyte comprises contacting the target
2 molecule with a first probe having a first probe specificity for recognition sites of the target
3 molecule to form a first plurality of local ternary complexes, the first probe having a first
4 predicted recognition site sequence.

1 36. The method of claim 35, further comprising using the voltage signal to determine
2 positional information of the first plurality of local ternary complexes.

1 37. The method of claim 36, wherein the positional information includes a parameter
2 corresponding to a spatial distance between two local ternary complexes.

1 38. The method of claim 36, wherein preparing the analyte further comprises contacting the
2 target molecule with a second probe having a second probe specificity for recognition sites of
3 the target molecule to form a second plurality of local ternary complexes, the second probe
4 having a second predicted recognition site sequence.

1 39. The method of claim 38, further comprising using the voltage signal to determine
2 positional information of the second plurality of local ternary complexes.

1 40. The method of claim 39, further comprising aligning positional information of at least
2 the first and second plurality of local ternary complexes to determine a DNA sequence of the
3 target.

1 41. The method of claim 27, wherein the biopolymer comprises a double-stranded nucleic
2 acid target molecule having a plurality of binding sites disposed along the sequence thereof.

1 42. The method of claim 41, wherein preparing the analyte comprises adding a plurality of
2 probe molecules having a first sequence specificity to the double-stranded nucleic acid target
3 molecule.

1 43. The method of claim 42, further comprising incubating the probe molecules having the
2 first sequence specificity and the target molecule so as to effectuate preferential binding of the
3 first probe molecules to both a first binding site and a second binding site of the target
4 molecule.

1 44. The method of claim 43, further comprising using the voltage signal to measure a
2 parameter related to a distance between the first binding site and the second binding site.

1 45. The method of claim 27, wherein preparing the analyte comprises contacting the
2 biopolymer with a first probe to create at least one probe-target complex at a recognition site of
3 the biopolymer for which the first probe has a known specificity, while leaving uncomplexed,
4 regions of the biopolymer for which the first probe is not specific.

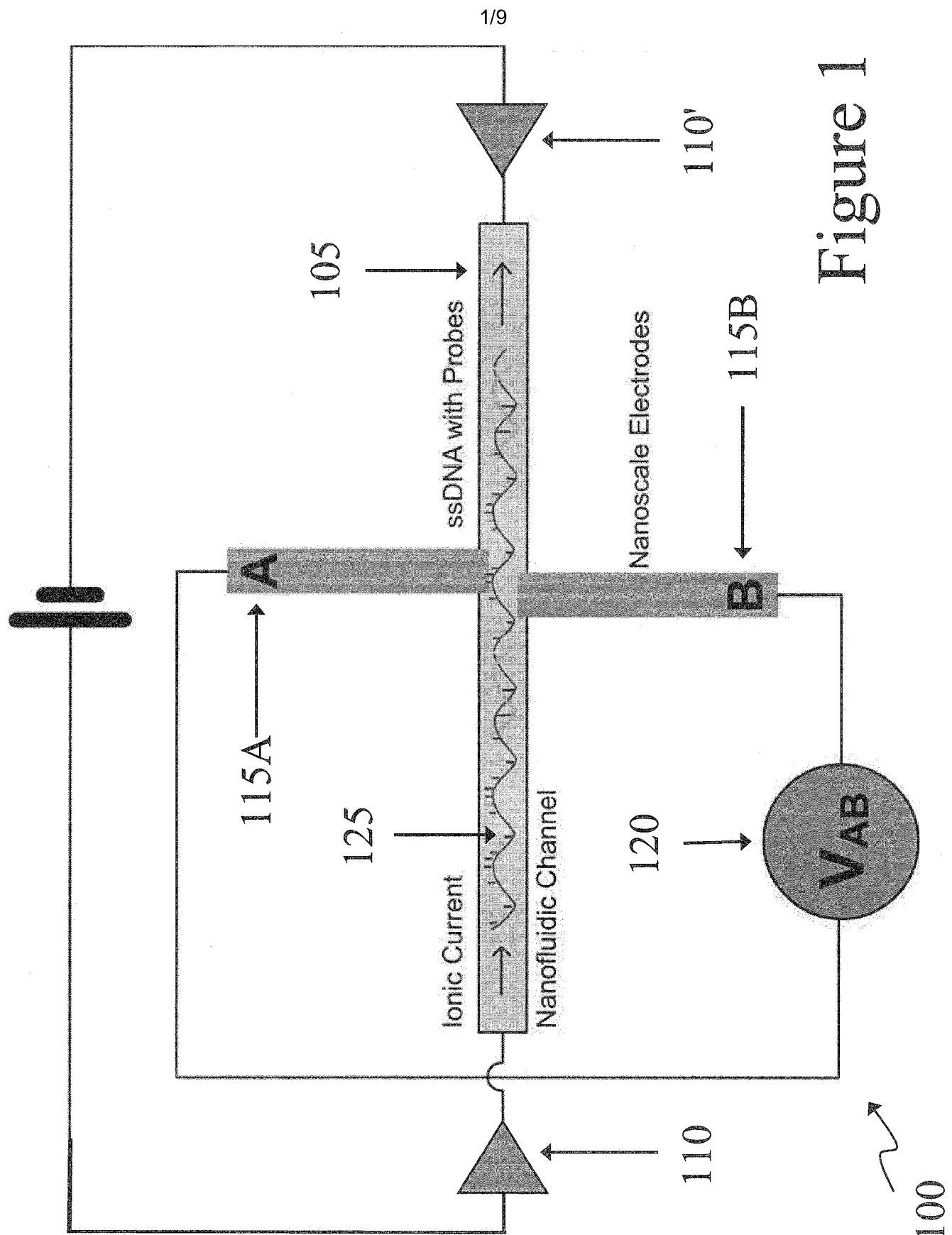
1 46. The method of claim 45, wherein preparing the analyte further comprises contacting the
2 biopolymer with a second probe to create at least one probe-target complex at a recognition site
3 of the biopolymer for which the second probe has a known specificity, while leaving
4 uncomplexed, regions of the target for which the second probe is not specific.

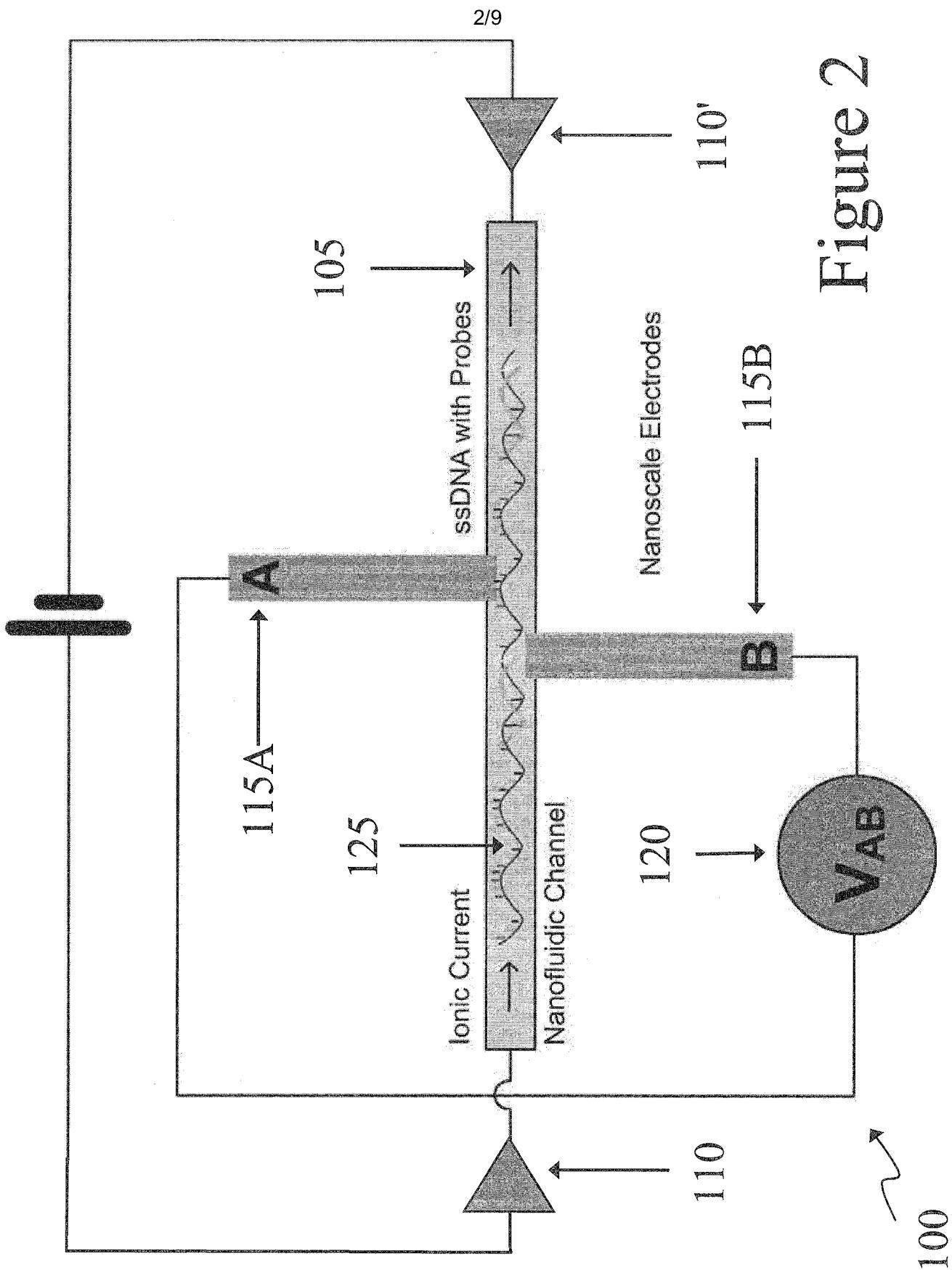
1 47. The method of claim 46, further comprising using the voltage signal to detect and
2 record complexed and uncomplexed regions of the biopolymer to create a first probe map of
3 the first probe and a second probe map of the second probe, the first probe map and the second
4 probe map incorporating information on the relative position of the hybridization of the probes.

1 48. The method of claim 47, further comprising determining a candidate sequence by
2 ordering at least two probe sequences using positional information or a combination of
3 overlapping sequences of the probe molecules and positional information.

1 49. The method of claim 46, wherein the first and second probe maps further incorporate
2 information on an error of the positional information for each probe.

1 50. The method of claim 49, further comprising determining a candidate sequence by
2 ordering at least two probe sequences using positional information and parameters relating to
3 the error in positional information or a combination of overlapping sequences of the probe
4 molecules and positional information and error in positional information.





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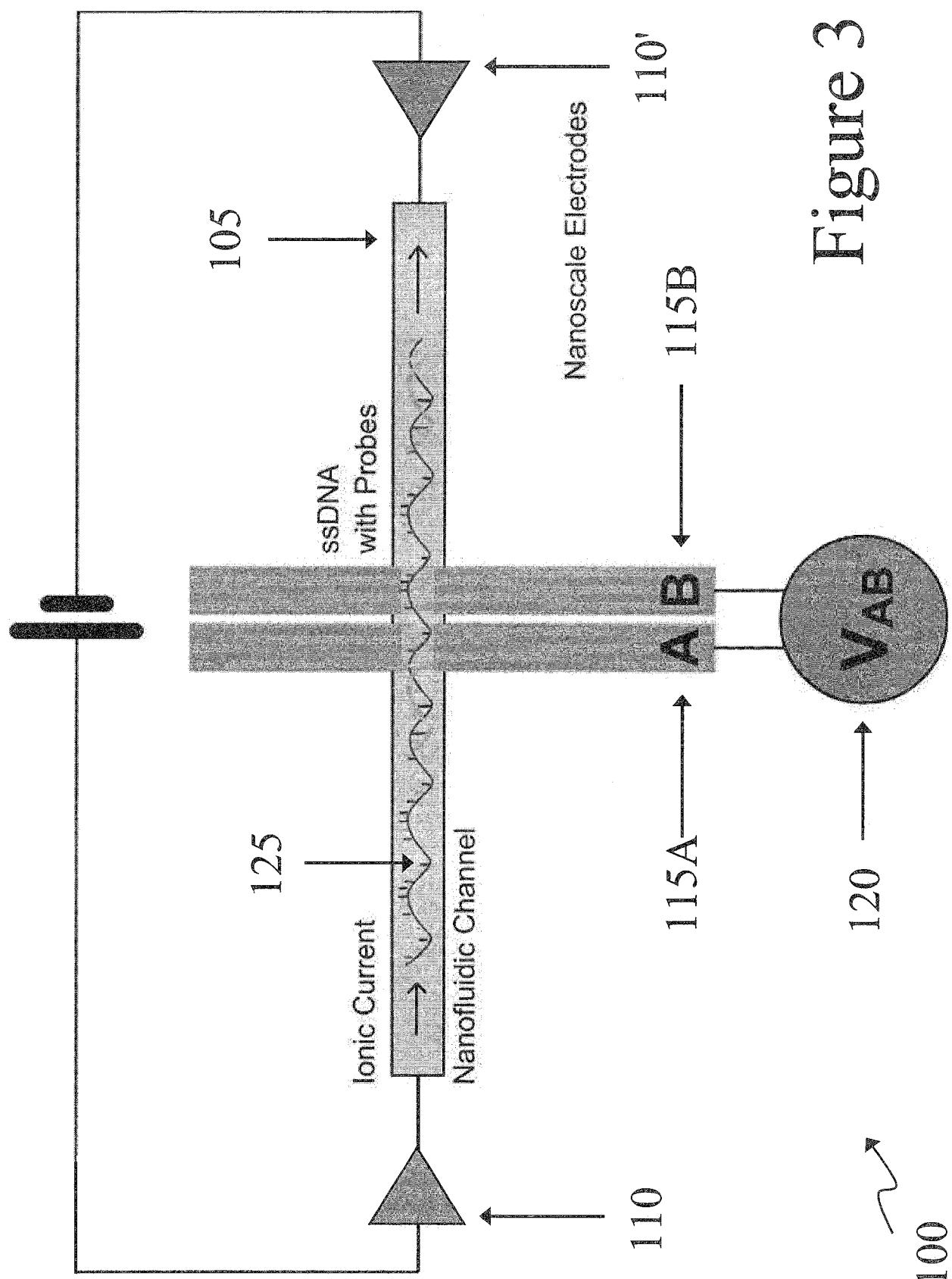


Figure 3

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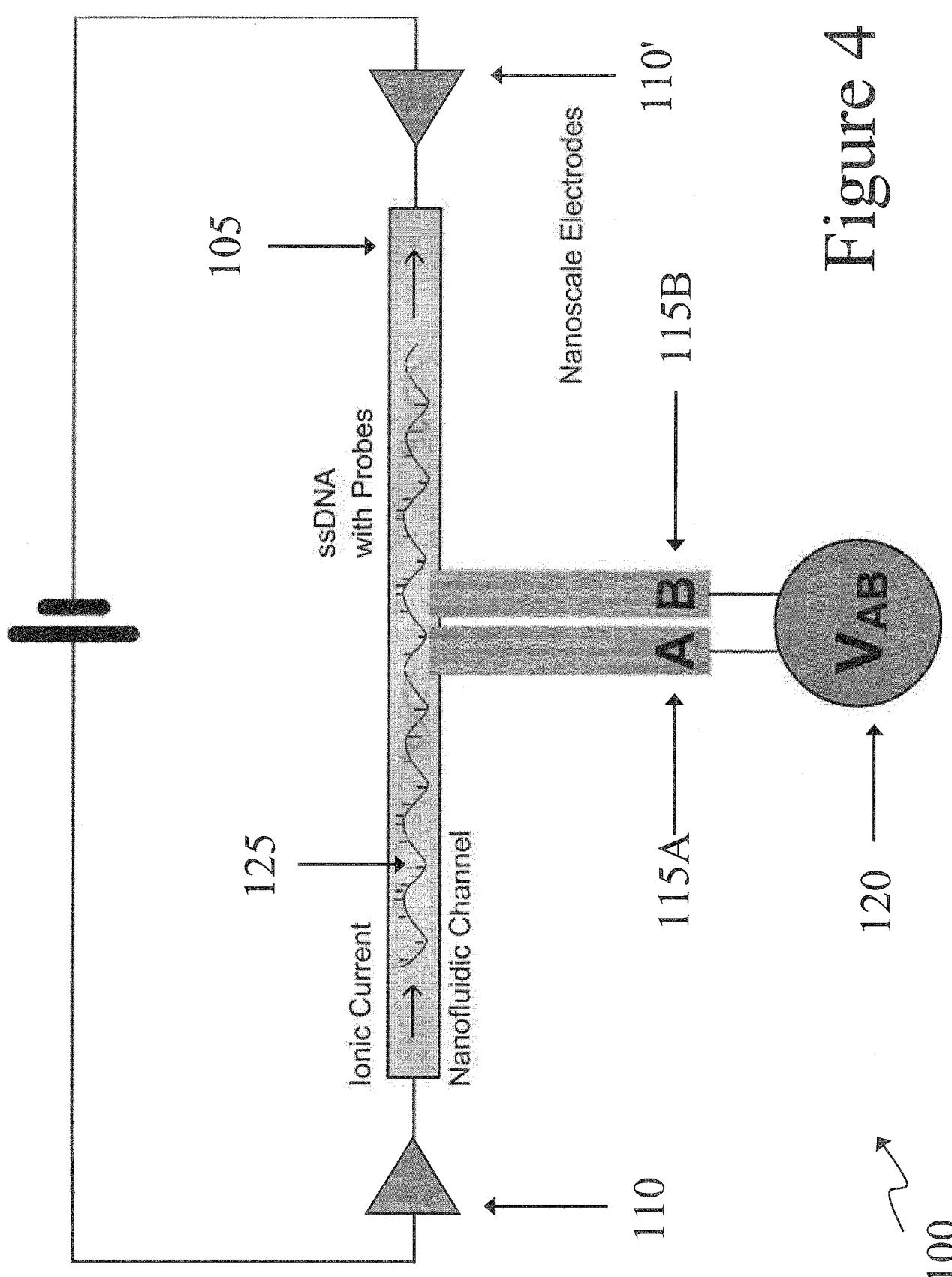


Figure 4

Figure 5

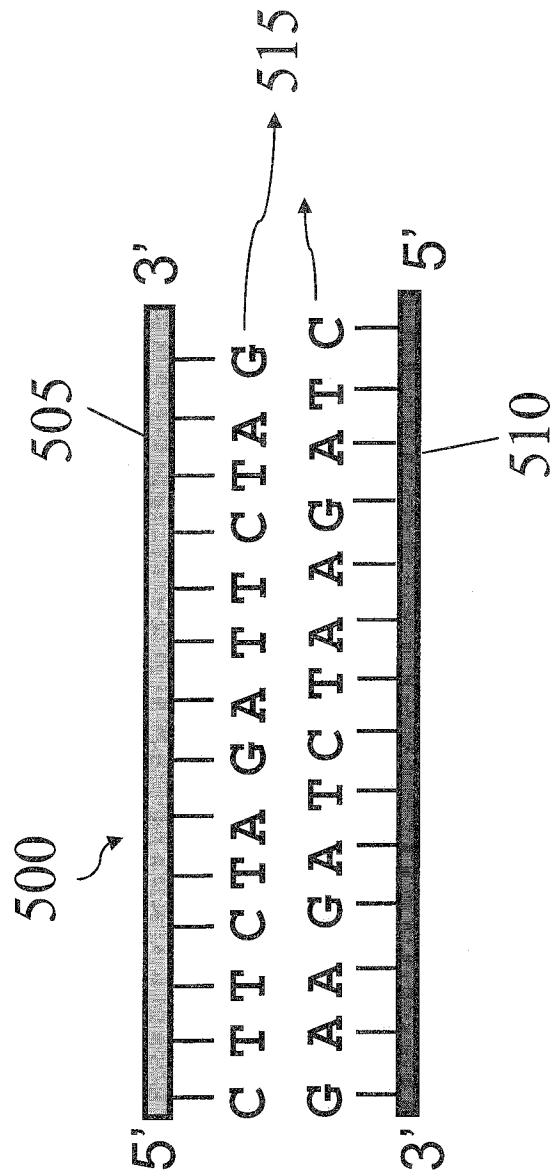


Figure 6

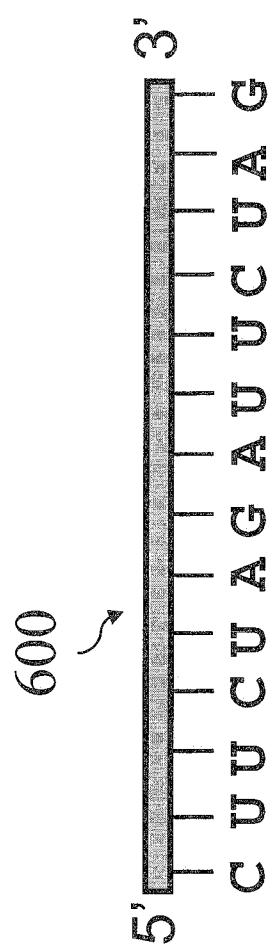


Figure 7

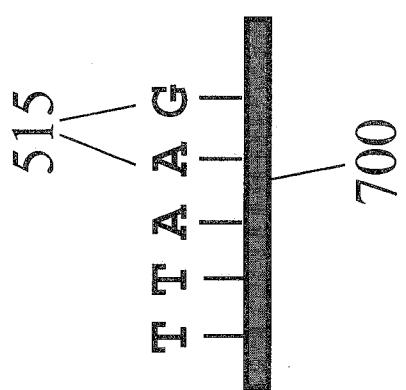


Figure 8

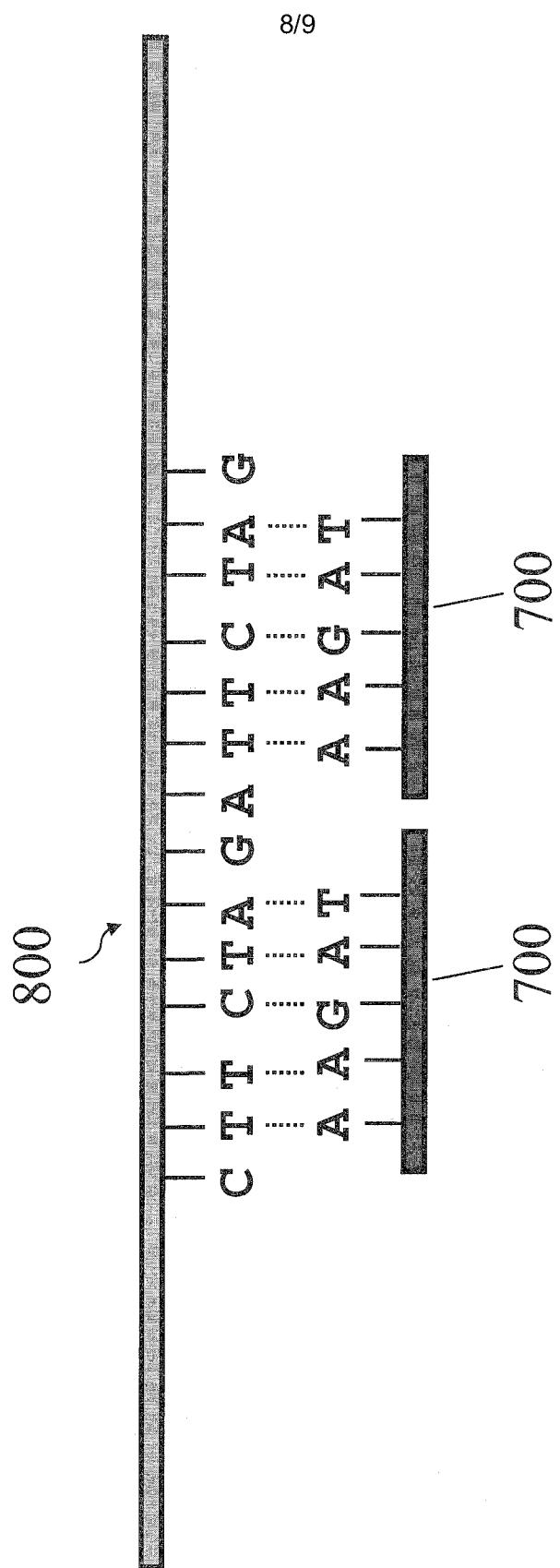


Figure 9

