



US006060023A

United States Patent [19]
Maracas

[11] **Patent Number:** **6,060,023**
[45] **Date of Patent:** **May 9, 2000**

- [54] **MOLECULAR SENSING APPARATUS**
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- [21] Appl. No.: **09/052,559**
- [22] Filed: **Mar. 31, 1998**
- [51] **Int. Cl.⁷** **G01N 15/06**; G01N 1/14; G01N 33/53; G01N 25/18; A61K 38/00
- [52] **U.S. Cl.** **422/68.1**; 422/50; 422/55; 422/56; 422/58; 422/63; 422/69; 422/76; 422/81.02; 422/81.03; 422/81.05; 422/81.07; 422/91.2; 435/4; 435/5; 435/6; 435/7.1; 435/7.2; 435/7.9; 435/90; 435/91.1; 435/91.3; 435/91.5; 435/91.51; 435/173.1; 436/149; 436/150; 436/806; 436/807; 530/300; 530/350; 530/333; 530/388.1
- [58] **Field of Search** 422/50, 55, 56, 422/68.1, 57, 58, 63, 69, 76, 81.02, 81.03, 81.05, 81.07, 91.2; 435/4, 5, 6, 7.1, 7.2, 7.9, 90, 91.1, 91.3, 91.5, 91.51, 173.1; 436/149, 150, 806, 807; 530/300, 350, 333, 388.1

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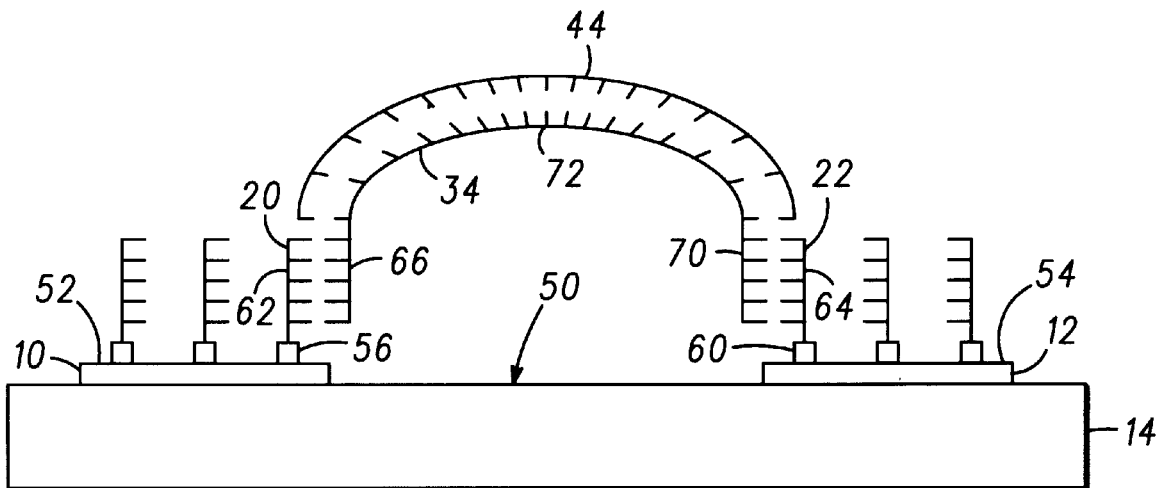
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Attorney, Agent, or Firm—James E. Gauger

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[57] **ABSTRACT**

A molecular sensing apparatus comprises a first electrode (10), a second electrode (12), a first molecule (20), a second molecule (22), and a third molecule (34). The first molecule (20) has a first chain of nucleic bases (30) and a first group (24). The first group (24) is bound to the first electrode (10). The second molecule (22) has a second chain of nucleic bases (32) and a second group (26). The second group (26) is bound to the second electrode (12). The third molecule (34) is bound to the first molecule (20) and the second molecule (22). A method which uses the molecular sensing apparatus is disclosed.

17 Claims, 4 Drawing Sheets



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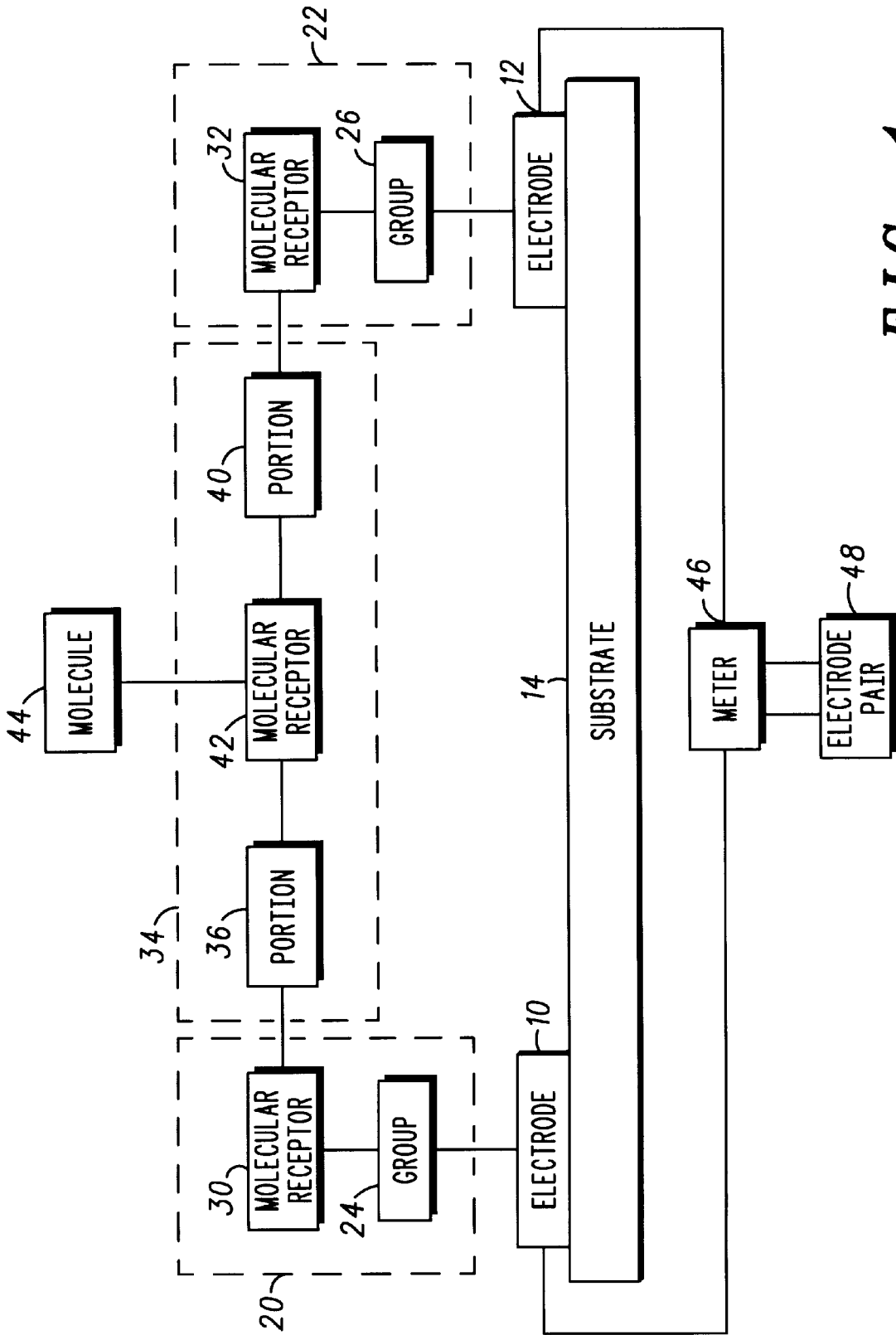


FIG. 1

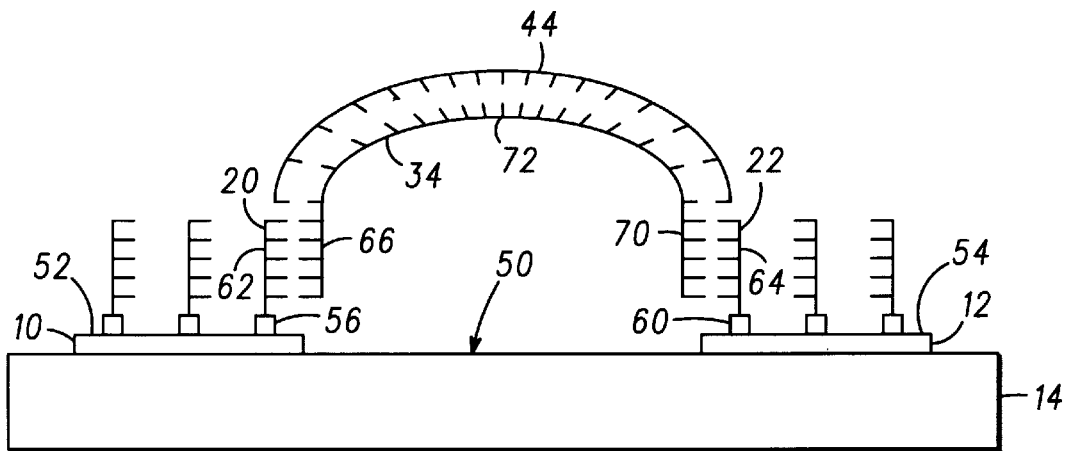


FIG. 2

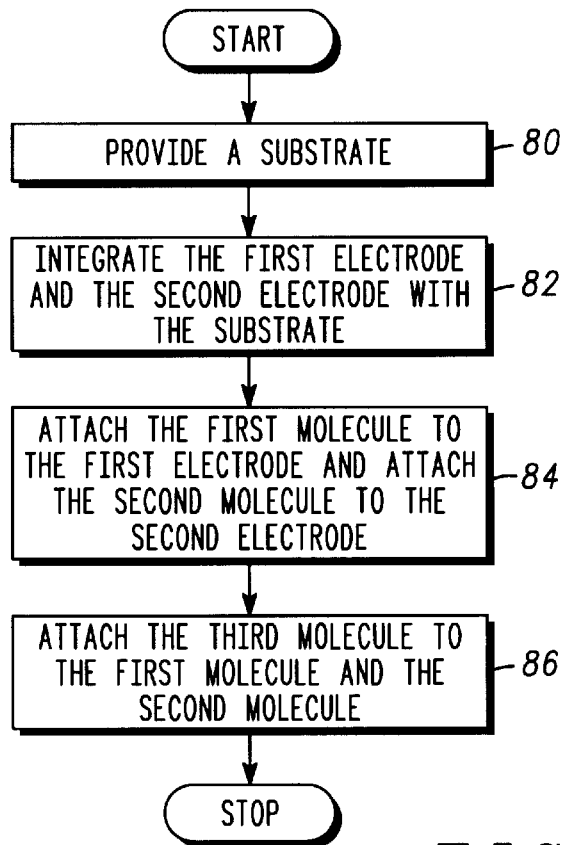


FIG. 3

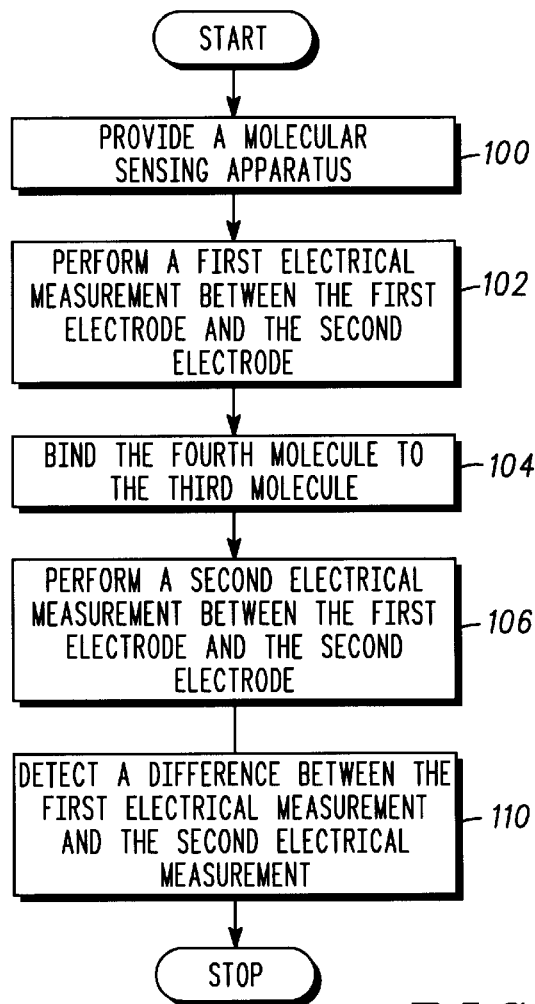


FIG. 4

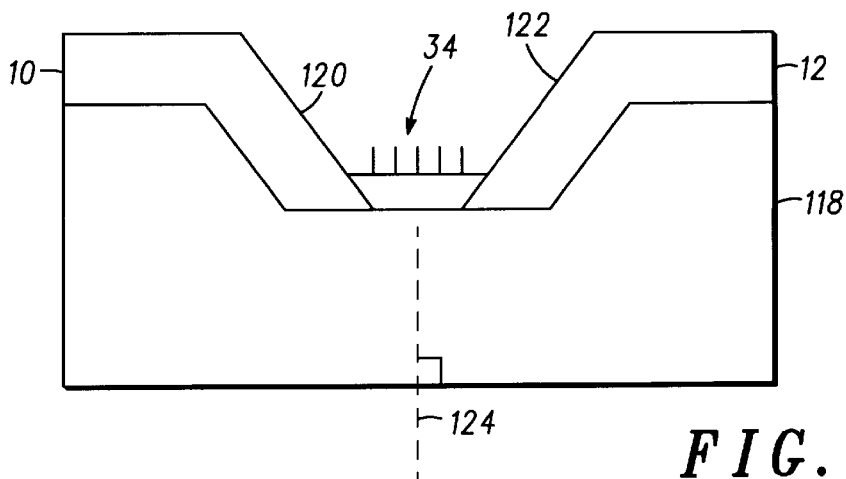


FIG. 5

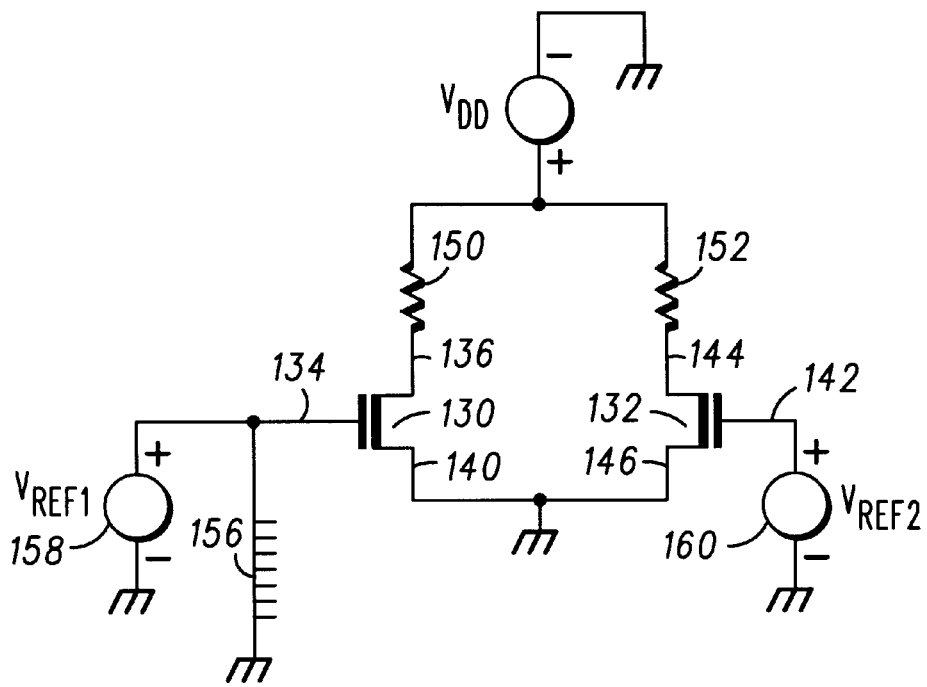


FIG. 6

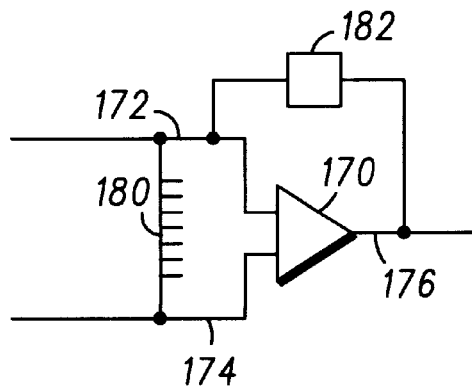


FIG. 7

MOLECULAR SENSING APPARATUS

TECHNICAL FIELD

The present invention relates to molecular sensing methods and systems.

BACKGROUND OF THE INVENTION

Recent efforts have been directed in developing chips for molecular detection. Of particular interest are DNA chips for sequencing and diagnostic applications. A DNA chip includes an array of chemically-sensitive binding sites having single-stranded DNA probes or like synthetic probes for recognizing respective DNA sequences. A sample of single-stranded DNA is applied to all of the binding sites of the DNA chip. The DNA sample attaches to DNA probes at one or more of the binding sites. The sites at which binding occurs are detected, and one or more molecular structures within the sample are subsequently deduced.

In sequencing applications, a sequence of nucleotide bases within the DNA sample can be determined by detecting which probes have the DNA sample bound thereto. In diagnostic applications, a genomic sample from an individual is screened with respect to a predetermined set of probes to determine if the individual has a disease or a genetic disposition to a disease.

Present molecular detection devices are better equipped to sense an aggregate sample of DNA/RNA rather than individual DNA/RNA strands. The ability to sense individual DNA/RNA strands (which may be either single-stranded or double-stranded) would advantageously reduce the DNA/RNA sample size that is applied to the device for detection purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is pointed out with particularity in the appended claims. However, other features of the invention will become more apparent and the invention will be best understood by referring to the following detailed description in conjunction with the accompanying drawings in which:

FIG. 1 is a block diagram of a molecular sensing apparatus in accordance with the present invention;

FIG. 2 is an illustration of a preferred embodiment of the molecular sensing apparatus of FIG. 1;

FIG. 3 is a flow chart summarizing steps performed in making a molecular sensing apparatus;

FIG. 4 is a block diagram of an embodiment of a molecular sensing method;

FIG. 5 is an illustration of an alternative embodiment of the molecular sensing apparatus of FIG. 1;

FIG. 6 is a schematic diagram of an embodiment of a circuit used in the meter for molecular sensing; and

FIG. 7 is schematic, block diagram of another embodiment of a circuit used in the meter for molecular sensing.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

FIG. 1 is a block diagram of a molecular sensing apparatus in accordance with the present invention. The molecular sensing apparatus includes a first electrode 10 and a second electrode 12 supported by a substrate 14. A first molecule 20 is bound to the first electrode 10. A second molecule 22 is bound to the second electrode 12.

The first molecule 20 includes an electrically-conductive group 24 which binds to a surface of the first electrode 10.

Preferably, the group 24 includes an end group comprising either sulfur, selenium, or tellurium, to bind to the first electrode 10, or a silane group for binding to oxide surfaces.

Similarly, the second molecule 22 includes an electrically-conductive group 26 which binds to a surface of the second electrode 12. Preferably, the group 26 includes an end group comprising either sulfur, selenium, or tellurium, to bind to the second electrode 12, or a silane group for binding to oxide surfaces.

The first molecule 20 further includes a molecular receptor 30 coupled to the group 24. The molecular receptor 30 is receptive to a molecule having a predetermined and/or a preselected molecular structure. The molecular receptor 30 can include either a biological molecule or a synthetic molecule having a specific affinity to its corresponding molecule.

Preferably, the molecular receptor 30 includes a first chain of nucleic bases to hybridize with a molecule having a complementary chain of nucleic bases. In this case, the molecular receptor 30 can include a single strand of DNA (deoxyribonucleic acid), a single strand of PNA (peptide nucleic acid), a single strand of RNA (ribonucleic acid), an oligonucleotide, or a polynucleotide. Preferably, the molecular receptor 30 includes a PNA receptor because of its higher binding stability per unit length in comparison to DNA.

The second molecule 22 further includes a molecular receptor 32 coupled to the group 26. As with the molecular receptor 30, the molecular receptor 32 is receptive to a molecule having a predetermined and/or a preselected molecular structure, and can include either a biological molecule or a synthetic molecule having a specific affinity to its corresponding molecule.

Preferably, the molecular receptor 32 includes a second chain of nucleic bases to hybridize with a molecule having a complementary chain of nucleic bases. In this case, the molecular receptor 32 can include a single strand of DNA, a single strand of PNA, a single strand of RNA, an oligonucleotide, or a polynucleotide. Preferably, the molecular receptor 32 includes a PNA receptor because of its higher binding stability per unit, length in comparison to DNA.

The first molecule 20 and the second molecule 22 can be equivalent. Here, the group 24 is the same as the group 26, and the molecular receptor 30 is the same as the molecular receptor 32. In this case, it is preferred that the molecular receptor 30 has a sequence of the nucleic bases equivalent to a sequence of the nucleic bases for the molecular receptor 32.

Alternatively, the first molecule 20 and the second molecule 22 can differ. In this case, it is preferred that the molecular receptor 30 differs from the molecular receptor 32 while the group 24 is the same as the group 26. Preferably, the molecular receptor 30 has a sequence of nucleic bases that differs from a sequence of nucleic bases for the molecular receptor 32.

A third molecule 34 is bound to the first molecule 20 and the second molecule 22. To bind to the first molecule 20, the third molecule 34 includes a first portion having an affinity to the molecular receptor 30. Preferably, the third molecule 34 includes a chain of nucleic bases 36 complementary to the first chain of nucleic bases in the molecular receptor 30. Similarly, to bind to the second molecule 22, the third molecule 34 includes a second portion having an affinity to the molecular receptor 32. Preferably, the third molecule 34 includes a chain of nucleic bases 40 complementary to the second chain of nucleic bases in the molecular receptor 32.

The third molecule 34 further includes a molecular receptor 42 interposed between the chain of nucleic bases 36 and

the chain of nucleic bases **40**. In general, the molecular receptor **42** is receptive to a molecule having a predetermined and/or a preselected molecular structure, and can include either a biological molecule or a synthetic molecule having a specific affinity to its corresponding molecule.

Preferably, the molecular receptor **42** includes a third chain of nucleic bases to hybridize with a molecule having a complementary chain of nucleic bases. In this case, the molecular receptor **42** can include a single strand of DNA, a single strand of RNA, an oligonucleotide, or a polynucleotide.

The apparatus can be used to electrically sense for a binding event between a fourth molecule **44** and the molecular receptor **42** of the third molecule **34**. Of particular interest are cases in which the fourth molecule **44** includes a single strand of DNA, a single strand of PNA, a single strand of RNA, an oligonucleotide, or a polynucleotide. For a specific binding event, the fourth molecule **44** has a chain of nucleic bases complementary to the third chain of nucleic bases.

A meter **46** is electrically connected to the first electrode **10** and the second electrode **12**. The meter **46** is used to electrically detect a binding event between the fourth molecule **44** and the molecular receptor **42** of the third molecule **34**. Preferably, the meter **46** includes an impedance meter, such as a resistance meter, a conductance meter, a capacitance meter or an inductance meter, to measure and/or detect a change in impedance between the first electrode **10** and the second electrode **12** resulting from the binding event.

Generally, the meter **46** can detect a change in an electrical quantity (measured between the first electrode **10** and the second electrode **12**) between two instances of time (e.g. a pre-binding time and a post-binding time). Examples of the electrical quantity include, but are not limited to, charge, current, voltage, and impedance. The electrical quantity can be an AC (alternating current) quantity, a DC (direct current) quantity, or a combination of AC and DC.

Alternatively, the meter **46** can detect a change in an electrical quantity between a first electrode pair (comprising the first electrode **10** and the second electrode **12**) and a second electrode pair **48**. The second electrode pair **48** can have molecules equivalent to the first electrode **20**, the second molecule **22**, and the third electrode **34**. However, a molecule such as the fourth molecule **44** is not exposed to these like molecules.

The meter **46** can be either integrated entirely to the substrate **14** (e.g. in a form an on-chip circuit), or entirely external to the substrate **14** (in a form of an off-chip circuit), or partially integrated to the substrate **14** and partially external to the substrate (e.g. using both on-chip and off-chip circuits).

In some cases, it may be preferred that the binding energy of the third molecule **34** to each of the first molecule **20** and the second molecule **22** be greater than the binding energy of the fourth molecule **44** to the third molecule **34**. For this purpose or to satisfy other stability requirements, the length (based upon a number of bases) of each of the molecular receptors **30** and **32**, and the portions **36** and **40** may be selected to be greater than the length of the molecular receptor **42** and optionally the length of the molecule **44**. The use of PNA/PNA binding between the third molecule **34** and each of the first molecule **20** and the second molecule **22**, and PNA/DNA or PNA/RNA binding between the third molecule **34** and the fourth molecule **44** also serve the aforementioned purpose. It is also preferred in some cases that the binding energy between the first molecule **20** and the

electrode **10** and between the second molecule **22** and the electrode **12** be greater than the binding energy of the fourth molecule **44** to the third molecule **34**. Satisfying all of these binding energy conditions is beneficial in cases where dehybridization of the fourth molecule **44** from the third molecule **34** is to be performed without detaching elements of the molecular sensor.

FIG. 2 is an illustration of a preferred embodiment of the molecular sensing apparatus of FIG. 1. The first electrode **10** and the second electrode **12** are integrated at a face **50** of the substrate **14**. Preferably, the substrate **14** for thiol bind chemistries is metalized with a layer of gold to form the first electrode **10** and the second electrode **12**. A standard mask such as an FET (field-effect transistor) mask can be used to fabricate the first electrode **10** and the second electrode **12** in this manner. Electrode contact patterns can be formed by several techniques, including but not limited to photolithography, electron beam lithography, scanning tunneling microscopy, and elastomeric contact printing.

A plurality of like molecules including the first molecule **20** are attached to the first electrode **10**. Similarly, a plurality of like molecules including the second molecule **22** are attached to the second electrode **12**.

The first electrode **10** has a surface **52** at which the first molecule **20** is bound. The second electrode **10** has a surface **54** at which the second molecule **22** is bound. Preferably, the surface **52** and the surface **54** are generally coplanar. Generally, the surface **52** and the surface **54** are closer to parallel than perpendicular, and have portions offset the same distance from the substrate **14**. It is noted that the surface **52** and the surface **54** need not be generally coplanar. A non-coplanar alternative is subsequently described with reference to FIG. 5.

The first molecule **20** has an end group **56** of either sulfur, selenium, or tellurium to form a conjugate with the surface **52** of the first electrode **10**. Similarly, the second molecule **22** has an end group **60** of either sulfur, selenium, or tellurium to form a conjugate with the surface **54** of the second electrode **12**.

The first molecule **20** has an oligonucleotide **62** coupled to the end group **56**. Similarly, the second molecule **22** has an oligonucleotide **64** coupled to the end group **60**. Preferably, each of the oligonucleotides **62** and **64** has a length of about 8 to 10 bases, but can be longer depending on the desired temperature stability requirements.

The third molecule **34** has a first end portion **66** complementary to the oligonucleotide **62** and a second end portion **70** complementary to the oligonucleotide **64**. Interposed between the first end portion **66** and the second end portion **70** is a polynucleotide **72**. The polynucleotide **72** has a base sequence complementary to a sequence to be detected in the fourth molecule **44**.

It is noted that the use of the first molecule **20** and the second molecule **22** is not necessary in alternative embodiments. In this case, the third molecule **34** is absent of the portions **36** and **40**, but include end groups such as the end groups **24** and **26** to bind directly to the electrodes **10** and **12**, respectively.

The molecules **20**, **22**, and **34** may be connected across various terminals of an active device such as either a field effect transistor, a bipolar junction transistor, or a single electron device to detect a binding event with the fourth molecule **44**. For example, the electrodes **10** and **12** can be electrically connected to a drain and a source, respectively, or to a gate and a drain, respectively, of a field effect transistor. Alternatively, the electrodes **10** and **12** can be

electrically connected to a base and an emitter, respectively, or to an emitter and a collector, respectively of a bipolar junction transistor. The selection of the active device is dependent upon the desired detection sensitivity and the external detection circuitry.

One or more of the molecules **20**, **22**, and **34** can be integrated directly onto terminals of the aforementioned transistors. The use of end groups **24** and **26** that bind to oxides and other insulators facilitate integration with MOS (metal oxide semiconductor) devices. A front-end comprised of the molecules and the transistor can be integrated with either an on-chip circuit or to off-chip circuits included in the meter **46**. On-chip integration of one or more of the molecules **20**, **22**, and **34** with active transistors may enable low-noise, high-sensitivity detection of molecular events.

FIG. **3** is a flow chart summarizing steps performed in making a molecular sensing apparatus. The steps are described for the elements described with reference to FIG. **1** and FIG. **2**.

As indicated by block **80**, a step of providing the substrate **14** is performed. Thereafter, a step of integrating the first electrode **10** and the second electrode **12** with the substrate **14** is performed as indicated by block **82**.

As indicated by block **84**, steps of attaching the first molecule **20** to the first electrode **10** and attaching the second molecule **22** to the second electrode **12** are performed. Either the first molecule **20** is attached first, the second molecule **22** is attached first, or the first molecule **20** and the second molecule **22** are attached substantially simultaneously. The first molecule **20** and the second molecule **22** can be included in a common solution applied to the electrodes **10** and **12**. Alternatively, the first molecule **20** and the second molecule **22** can be included in separate solutions.

As indicated by block **86**, a step of attaching the third molecule **34** to the first molecule **20** and the second molecule **22** is performed. Preferably, the step of attaching the third molecule **34** is performed after the first molecule **20** is attached to the first electrode **10** and after the second molecule **22** is attached to the second electrode **12**.

FIG. **4** is a block diagram of an embodiment of a molecular sensing method. As indicated by block **100**, the method includes a step of providing a molecular sensing apparatus. Preferably, the molecular sensing apparatus is in accordance with embodiments described with reference to FIG. **1**, FIG. **2**, and FIG. **5**.

As indicated by block **102**, the method includes a step of performing a first electrical measurement between the first electrode **10** and the second electrode **12**. The first electrical measurement is performed using the meter **46**. Preferably, this step includes measuring a first conductance between the first electrode **10** and the second electrode **12**.

As indicated by block **104**, a step of binding the fourth molecule **44** to the third molecule **34** is performed. This step can include applying a solution including the fourth molecule **44** to the apparatus. Preferably, the step of binding the fourth molecule **44** to the third molecule **34** is performed after the step of performing the first electrical measurement.

As indicated by block **106**, the method includes a step of performing a second electrical measurement between the first electrode **10** and the second electrode **12**. The second electrical measurement is performed using the meter **46**. Preferably, this step includes measuring a second conductance between the first electrode **10** and the second electrode **12**. It is also preferred that the second electrical measurement be performed after the fourth molecule **44** is bound to the third molecule **34**.

As indicated by block **110**, a step of detecting a difference between the first electrical measurement and the second electrical measurement is performed. Preferably, this step can include detecting a difference in conductance that exceeds a predetermined threshold. The difference is indicative of a binding event between the fourth molecule **44** and the third molecule **34**.

FIG. **5** is an illustration of an alternative embodiment of the molecular sensing apparatus of FIG. **1**. In this embodiment, the first electrode **10** and the second electrode **12** have a V-shaped configuration. The V-shaped configuration is fabricated by reactive ion etching, selective crystallographic plane etching, or selective patterned epitaxial growth to a substrate **118** such as Si or GaAs. Preferably, the first electrode **10** and the second electrode **12** are formed of gold or are comprised of a semiconducting material such as indium arsenide.

The first electrode **10** has a surface **120** to which a first end of the third molecule **34** is attached. The first end of the third molecule **34** have an end group which is directly attached to the surface **120**. Alternatively, the third molecule **34** is bound to the first molecule **20** which is directly attached to the surface **120** as described earlier. The second electrode **12** has a surface **122** to which a second end of the third molecule **34** is attached. The second end of the third molecule **34** have an end group which is directly attached to the surface **122**. Alternatively, the third molecule **34** is bound to the second molecule **22** which is directly attached to the surface **122** as described earlier.

Both the surface **120** and the surface **122** are transverse to a generally planar orientation of the substrate **118**. The planar orientation of the substrate **118** is represented a normal axis **124**.

The embodiment described with reference to FIG. **5** can be used in performing the molecular sensing method of FIG. **4**.

FIG. **6** is schematic diagram of an embodiment of a circuit used in the meter **46** for molecular sensing. The circuit comprises a first transistor **130** and a second transistor **132**. The first transistor **130** includes a gate **134**, a drain **136**, and a source **140**. The second transistor includes a gate **142**, a drain **144**, and a source **146**. A first resistor **150** is coupled between the drain **136** and a voltage supply VDD. A second resistor **152** is coupled between the drain **144** and the voltage supply VDD. The source **140** and the source **146** are coupled to a voltage level such as ground **154**.

The gate **134** is coupled to the first electrode **10** and ground **154** is coupled to the second electrode **12**. Hence, a combination of one or more molecules **156** as described earlier is connected between the gate **134** and ground **154**.

A first reference voltage **158** is applied to the gate **134**. A second reference voltage **160** is applied to the gate **142**. An electrical measurement to sense for a binding event can be performed by sensing a voltage at the drain **136**, a voltage at the drain **144**, or a voltage difference between the drain **136** and the drain **144**.

FIG. **7** is a schematic, block diagram of another embodiment of a circuit used in the meter **46** for molecular sensing. The circuit includes a differential amplifier such as an operational amplifier **170** having a first input **172**, a second input **174**, and an output **176**. A combination of one or more molecules **180** as described earlier can be connected between the first input **172** and the second input **174**. Optionally, an impedance element **182** such as a resistor is connected between the first input **172** and the output **176**. A signal indicative of a binding event is produced at the output

176 in response to an input signal (e.g. a voltage or a current) applied to at least one of the first input 172 and the second input 174.

As an alternative to the embodiment of FIG. 7, the combination of one or more molecules 180 can be electrically connected in a feedback loop between the first input 172 and the output 176, or can be electrically connected between a signal source and one of the first input 172 and the second input 174.

Thus, there has been described herein several embodiments including preferred embodiments of a molecular sensing apparatus and method.

Because the various embodiments of the present invention use molecules which are both bound to and electrically coupled to sensing electrodes, they provide a significant improvement in that a direct conductance measurement can be made across the sensing electrodes to sense for single-stranded DNA/RNA and double-stranded DNA/RNA.

It will be apparent to those skilled in the art that, the disclosed invention may be modified in numerous ways and may assume many embodiments other than the preferred form specifically set out and described above.

Accordingly, it is intended by the appended claims to cover all modifications of the invention which fall within the true spirit and scope of the invention.

What is claimed is:

1. A molecular sensing apparatus comprising:
 - a first electrode;
 - a second electrode;
 - a first molecule having a first chain of nucleic bases and a first group, the first group bound to the first electrode;
 - a second molecule having a second chain of nucleic bases and a second group, the second group bound to the second electrode; and
 - a third molecule bound to the first molecule and the second molecule.
2. The molecular sensing apparatus of claim 1 wherein the first group and the second group are electrically conductive.
3. The molecular sensing apparatus of claim 1 wherein at least one of the first group and the second group comprises sulfur.
4. The molecular sensing apparatus of claim 1 wherein the third molecule comprises a first complementary chain of nucleic bases bound to the first chain of nucleic bases and a second complementary chain of nucleic bases bound to the second chain of nucleic bases.
5. The molecular sensing apparatus of claim 4 wherein the third molecule comprises a third chain of nucleic bases disposed between the first complementary chain of nucleic bases and the second complementary chain of nucleic bases.
6. The molecular sensing apparatus of claim 5 further comprising a fourth molecule bound to the third molecule, the fourth molecule having a third complementary chain of nucleic bases bound to the third chain of nucleic bases of the third molecule.

7. The molecular sensing apparatus of claim 1 wherein the first electrode and the second electrode are formed of a metal.

8. The molecular sensing apparatus of claim 7 wherein the first electrode and the second electrode are formed of gold.

9. The molecular sensing apparatus of claim 1 further comprising a substrate to support the first electrode and the second electrode, wherein the first electrode and the second electrode are integrated with the substrate.

10. The molecular sensing apparatus of claim 1 wherein the first electrode comprises a first surface to which the first molecule is bound, wherein the second electrode comprises a second surface to which the second molecule is bound, and wherein the first surface and the second surface are coplanar.

11. The molecular sensing apparatus of claim 1 further comprising a meter electrically coupled to the first electrode and the second electrode.

12. The molecular sensing apparatus of claim 11 wherein the first electrode and the second electrode provide a first electrode pair, the molecular sensing apparatus further comprising a second electrode pair similar to the first electrode pair, wherein the meter is electrically coupled to the second electrode pair.

13. The molecular sensing apparatus of claim 1 wherein at least one of the first electrode and the second electrode comprises a semiconducting material.

14. The molecular sensing apparatus of claim 1 wherein at least one of the first group and the second group is a silane group.

15. A molecular sensing apparatus comprising:

- a first electrode having a first surface;
- second electrode having a second surface coplanar to the first surface;
- a first molecule having a first chain of nucleic bases and a first end group, the first end group bound to the first surface of the first electrode;
- a second molecule having a second chain of nucleic bases and a second end group, the second end group bound to the second surface of the second electrode; and
- a third molecule having a first complementary chain of nucleic bases bound to the first chain of nucleic bases of the first molecule, a second complementary chain of nucleic bases bound to the second chain of nucleic bases of the second molecule, and a third chain of nucleic bases disposed between the first complementary chain of nucleic bases and the second complementary chain of nucleic bases.

16. The molecular sensing apparatus of claim 1 wherein at least one of the first group and the second group comprises selenium.

17. The molecular sensing apparatus of claim 1 wherein at least one of the first group and the second group comprises tellurium.