

(19) Weltorganisation für geistiges Eigentum
Internationales Büro(43) Internationales Veröffentlichungsdatum
3. Mai 2001 (03.05.2001)

PCT

(10) Internationale Veröffentlichungsnummer
WO 01/31057 A3

(51) Internationale Patentklassifikation: C12Q 1/68

[DE/DE]; Magnolienstrasse 3, 65929 Frankfurt am Main
(DE). WINDHAB, Norbert [DE/DE]; Akazienstrasse 28,
65795 Hattersheim (DE).

(21) Internationales Aktenzeichen: PCT/EP00/10209

(22) Internationales Anmeldedatum:
17. Oktober 2000 (17.10.2000)(74) Anwalt: ACKERMANN, Joachim; Postfach 11 33 26,
60048 Frankfurt am Main (DE).

(25) Einreichungssprache: Deutsch

(81) Bestimmungsstaaten (national): AU, BR, CA, JP, KR,
US.

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
199 50 969.7 22. Oktober 1999 (22.10.1999) DE(84) Bestimmungsstaaten (regional): europäisches Patent (AT,
BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
NL, PT, SE).

NANOGEN RECOGNOMICS GMBH

(71) Anmelder (für alle Bestimmungsstaaten mit Ausnahme
von US): AVENTIS RESEARCH & TECHNOLOGIES
GMBH & CO KG [DE/DE]; 65926 Frankfurt am MainVeröffentlicht:
mit internationalem Recherchenbericht

DE: Industriepark Höchst

65926 Frankfurt am Main

(72) Erfinder; und Germany

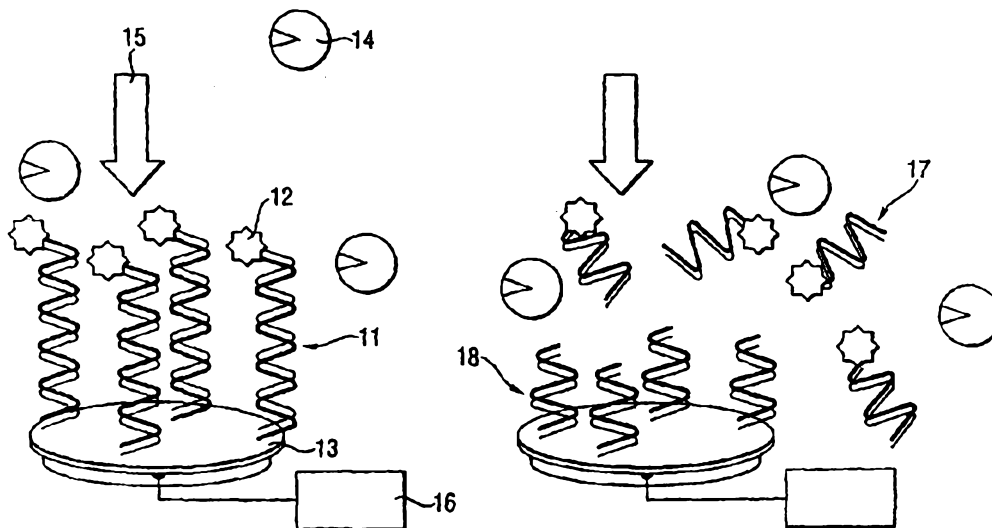
(75) Erfinder/Anmelder (nur für US): MUTH, Jochen

(88) Veröffentlichungsdatum des internationalen
Recherchenberichts: 27. Dezember 2001

[Fortsetzung auf der nächsten Seite]

(54) Title: DOUBLE-STRAND NUCLEIC ACID PROBES AND THE USE THEREOF

(54) Bezeichnung: DOPPELSTRANG-NUKLEINSÄURE-SONDEN UND DEREN VERWENDUNG



(57) Abstract: The invention relates to electronically-isolable double-strand nucleic acid probes and use thereof for rapid and easy detection of interactions between double-stranded nucleic acids and factors which interact with them either by mediated or direct means. The invention further relates to the production of said double-strand nucleic acids.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft elektronisch auslesbare Doppelstrang-Nukleinsäure-Sonden und deren Verwendung zum schnellen und einfachen Nachweis von Wechselwirkungen zwischen doppelsträngigen Nukleinsäuren und mit ihnen unmittelbar oder mittelbar interagierenden Faktoren, sowie Verfahren zur Herstellung der Doppelstrang-Nukleinsäure-Sonden.



Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

Double-stranded nucleic acid probes and their use

Description

- 5 The present invention relates to electronically readable double-stranded nucleic acid derivatives and to their use for rapidly and simply detecting interactions between double-stranded nucleic acids and factors which interact directly or indirectly with them, in particular with proteins, peptides, single-stranded or double-stranded nucleic acids, or nucleic acid-damaging
10 substances.

Double-stranded nucleic acids play a crucial role, in particular as carriers of the hereditary factors, in the living cell and also in many viruses. In this connection, they are subject, in natural systems, such as a cell, to a great
15 variety of internal and external influences. In particular interaction between proteins and double-stranded DNA are of great interest since such interactions have a crucial influence on the transcription or repression of individual genes and consequently on the phenotype of the corresponding organisms. However, the replication of the hereditary factors in mitosis or
20 meiosis, the restriction of viral nucleic acids, for example, or the packaging and unpackaging of eukaryotic nucleic acids in the chromosomes, are also other important processes in living cells which are controlled by the complex interplay of proteins and nucleic acid. In addition to this, other chemical substances are also able to interact with double-stranded nucleic acids,
25 with the class of carcinogenically active intercalating or nucleic acid-damaging substances only being mentioned here by way of example. Nucleic acids themselves, whether single-stranded or double-stranded, can also interact with their double-stranded cognates, for example in association with recombination, insertion or transposition.

- 30 When investigating such processes, a knowledge of molecule/molecule interactions plays a central role, irrespective of whether it is a matter of identifying the individual biologically active building blocks or a matter of elucidating the mechanisms, that is the interplay of these building blocks.
35 Consequently, the development of novel and efficient methods for detecting such interactions is at the center of interest.

Napier, M. E. et al. "Probing Biomolecule Recognition with Electron Transfer: Electrochemical Sensors for DNA Hybridization" *Bioconjugate Chem.* (1997), 8(6), 906-913 have already described measurement arrangements which make it possible to measure hybridization events
5 directly on single-stranded nucleic acids. This detection method is based on guanosine-bound DNA being oxidized by way of ruthenium complexes and on it being possible to detect this process by means of cyclic voltammetry.

The documents WO 95/15971, WO 96/40712 and DE 19901761 A1
10 describe methods which exploit the electrical conductivity of double-stranded nucleic acid hybrids as compared with single-stranded nucleic acids. Both use their methods exclusively for detecting single-stranded nucleic acid sequences. For this, complementary single-stranded nucleic acid probes, which contain, covalently bound, at least one electron donor
15 unit and at least one electron acceptor unit, preferably in the form of an electrode, are used for detecting the presence of single-stranded nucleic acid target sequences. A detectable electron flow is generated thermally or by way of a current-inducing signal, such as light, in the electrically conductive probe-target sequence hybrid.

20 The above-described methods are limited to detecting hybridized single-stranded nucleic acids. The detection is restricted to the mere presence of a target sequence in the medium to be investigated and, in addition to this, does not permit any conclusions to be drawn with regard to the biological
25 activity of the interacting nucleic acid.

If interactions of double-stranded nucleic acid sequences are to be investigated, it is necessary to have recourse to other methods, e.g. to gel-electrophoretic methods.

30 In the gel shift method (Fried, M. & Crothers, D. M. "Equilibrium and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis" (1981) *Nucleic Acid Res.* 9, 6505-6525; Garner, M. M. & Revzin, A. "A gel electrophoresis method for quantifying the binding of
35 proteins to specific DNA regions: application to components of the *Escherichia coli* lactose operon regulator system" (1981) *Nucleic Acid Res.* 9, 3047-3060), a radioactive DNA fragment is incubated with a protein extract which is to be investigated. If the protein extract contains a protein

which binds the DNA fragment, two bands can then be detected by gel electrophoresis, with one of the bands representing the free DNA fragment and the second, displaced band containing the DNA fragment/protein complex.

5

The footprint technique represents another in vitro method for detecting DNA-protein binding (Watson, J. D. et al., Rekombinierte DNA (Recombinant DNA), 2nd edition 1993, pp. 143-146, Spektrum Akademischer Verlag). In this method, a radioactively labeled DNA sequence is subjected to incomplete restriction degradation, e.g. using DNase I. A protein which is bound protects the sequence, at the corresponding site, from degradation, resulting in the corresponding bands being missing, as compared with the free sequence, when the fragments are detected by electrophoresis.

15

These methods involve preparing the DNA fragments and the protein extracts, labeling the DNA fragments, preparing a reaction mixture and carrying out a gel-electrophoretic separation. In practice, the implementation of these essential detection steps is very laborious and time-consuming. In addition, it is not possible to perform the detection throughout under native conditions and the use, for further investigations, of reaction mixture constituents which have been fractionated by gel electrophoresis proves to be difficult.

25 Using this as a basis, the object underlying the present invention is to make available novel probes for detecting and investigating interactions of double-stranded nucleic acids and methods for rapidly and simply detecting such interactions.

30 The object is achieved using novel, electronically readable double-stranded nucleic acid probes.

The probes contain nucleic acids, which are at least partially double-stranded, or single-stranded nucleic acids having self-recognizing domains which are bound to a conductive surface, preferably to an electrode, e.g. to field effect transistors. The nucleic acid segment which is present as a duplex is linked to at least one electron donor unit or, alternatively, to at least one electron acceptor unit, with at least one constituent section of the

35

nucleic acid region to be investigated being located between the electrode and the electron acceptor unit or electron donor unit. This constituent section forms the detection site. The individual probe subunits can be bound covalently or by way of stable, supramolecular interactions, such as
5 van der Waals interactions, dipole interactions, in particular hydrogen bonds or ionic interactions.

All single-stranded or double-stranded nucleic acids of any arbitrary sequence can be used for constructing the probe. In this connection, it is
10 immaterial whether natural, synthetic or modified nucleic acid sequences are employed. When a single-stranded nucleic acid is bound on, the corresponding double strand can be formed simply by hybridizing with a complementary single-stranded nucleic acid (e.g. McCarthy, B. J. et al., "Specificity of molecular hybridization reaction" Annu. Rev. Biochem.
15 (1970), 39, 131-150), with the formation of a double strand being elicited by intramolecular folding when the single-stranded nucleic acids are self-complementary. When such single-stranded nucleic acids are synthesized, two domains having complementary sequences are advantageously linked covalently by way of a bridge comprising at least one nucleotide, preferably
20 a bridge consisting of from 4 to 6 nucleotides, in particular consisting of thymine nucleotides, or by way of an artificial, at least monoatomic bridge. Examples of such artificial bridges are disulfide bridges (Hetian Gao et al., "Stabilization of double-stranded oligonucleotides using backbone-linked disulfide bridges", Nucleic Acid Research, 1995, Vol. 23, No. 2, "85-292),
25 stilbene dicarboxamide bridges (Lewis, F. D. et al., "Distance-depending electron transfer in DNA Hairpins" Book of abstract 215th ACS National Meeting, Dallas, vol. 29, p. Physics 255, 1988), Ru complex bridges (Lewis, F. D. et al., "Synthesis and spectroscopy of Ru(II)-bridged DNA hairpins", Chem. Commun., vol. 4, p. 327, 1999), hexaethylene glycol
30 bridges (Durand, M. et al., "Circular dichroism Studies of an Oligonucleotide containing Hairpin Loop made of a Hexaethylen Glycol Chain: Conformation and Stability.", (1990) Nucl. Acids Res. 18, 6353-6359), aromatic terephthalimide bridges (Salunkhe, M. S. et al., (1992), Control of Folding and Binding of Oligonucleotides by Use of a Non-
35 Nucleotide Linker", J. Am. Chem. Soc. 114, 8768-8772), unbranched or branched diol bridges, 3'-aminomodifier-CPG (GlenResearch), which is preferably synthesized on a thiol support, or branched phosphoramidite bridges. A great advantage of such nucleic acids, which contain self-

complementary sequence segments, lies in the simple, fully synthetic availability of the single-stranded nucleic acid and the ability to simply prepare the double-stranded probe from a single molecular nucleic acid subunit. In addition to this, it has been found that, when the double-stranded nucleic acid probes are prepared, the intramolecular hybridization of the single-stranded nucleic acids containing self-complementary sequence segments proceeds independently of the nucleic acid concentration. Another advantage of this method is that the preparation can be effected without using enzymes. A further advantage is consequently that the double-stranded nucleic acid probes can also be prepared under denaturing conditions. A hairpin-forming, single-stranded sequence having a free 3'OH end can also be completed, to form the corresponding nucleic acid duplex, using a DNA or RNA polymerase, e.g. a Klenow or Taq polymerase.

Natural or synthetic DNAs, cDNAs or RNAs can, for example, be used for preparing the probes according to the invention. It is likewise possible to use their hybrids and their modified derivatives. The modified derivatives include, in particular, nucleic acids which contain nucleotides which are modified at the sugar, such as 2'-O-methyl nucleotides or 2'-O-allyl nucleotides. It is likewise possible to modify the phosphate group, for example to give the phosphoramidate, the phosphorothioate or the methylphosphonate. It is also possible to use nucleic acids which either do not contain any sugar or contain a sugar which does not occur in natural nucleic acids, for example peptidyl nucleic acids or pyranosyl nucleic acids. In addition to the nucleic acids whose sugar-phosphate backbone is altered, it is also possible to use, as probe constituents, nucleic acids which contain nucleotides which do not occur naturally, such as xanthine, hypoxanthine or inosine.

Preference is given to incorporating double-stranded DNA or RNA nucleic acid sequences into the probes. DNA sequences which bind naturally occurring proteins or peptides, in particular DNA sequences which are involved in gene regulation, such as operator or promoter sequences, are of particular interest. Depending on the application problem, it can be expedient to use consensus sequences or sequences which are allelic-specific or organism-specific.

The length of the nucleic acid sequence between the electrode and the electron donor units or electron acceptor units is preferably between 2 and 100 base pairs, particularly preferably between 5 and 50 base pairs. The double-stranded region has to extend as far as the nucleic acid electrode linker in order to ensure an electron flow. Single-stranded regions can extend beyond the double-stranded detection region which lies between the electrode and the electron donor units or electron acceptor units. The double-stranded detection region can contain single-stranded breaks in the sugar-phosphate backbone.

10

The form of a double strand can be chemically stabilized by chemical modifications which lead to intermolecular or intramolecular bridge bonds, such as disulfide bridges, or other unnatural strand subunits. Another possibility of stabilizing the nucleic acid duplex segments is provided by the selective introduction of thymine nucleotides at two opposing positions in the duplex and subsequent radiation-induced thymidine bridge formation. It is also possible to use azido nucleotides or psoralene derivatives in an analogous manner for the photoinduced formation of bridges (Fabrega, C. et al., "Studies of the Synthesis of Oligonucleotides Containing Photoreactive Nucleosides: 2-Azido-2'-Deoxyinosine and 8-Azido-2'-Deoxyadenosine", Biol. Chem., Vol. 379, 527-433, 1998; Pies, U. et al., Nucleic Acid Research 1989, 17, 285). This can, for example, prevent exonucleases from degrading the nucleic acid duplex. The sensitivity of the double-stranded nucleic acids toward undesirable, method-associated influences, such as the temperature of the medium to be investigated, is likewise decreased.

All molecules, molecule parts or surfaces which are able to emit electrons from their basic state or from an excited state, or to take up electrons in the basic state or in an activated state, can act as electron donor units or electron acceptor units. An electron donor unit can be excited, for example, by irradiation with light or ionization, while an electron acceptor can be activated, for example, by ionization, and likewise by means of light or by means of chemical ionization. Examples of electron donors and electron acceptors which have proved to be of value are metal complexes or organic redox-active compounds, as are described in patent application WO 96/40712, or naturally occurring or modified photoinducible redox-active reaction centers, as are used, for example, in patent application DE

19901761 A1. However, electrically conductive surfaces, in particular electrodes, can also be used as the donor or acceptor unit. In particular, it has been found worthwhile experimentally to use ferrocene or PQQ as electron donor units.

5

The covalently bound electron donor units or electron acceptor units can be integrated into multimolecular electron transfer systems. On the one hand, the electron donor unit (electron acceptor units) which is/are active in the double-stranded nucleic acid probe can be charged, in a regeneratable manner, by way of dissolved electron donors such as anions (cations), with a closed circuit being constructed using the double-stranded nucleic acid probes as a resistance. On the other hand, in analogy with the naturally occurring reaction centers, such as chlorophyll, it is also possible to associate other redox-active electron transfer units with the covalently bound electron donor unit or electron acceptor unit.

10
15

Preference is given to inducible and/or regenerative donor units or acceptor units. Regenerative systems make it possible for there to be a continuous electron flow through the nucleic acid duplex, whereas the electron flow can be controlled in time by the inducer in the case of inducible systems.

20

If, for example, chlorophyll or bacteriochlorophyll or its modified derivatives are used as electron donor systems, the electron flow can be induced by light irradiation, in analogy with the natural processes of photosynthesis.

25

The reaction centers can be regenerated once again by way of suitable dissolved substances, such as $\text{Fe}(\text{CN})_4^{3-}$ or $\text{Fe}(\text{CN})_6^{4-}$, which donate electrons. The induction can also be effected directly by way of chemical substances which transfer electrons to the electron donor units or remove electrons from the electron acceptor units.

30

The nucleic acids can, for example, be bound to the electrode by way of a thiol group (Chidsey, C.E.D., Science, vol. 251, p. 919 (1991)), or by way of phosphorothioates or phosphorodithioates to a gold surface. However, it is also possible for them to be bound by way of molecular linkers (e.g. described by Kayyem et al. in WO 98/20162) which conduct well and which preferably contain conjugated double bond systems and possess a higher conductivity than the nucleic acid which is bound on. The use of linkers containing conjugated double bond systems enables electrons to be

35

conducted along the linker structure onto the electrode surface. This makes it possible to passivate the electrode surface which remains free in an elegant manner. The double-stranded nucleic acid probes can be bound to an unstructured, continuous electrode surface or to a structured, for example matrix-shaped, electrode array surface. The surface is structured such that the individual array positions of the electrode can be read independently of each other. Nucleic acid probes containing specific sequences can be assigned to the individual array positions or the reaction space can be compartmentalized. In this way, it is possible to achieve a high degree of parallelization when reading the electrode electronically.

It has been found, surprisingly, that the double-stranded nucleic acid probes according to the invention offer the possibility of using a change in conductivity to detect factors which interact with nucleic acid double strands. Interacting factors are chemical substances or radiation which enter into interaction with a nucleic acid double strand and induce a change in the primary, secondary, tertiary or quaternary structure of the double strand. These structural changes lead to a significant change in the electrical resistance of the duplex structure and consequently to a change in the electron flow along the double-stranded nucleic acid. Presumably, in addition to the structural changes in the double-stranded nucleic acids brought about by interacting factors, other effects, such as the insulating effect of nucleic acid-bound proteins or peptides, or the direct participation of intercalating substances, such as aromatic molecules, in the electron conduction through the nucleic acid double strand, also have an influence on the resulting electron flow. As a rule, an aggregate is formed between the probes and the interacting substances, with the aggregates being stabilized by way of intermolecular forces or even by way of covalent bonds.

Changes in the electrical conductivity of double-stranded nucleic acids are brought about, for example, by the binding of proteins or peptides, such as antibodies, polymerases, transcription factors, enhancers or repressors, whose activity can, in turn, be modulated by substances having an indirect effect, such as by way of inducers or enzymes having a modifying action. Organic molecules, such as some hormones, can also interact with double-stranded nucleic acids, either directly or in combination with proteins or peptides, such as hormone receptors. Single-stranded nucleic acid can

likewise interact with double-stranded nucleic acids, for example with the formation of a triplex. However, salt-containing solutions, mimetics or substances which intercalate into the nucleic acid duplex, including cytostatic agents such as the anthracyclins, also induce measurable changes in the structure of the double strand.

The effects which have thus far been described leave the covalent bonds in the double strand intact. Other proteins or nucleic acid-damaging substances directly attack the primary structure of the nucleic acids. Endonucleases cleave nucleic acids, exonucleases degrade nucleic acids from a free strand end, ligases create covalent bonds between terminal nucleotides, while topoisomerases bring about single-strand breaks and single-strand linkages. However, mimetics, nucleic acid-damaging substances and cytostatic agents which, for example, have an alkylating or crosslinking effect on nucleic acids, such as platinum complexes, for example cisplatin, methanesulfonates, for example busulfan, or n-nitroso compounds, for example carmustin, can also attack their covalent bonds and/or create new covalent bonds. Radioactive and electromagnetic radiation, such as α , β , γ or UV radiation, can likewise lead to such structural changes and consequently to a measurable change in the electron flow within the double-stranded nucleic acid.

The use of the double-stranded nucleic acid derivatives according to the invention as probes offers diverse advantages. All the interactions to which a double-stranded nucleic acid probe is subjected and which have an effect on the electrical conductivity can be detected. The measurement can be effected coulometrically, impedometrically, preferably frequency-dependently resistively or capacitively, voltametrically, potentiometrically or amperometrically. Surprisingly, the disruptive effect of ions from the reaction solution which are discharged directly on the electrode is very small. In order to increase the measurement sensitivity still further, the electron flow through the double-stranded nucleic acid probe can be frequency-modulated. When photoinducible electron donor units are used, the modulation can be effected by way of light flashes. Very small changes in conductivity can also be detected by the number of probes applied or by extending the period of measurement. The number of probes is preferably selected such that the current change which results from the interaction between the double-stranded nucleic acid and the factors which interact

with it is at least in nA range. Due to their particularly high sensitivity, cyclovoltametry, difference pulse voltametry and square wave voltametry have proved to be particularly suitable methods of measurement.

- 5 Another advantage of the present probes lies in their high degree of compatibility with a very wide variety of reaction media. The use of crude extracts as a reaction medium, or the use of reaction media containing a salt concentration corresponding to the natural conditions, is of particular interest. Furthermore, it is possible to investigate the kinetics of double-
10 stranded nucleic acid interactions, and equilibrium constants of double-stranded nucleic acids with interacting substances can be determined. This offers the possibility of finding antagonists and allosteric interactions with other factors and determining their activity. In this connection, it is possible to carry out investigations as a multistep reaction in which individual factors
15 are pipetted into the reaction solution, one after another, as the measurement is proceeding.

- Other parts of the subject-matter of the invention are methods for detecting interactions between double-stranded nucleic acids and factors which
20 interact with them and the use of the double-stranded nucleic acid probes according to the invention for this purpose.

- The probes according to the invention are particularly suitable for investigating gene regulation, in particular the transcription or repression of
25 genes. A large number of substances which interact directly with the double-stranded DNA, such as RNA polymerases, transcription factors, repressors or enhancers, or which only display an indirect effect, such as inducers, enzymes, such as phosphorylases, second messengers, such as cAMP or cGMP, or receptors and their binding partners, such as hormones,
30 play an important role in gene regulation. In this connection, the signal chains which lead to the transcription or repression of individual genes can be extremely complex. The probes according to the invention can provide important insights in clarifying the interplay of the individual factors.

- 35 For this, known regulatory DNA sequences in a gene, for example, are incorporated into the probes. The probes are applied to an electrode surface such that the sum of the change in the electrical conductivity of the double-stranded DNA probes is measured. In the free, non-associated

state, the reference electron flow through the probes is determined in the presence of a reference solution. Alternatively, the reference value can also be determined using an electrochemical standard cell/electrode. Ideally, the reference solution contains all the components of the reaction solution apart from the factors to be tested. The reference electron flow is modulated by successively adding the individual substances to be tested. The change in the electron flow then provides information about an interaction which has taken place between the test substance and the probe sequence, with the strength of the change in the electron flow providing information about the strength of the interaction and about the equilibrium constant of the binding reaction. In this way, it is possible to measure all direct interactions of the DNA with the directly interacting substances, such as polymerases, transcription factors or repressors. It is thereby possible to identify substances which interact directly with the DNA duplex. Consequently, this also opens up a novel possibility for rapidly finding transcription factors which possess tissue-specific activity by using probes which contain known, tissue-specific promoter sequences.

The effect of other factors which influence the activity of these substances, that is factors having an indirect effect, can be determined indirectly by way of the change in the equilibrium constants of RNA polymerases, transcription factors or repressors. For this, either reaction solutions containing different factors can be tested or the indirectly acting factors are introduced successively into the reaction solution. In this way, it is also possible to determine interactions between individual substances within the regulatory signal chains.

If, for example, a strong inducer which acts on the repressor is added to an operator-repressor complex in the reaction solution, the equilibrium constant approaches zero and the measured electron flow comes into line with the reference electron flow.

The *Escherichia coli* lactose operon provides an example of such an induction mechanism. The effect of the lac repressor is abolished by the presence of lactose, the repressor loses its affinity for the operator sequence and the electron flow will come into line with the reference value. If β -galactosidase, which is a lactose-degrading enzyme, is added to the reaction solution, the lac repressor is reactivated.

Another example is the *E. coli* *lexA* repressor, which regulates a large number of genes which are involved in DNA repair. *lexA* is proteolytically cleaved by single-stranded DNA-bound *recA* proteins and consequently detached from the appurtenant operator. As a consequence, the electron
5 flow within the double-stranded DNA probe will once again come into line with the reference value.

At a given inducer concentration, the degree to which the electron flow comes into line with the reference electron flow is a measure of the
10 strength of the inductive effect. On the other hand, by varying the inducer concentration, it is possible to investigate concentration-dependent effects of the inducer on the repressor or a linear dependence of the induction on the concentration of the inducer.

15 If the repressor is inactive in the reaction mixture, the electron flow remains at the reference value. By adding other substances, it is possible to test their repression-activating effect on the repressor. Such a regulatory mechanism exists, for example, in the *E. coli* tryptophan operon. The *trp* repressor is activated by the presence of tryptophan and the repressor-
20 tryptophan complex binds to the appurtenant operator sequence. The electron flow within the double-stranded DNA probes can be observed to diverge from the reference value.

In an analogous manner, there is the possibility of determining the
25 equilibrium constant of the binding of RNA polymerases or transcription factors to DNA promoters, and the effect of their cofactors and their modifications. Probes which contain a promoter sequence can be used for this purpose. A reference value can then be determined, with this value being modulated by adding individual factors. A change in the reference
30 value indicates that the added substance has interacted. In addition to this, the RNA polymerases and transcription factors which bind in the promoter region can be identified.

If, for example, the SV 40 promoter is used for preparing the double-
35 stranded nucleic acid probes, it is then possible to investigate the binding of the transcription factor Sp1, which binds specifically to this promoter.

By adding other protein or peptide cofactors, second messengers or enzymes having a modifying effect, such as phosphorylases, or transcription-influencing molecules, such as some antibiotics, their activating or inhibiting effect on the transcription factor-DNA binding can be measured.

For example, in the case of the transcription factor Sp1, the binding of this factor to the SV 40 promoter is inhibited by the antibiotic actinomycin D.

10 The transcription factor p53, which is the product of a tumor suppressor gene, is another example. P53 can be phosphorylated, and thereby activated, by adding a phosphorylase in the presence of a suitable phosphate donor, such as ATP. As a result of the phosphorylated p53 binding to the probe sequence, the electron flow changes steadily until a
15 concentration-dependent saturation value is reached. Aside from the pure interaction context, it is then possible to obtain information about the reaction rate from the slope of the electron flow-time curve. The slope of the curve is directly proportional to the reaction rate, i.e. of the phosphorylation reaction in the case of this present example.

20 If the DNA sequence in the probes comprises the operator and the promoter, it is also possible to measure competitive effects between RNA polymerases, promoter-binding transcription factors and repressors. For example, if a repressor displaces a transcription factor which has already
25 been bound, the electron flow then changes correspondingly. The electron flows which are measured lie between the values for the reaction solutions which contain only an RNA polymerase and/or the transcription factor and only the repressor. In this way, it is possible, for example, to investigate the direct effect of the lac repressor, the trp repressor or the lexA repressor on
30 the binding of RNA polymerases to the promoter.

A particular advantage of the method which is described here is the possibility of investigating allele-specific interactions between substances which possess regulatory activity and the appurtenant DNA regulation
35 sequences. In this way, it is possible to investigate the different activity of DNA sequences and DNA-binding proteins or peptides of the wild type and mutated DNA sequences, proteins or peptides in separate assays

independently of phenotypically recognizable changes. In particular, it becomes possible to investigate features which are expressed recessively.

5 In addition to this, it is possible to investigate mixtures of the gene products from different alleles of a gene which interact directly or indirectly with double-stranded DNA. For example, if a wild-type transcription factor binds to a promoter but not a mutant of the promoter, a transcription factor extract from a homozygous wild-type organism gives rise to the greatest change in electron flow through the double-stranded nucleic acid probe while a
10 transcription factor extract from a homozygously mutant organism does not bring about any change in the conductivity of the probe. Organisms which are heterozygous with regard to the transcription factor give rise to an electron flow through the double-stranded nucleic acid probe which lies between the two extracts from the homozygous organisms; in the case of
15 this experiment, it is necessary to use extracts which are at a defined concentration.

The method is not restricted to identifying substances which participate in gene regulation; thus, histones, helicases, topoisomerases or ligases, for
20 example, are also able to bind to double-stranded DNA. Whereas helicases, topoisomerases and chromatin-remodulating enzymes (e.g. nucleosomal ATPase ISWI) are as a rule able to decrease the electron flow through the probes, they can also lead to an increase in the electron flow if use is made of double-stranded nucleic acid probes which contain one or
25 more bond breaks in the sugar-phosphate backbone which are ligated.

The loss of conductivity is observed in the case of nucleases which recognize and degrade double-stranded structures. The electron flow through the probes falls steadily and approaches zero when the duration of
30 the reaction is sufficiently long. Adding nuclease-blocking substances prevents such an effect being observed.

When bound RNA-DNA hybrid probes are used, there is then the possibility, for example, of investigating RNAses which recognize and
35 degrade the RNA moieties within the double strand. The loss of the RNA building blocks generates a remaining single strand, which can now be detected by the loss in conductivity. It is not necessary for the whole of the RNA to be degraded to achieve this; even the loss of from one to two

ribonucleotides between the electron donor units or electron acceptor units and the electrode results in a drastic decline in conductivity in the double strand. In an analogous manner, it is possible to detect DNAses using DNA double-strand probes.

5

Restriction enzymes, such as Hind III, which catalyzes a smooth double-strand break, or EcoR I or PST I, which produces a staggered double-strand break, have the most significant effect on the conductivity of double-stranded nucleic acid probes, provided a suitable restriction site is present in the double-strand sequence. As a result of the two nucleic acid strands between the electron donor units or electron acceptor units and the electrode being ruptured, the electron flow through the double strand comes to a complete standstill.

15 It is also possible to use the double-stranded nucleic acid probes according to the invention to determine the binding sequences of individual nucleic acid-binding substances. Use is advantageously made, for this purpose, of electrode arrays in which each field is connected to a group of double-stranded nucleic acids of precisely defined sequence. In the ideal case, all possible 4^n sequences, with n being the number of the base pairs, are applied to the electrode in 4^n fields which can be electronically read separately. Factors binding double-stranded nucleic acids modulate the conductivity of the array positions which contain a sequence which is recognized by the factor. The appurtenant, recognized sequence is determined from the corresponding array position. Using the sequences which have been isolated, it is possible to prepare probes which can in turn be used to identify the appurtenant genes employing conventional hybridization methods. However, this method can also be used to assess modified nucleic acid sequences which have been prepared by site-specific mutagenesis or transcription factors. A strong nucleic acid-transcription factor/RNA polymerase binding is a sign of a highly active transcription-promoting complex. It is consequently possible to select highly active promoter sequences and the appurtenant highly active transcription factors. Such promoter/transcription factor complexes can be used for constructing novel expression vectors.

35

It is also possible to incorporate extracts of regulatorally active DNA fragments of a particular gene, which fragments do not have precisely

specified sequences, into the double-stranded nucleic acid probes. It is then possible to use known factors, which bind to these sequences and which are at a specified concentration, to determine whether homozygously binding sequences, homozygously non-binding sequences or heterozygous sequence mixtures are present in the probes.

Alternatively, a nucleic acid fragment extract can be added directly to a double-stranded nucleic acid probe/probe-binding protein. The competitive effect of the nucleic acid fragment extract on the measuring system is then determined.

In addition to the previously mentioned substance classes which recognize double-stranded nucleic acid, it is also possible for single-stranded or double-stranded nucleic acids to interact with the probe sequences.

The at least partial separation of the nucleic acid double strand of the probe, due to the hybridization of the single-stranded nucleic acids, which compete as binding partners, with a complementary probe sequence, leads either to triplex structures or to a dissolution of the probe duplex due to rehybridization. The conductivity of the probe is consequently interrupted or altered. Consequently, it is possible to use this method to detect hybridization events.

Using a destabilized double-stranded nucleic acid sequence in the probe facilitates hybridization with a single-stranded nucleic acid which is present in solution. Destabilization as compared with a DNA double strand can be achieved, for example, by using DNA/RNA hybrids. The DNA/RNA hybrid pairing can additionally be weakened by modifying the DNA strand to form a phosphorothioate nucleic acid.

It is likewise possible to achieve easier hybridization with a single strand present in solution by using a single strand end which protrudes from the double strand probe. The hybridization then takes place with the single strand end and parts of the double-stranded structure. If a break has been introduced in the nucleic acid backbone in the recognized double-stranded structure, the newly formed hybrid can then even become completely detached from the probe and interrupt the electron flow within the probes.

- In addition to this, it is also possible to detect recombination events and identify factors, in particular enzymes, which are involved in the recombination. When a double-stranded nucleic acid recombines completely with the probe sequence, the electron donor units or electron acceptor units can be separated from the electrode. There can no longer be any electron flow through the probe. However, the recombination event does not have to have been completely concluded; the formation of a triplex structure, which has an effect on the double-stranded probe structure, is sufficient in order to measure a decrease in the electrical conductivity. If recombination-promoting factors, such as the *E. coli* recA protein, are added to the reaction solution, it is then possible to measure a more rapid decline in the electron flow or an increase in the total decline in the electron flow.
- 15 If the probes contain sites for integrating transposable elements, it is then also possible to determine transposition events. Since the strength of the electron flow in the double strand of the probe decreases with increasing distance, that is an increasing number of base pairs, between the electron donor unit or electron acceptor unit and the electrode, the electron flow also decreases when a transposon is integrated into the probe sequence. A corresponding increase in the electron flow can be detected when the reverse reaction takes place.
- 25 It is also suitable to use the double-stranded nucleic acid probes for detecting nucleic acid-damaging substances or for detecting the nucleic acid-damaging effect of chemical substances. The damage to a double-stranded nucleic acid can be caused in a variety of ways. Thus, some substances, in particular aromatic substances such as ethidium bromide or acridine, can intercalate into the nucleic acid duplex structure. Other substances are able to react with nucleic acids, for example strong bases, which hydrolyze the nucleic acids, benzpyrines, which add on to the nucleic acids, or peroxides, which readily form free radicals and trigger free radical chain reactions within the nucleic acid. The change in the nucleic acid structure due to these substances brings about a change in the conductivity of the double-stranded nucleic acid probes.

Such a test can be carried out in a compartmentalized reaction vessel in which the floor is formed by the electrode in array form. The probes are

applied at uniform density to the individual array positions. A reaction solution containing a different test substance can be added to each reaction compartment. It is also possible to add reaction solutions which contain the same test substance at different concentrations. In this way, it is possible to create a simple test to identify DNA-damaging substances and consequently substances having a carcinogenic effect.

Double-stranded nucleic acids also interact with substances which are dissolved in the reaction medium, in particular with ionic compounds which have a destabilizing effect. However, interactions with the solvent itself can also influence the conductivity of the probes. For example, on dehydration, the DNA double helix of the B type changes in the A type. The novel probes can be used to measure this change.

The reaction media or reaction solutions can contain any arbitrary solvents and additives. Preference is given to using, as reaction solutions, aqueous, ionic buffer solutions which can contain chemical substances which interact with double-stranded nucleic acids. It is likewise possible to use crude extracts directly as the reaction medium. The detection of the interaction between the double-stranded nucleic acid probe sequences and the substances which interact with them preferably takes place under physiological conditions, that is in 10 to 200 mM aqueous, salt-containing solutions at pH 7-9. Preference is given to using a phosphate buffer as the buffer. The measurement temperature is preferably between 4 and 40°C. However, it may be appropriate to deviate from these measurement conditions in individual cases. For example, a higher measurement temperature can be used for finding proteins whose activity is temperature-stable, such as the Taq polymerase which is used for PCR.

It follows from this that the double-stranded nucleic acid probes can be used in a wide variety of ways for analyzing active factors which interact with nucleic acids or for analyzing active double-stranded nucleic acid sequences.

The double-stranded nucleic acid probes according to the invention can be combined, in analytical appliances, with classical separation methods, such as chromatography. Active constituents which bind to a double-stranded nucleic acid can be tracked down rapidly through fractionating cell extracts

or substance libraries. The process can be operated continuously in an online measurement method.

5 In order to clarify the invention, possible methods for preparing the double-stranded nucleic acid probes according to the invention, and individual methods for measuring interactions between double-stranded nucleic acids and factors which interact with them, are described below by way of example.

10 Methods which are known to the skilled person can be used for preparing suitable double-stranded nucleic acid probes. For this, thiol groups, for binding to a gold electrode, and amino groups, for binding on the electron donor units, are introduced into the nucleotide sequences which are to be incorporated into the probe.

15 If they are not commercially available, the oligonucleotides which are employed can, for example, be prepared using a DNA synthesis method, i.e. what is termed the phosphoramidite method (Beaucage, S. L.; Caruthers, M. H. Deoxynucleoside phosphoramidites. A new class of key
20 intermediates for deoxypolynucleotide synthesis. Tetrahedron Lett. (1981), 22(20), 1859-62).

This is a method which can be used for selectively preparing both unmodified nucleic acids and modified nucleic acids, for example RNA's phosphorothioates and PNAs, in high purity and yield, and is nowadays
25 used routinely.

For the synthesis, the desired synthesis building blocks are built up, on the 1 μ mol scale, in a predetermined sequence on solid CPG support material using an Expedite Synthesizer of the Expedite 8909 type supplied by
30 PerSeptive Biosystems. 0.1 M solutions of appropriate phosphoramidite building blocks and an 0.5 M tetrazole solution are used for this purpose. Because of their lability, the building blocks are dissolved in dry acetonitrile (water content < 30 ppm).

The necessary modifications are synthesized using amino-, SH- or phosphate-On phosphoramidite building blocks (Eurogentec, Clontech) and
35 support materials ("SH-CPG"-Chemgenes, Glen Research, 3'-phosphate CPG Glen Research).

In order to introduce the thiol modification, the desired sequence is synthesized, on a 1 μ Mol scale, on thiol-CPG support material (Chemgenes) at the 3' end of the oligonucleotide. In order to avoid the sulfur being oxidized, an 0.02 M solution of iodine is used during the DNA synthesis (Kumar, A. et al., Nucleic Acids Research 1991, 19, 4561).

The amino group can be introduced selectively, at chosen positions which are not located on the electrode side of the double-strand structure, using an 0.2 M amino modifier II solution (Clontech) or an 0.1 M amino-modifier C6 dT solution (Glen Research) in dry acetonitrile (water < 30 ppm).

After the syntheses have been concluded, the modified oligonucleotides are worked up; for this, the oligomers are cleaved off the CPG support under basic conditions, using an aqueous ammonia solution (25-32%), and incubated at 55°C for several hours. When thiol groups are present, DTT is added up to a concentration of 50 mM. Subsequently, the solution is concentrated and freed of ammonia and coevaporated several times with water. Oligonucleotides which are to contain a modified thymidine building block, are coupled to make them longer. After the absorption has been determined at 260 nm, RP-HPLC (Lichrochart 100 RP-C18) is used to free the crude products from termination products and cleaved-off protecting groups. A gradient of acetonitrile in 0.1 triethylammonium acetate buffer, of from 0-60% in 60 minutes, is used as the mobile phase. The main peak is isolated, quantified spectrometrically at 260 nm, lyophilized and dissolved in 500 μ l of 50 mM DTT. The solution is incubated at 37°C for 30 minutes and subsequently desalted using a PD-10 column in accordance with the manufacturer's (Pharmacia) instructions and pipetted onto the gold surface for being bonded covalently to the electrode. Before being used, the buffer solutions are saturated with argon.

In an alternative method for modifying the oligonucleotides with a thiol group, the sequence containing phosphoramidite nucleotide building blocks is synthesized, in the desired order, on a 3'-phosphate CPG as described above. An AminoModifier II (Clontech) can be used as the amino-modified building block.

After the amino-modified 3'-phosphorylated oligonucleotide has been cleaved off, the oligonucleotide is esterified with $(\text{HO}-(\text{CH}_2)_2-\text{S})_2$, to give $\text{P-O}-(\text{CH}_2)_2-\text{SS}-(\text{CH}_2)_2-\text{OH}$, in order to introduce the requisite thiol function.

After the 3' end of the oligonucleotide has been esterified, the nucleic acid is added, at a concentration of $1-2 \times 10^{-4}$ M in application buffer (10 mM Tris, 1 mM EDTA, pH 7.5 in the added presence of 0.7 molar TEATFB) containing 10^{-4} - 10^{-1} molar 2-hydroxymercaptoethanol, to the cleaned gold surface of the electrode and incubated for 2-24 hours.

In order to prepare the gold surface of the electrode, freshly cleaved mica (Muscovite flakes), for example, can be cleaned in an electrical discharge chamber using an argon ion plasma, and gold (99.99%) can subsequently be applied by electrical discharge in a layer thickness of approx. 100 nm (Ron, Hannoch; Matlis, Sophie; Rubinstein, Israel. Self-Assembled Monolayers on Oxidized Metals. 2. Gold Surface Oxidative Pretreatment, Monolayer Properties and Depression Formation. Langmuir (1998), 14(5), 1116-1121; Golan, Yuval; Margulis, Lev; Rubinstein, Israel. Vacuum-deposited gold films. I. Factors affecting the film morphology. Surf. Sci. (1992), 264(3), 312-26).

The metal surface is then cleaned with a 30% H_2O_2 /70% strength H_2SO_4 solution and subsequently immersed in ethanol for 20 minutes. The surface is finally rinsed with double distilled water.

Alternatively, commercially available precious metal electrodes can be prepared by polishing the electrode surface and then rinsing it. The subsequent electrochemical cleaning of the electrodes can be effected, for example, cyclic voltammetrically in sodium hydroxide solution using a potentiostat, with the electrodes [lacuna] several times in a potential range of from -2.0 to +1.2 V against an Ag/Ag reference electrode (3M NaCl solution). A platinum rod can, for example, serve as the counterelectrode.

SH-modified oligonucleotides are bound on, for example, by incubating the cleaned Au electrodes with a solution of the corresponding oligonucleotide in phosphate buffer by adding the solution dropwise to the gold disk and incubating for several hours in a water-saturated atmosphere.

An electron donor unit or electron acceptor unit, such as pyrroloquinolinequinone, methoxatin, PQQ or ferrocene, can be bound to the monolayer-bound oligonucleotides or to the oligonucleotides present in solution.

The linkage of the redox units (PQQ, Sigma-Aldrich, Steinheim, Germany) to the primary amino group of the modified T building block (aminomodifier dT) in the hairpin on monolayer-bound oligonucleotides is achieved by activation of the PQQ carboxylate groups in buffer containing a soluble carbodiimide and subsequent amide formation.

The second method of obtaining redox units which contain oligonucleotide monolayers on gold is to modify the oligonucleotides with redox-active electron donor units or electron acceptor units, such as PQQ and ferroceneacetic acid, in solution and to subsequently immobilize them on the gold surface.

A redox unit is bound on by activating the carboxylate functions and then binding them to the primary amino group of the modified T building block (amino-modifier dT) in the oligonucleotide.

For this, PQQ is dissolved in water, after which N-hydroxysuccinimide and water-soluble carbodiimide are added. For the purpose of modifying the oligonucleotide, water and triethylammonium hydrogen carbonate solution are added to the activation solution. After the amino-modified oligonucleotide has been added, the solution is left to stand. The reaction mixture can be worked up by means of size exclusion chromatography.

The ferroceneacetic acid-modified oligonucleotides are synthesized by activating the ferroceneacetic acid (e.g. with o-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU)) and subsequently coupling it to the free amino group of the oligonucleotide. The activated ferroceneacetic acid is added to a solution of the modifying oligonucleotide in the presence of a base. After a reaction time of several hours, the oligonucleotide is purified.

A Zn-bacteriochlorophyll electron donor unit can be covalently bound on after the nucleic acid has been bound to the gold surface of the electrode and after a further washing step using water. For this, the modified nucleic acid substrate is moistened with a solution of 3×10^{-3} molar quinone 2-(CH₂-CH₂-CO₂H-UQ-50), 10^{-2} molar EDC (3'-dimethylaminopropyl)-N-carbodiimide and 10^{-2} molar N-hydroxysulfosuccinimide in 0.1 M HEPES buffer (2-(4-(2-hydroxyethyl)-1-piperazino)ethanesulfonic acid, pH 7.5). The quinone which is used is obtained by, in a standard manner, cleaving a

- methoxy group with HBr (ether cleavage), reacting the resulting free hydroxyl group with an equimolar quantity of Cl-CH₂-CH₂-CO₂H, and subsequently purifying by chromatography (Moore; H. B. and Folkers, K. Journal of American Chemical Society, 1966, 88, 5919-23; Daves, G. et al. 5 Journal of American Chemical Society, 1968, 90, 4487-4493).
- After having been washed with double distilled water, the modified substrate is incubated once again with an aqueous solution comprising 3×10^{-3} molar Zn-bacteriochlorophyll (as the free acid), 1.5×10^{-1} molar (3-dimethylaminopropyl)carbodiimide, 2.5×10^{-3} molar hydrazine 10 monohydrate and 1×10^{-1} molar imidazole at 23 °C for 16 hours. The Zn-bacteriochlorophyll is prepared as the free acid by incubating with trifluoroacetic acid.
- (Hartwich, Gerhard; Fiedor, Leszek; Simonin, Ingrid; Cmiel, Edmund; Schaefer, Wolfgang; Noy, Dror; Scherz, Avigdor; Scheer, Hugo. Metal- 15 Substituted Bacteriochlorophylls. 1. Preparation and Influence of Metal and Coordination on Spectra. J. Am. Chem. Soc. (1998), 120(15), 3675-3683).
- The hybridizing or back-folding of the nucleic acids to give the double-stranded probes can be effected before or after applying the modified 20 nucleic acid to the gold surface of the electrode. The hybridization can be effected, for example, in buffered solution in accordance with the method known to the skilled person. Preference is given to using nucleic acid duplexes which are already hybridized when preparing the probes. In this connection, the hybridization is effected under control conditions, while 25 cooling slowly in order if at all possible to avoid mispairings. Heating for a short while at 95°C and then cooling down slowly to room temperature in a waterbath has been found to be a satisfactory method.
- Applied, single-stranded nucleic acids, which are 5'-phosphorylated 30 (phosphate-On phosphoramidite building blocks (Horn, T. and Urdea, M., Tetrahedron Letter, 1986, 27, 4705)) and which have been esterified to give the thiol derivative, and which only possess a short hairpin having a free 3' OH end, can be synthesized enzymically into a double strand using a DNA polymerase. For this, a DNA structure is, for example, incubated 35 with a mixture of the four nucleotide triphosphates containing the Klenow fragment of DNA polymerase I. The desired nucleic acid duplexes are obtained.

It has proved to be advantageous to coat the free electrode surface, preferably with a monomolecular coating. After the double-stranded nucleic acid probes have been applied, the surface of a gold electrode can be saturated, for example, with alkyl disulfides, alkylthiols or hydroxyalkylthiols.

Individual assays which can be carried out using the double-stranded nucleic acid probes according to the invention are described below, by way of example, with the aid of the figures.

10

Fig. 1 describes an assay for investigating restriction enzymes or exonucleases.

A double-stranded DNA structure (11), possessing a restriction cleavage site and a light-inducible electron donor unit (12) is covalently bound on a gold electrode (13) as described in the preceding examples. The DNA double strand (11) of the probe is cut into two fragments (17, 18) in a reaction solution which contains a restriction enzyme, e.g. *Pst*1 (14). The activity is measured over time, under light irradiation (15), using an evaluation device (16).

In the reverse of this, the fragments can be linked together once again enzymically by ligation of on DNA-Zn-bacteriochlorophyll degradation products, e.g. obtained from the reaction solution produced above after the solution has been freed preparatively from the restriction enzyme by means of RP-HPLC. For this, the fragments can be ligated in an excess of a ligase and in a DNA ligase buffer, with the efficiency of the ligation being detected amperometrically by the generation of an electron flow through the double-stranded DNA which is synthesized.

30

Fig. 2 depicts an assay for investigating the binding of a transcription factor.

The transcription factor (21), e.g. Oct2A, TATA or Sp1, is added, in buffered solution, to a recipient structure consisting of a gold electrode (22) which possesses double-stranded nucleic acid probes (23) having the relevant binding site which is specific for the transcription factor. The double-stranded DNA probe sequences (23) are provided with a covalently bound light-inducible electron donor unit (24), e.g. Zn-bacteriochlorophyll.

35

Following incubation with the relevant transcription factor (21), the assay is read with an evaluation unit (26) while irradiating with light (25). As a control, it is possible to carry out analyses in parallel of experimental assays in which, for example, DNA fragments (27) which are not bound to the electrode surface but which contain binding sites for the relevant transcription factor, are included in excess as competitors.

In a somewhat modified method, it is possible, for example by adding the sp1 competitor actinomycin D before and after adding Sp1, to measure the DNA-protein interaction and the change in conductivity which is elicited.

Fig. 3 describes an assay for detecting a DNA-DNA interaction.

A self-complementary DNA (31) possessing a covalently bound light-inducible electron donor unit (34) is applied to the gold electrode (32) as described in the above examples. The region of nucleotides at the 3' end is theoretically considered to be present as a single strand. By means of hybridizing with a relatively long oligomer, e.g. a 38mer oligomer (33), which exhibits complementarity over a region of the other nucleic acid strand, the single strand structure is filled in selectively. For this, a buffered oligomer (33) is, for example, added in excess to the bound probes and the mixture is then incubated.

The excess of unhybridized oligonucleotides is subsequently washed out. After adding a buffered solution containing a second complementary oligomeric single-stranded nucleic acid, with it being possible for this nucleic acid in the present case to be, for example, a 38mer oligomer (35) which is complementary to the former oligomer (33), rehybridization, with the formation of a hybrid (37) which is formed from the oligomers (33) and (35), is measured, while irradiating with light (36), using an evaluation unit (38).

Fig. 4 describes an assay for investigating the intercalation of chemical substances into double-stranded DNA.

In such an assay, double-stranded nucleic acid probes (41), possessing a covalently bound light-inducible electron donor unit (46), are applied to a matrix-shaped gold electrode array surface (42) at the individual array position (47). The bound DNA probes (41) are incubated with different

concentrations of solutions of the substances A, e.g. propidium iodide (43), B, e.g. ethidium bromide (44) and C, e.g. Syber[®]green (45). (For reasons of clarity, the second dimension of the array surface is not depicted in Fig. 4). The effect of substances A to C (43) - (45) on the double strand structure is monitored, after irradiating with light (48), using an evaluation unit (49). The separately readable array positions produce substance-dependent and concentration-dependent signals (50).

Figures 5 to 13 show the measurement curves which were determined by means of difference pulse voltametry as described in the following implementation examples.

Implementation examples:

Example 1: Pretreating the gold electrodes

The Au electrodes are cleaned by polishing the electrode surface with a suspension of 0.3 μm argillaceous earth (LECO, St. Joseph, USA) and subsequently rinsing with Millipore water (10 M Ω cm). The subsequent electrochemical cleaning of the electrodes is effected by means of cyclic voltametry (potentiostat: EG&G PAR 273A, GB) in 0.2M NaOH, with the electrodes firstly being cyclized 5 times between potentials of -0.5 and -1.8 V (against an Ag/AgCl reference electrode (3M NaCl)) and subsequently cyclized 3 times between -0.8 and 1.0 V (rate of advance, 50 mV sec⁻¹). A platinum rod is used as the counterelectrode.

Example 2: Methods for modifying Au electrodes with redox-labeled oligonucleotides

Double-stranded nucleic acids were used for the modification. The hybridization takes place in the stock solution (1 mmol/l solution of the self-complementary single-stranded nucleic acids in water), which nucleic acids were heated at 95°C in a waterbath and slowly cooled down to room temperature within the space of from 2 to 4 hours.

35

Method A: Modifying oligonucleotide monolayers with a redox label

Binding 5'-SH-oligos to gold

The 5'-SH-modified oligonucleotides (Interactiva, Ulm, Germany) (sequences, see table 1) are bound on by incubating the cleaned Au electrodes with a 100 μ M solution of the corresponding oligonucleotide in 1M phosphate buffer (pH 6.6, Calbiochem; addition of 0.5 mM dithiothreitol (DTT, Sigma-Aldrich, Steinheim, Germany) assists the binding-on of ferrocene-modified oligonucleotides), by dripping 5 μ l of this solution onto the gold disk and incubating at 4°C for 4-5 hours in a water-saturated atmosphere. The characterization of the resulting oligonucleotide monolayers is checked by blocking the diffusion-controlled Fe(II/III) redox reaction in aqueous $K_3/K_4Fe(CN)_6$ solution (salts from Merck, Darmstadt, Germany) (20 mM in 20 mM phosphate buffer, pH 7) by means of cyclic voltametry (CV).

Back-filling the monolayer gaps with 1,6-mercaptohexanol

The gaps, which arise due to steric reasons, between the individual oligonucleotide molecules on the monolayer are back-filled by incubating the electrodes, at room temperature for 60-90 minutes, in a 1 mM solution of 6-mercaptohexanol (Aldrich, USA) in (degassed) Millipore water.

Binding the redox label (pyrroloquinolinequinone, Methoxatin or PQQ) to the monolayer-bound oligonucleotides

The linkage of the redox label (PQQ, Sigma-Aldrich, Steinheim, Germany) to the primary amino group of the modified T building block (aminomodifier dT) in the hairpin is achieved by activating the PQQ carboxylate groups using a water-soluble carbodiimide (EDAC, Sigma-Aldrich) and subsequent amide formation. This reaction takes place, at room temperature for 30 - 60 minutes, in a PQQ solution (approx. 1 mg/ml in 10 mM HEPES buffer (pH 8)) in the added presence of 50 mM EDAC.

30

Method B: Synthesizing redox-modified oligonucleotides

The second method of obtaining redox-labeled oligonucleotide monolayers on gold is that of modifying the oligonucleotides with redox-active groups (PQQ and ferroceneacetic acid) in solution and subsequently immobilizing them on the gold surface.

35

Binding-on PQQ:

The PQQ redox functions are linked on by activating the carboxylate functions and subsequently bonding them to the primary amino group of the modified T building block (amino-modifier dT) in the oligonucleotide.

For this, 1.65 mg of PQQ (Sigma-Aldrich, Steinheim, Germany) are dissolved in 25 μ l of Millipore water (0.2M), after which 1.15 mg of N-hydroxysuccinimide (NHS, 0.25M, Sigma-Aldrich) and 6 mg of a water-soluble carbodiimide, i.e. ethyldiaminopropylcarbodiimide (EDAC, 0.5M) are added. After 30 minutes at room temperature, the resulting precipitate is dissolved once again by adding 50 μ l of DMF. After a further 15 minutes, the solution is diluted with a further 145 μ l of DMF.

For modifying the oligonucleotide, 3.7 μ l of Millipore water and 3.2 μ l of triethylammonium hydrogen carbonate solution (TEC buffer, 1M, Fluka, Buchs, Switzerland) are added to 120 μ l of the activation solution. After 10 OD of the amino-modified oligonucleotide have been added, the solution is left to stand at room temperature for 24 hours. The reaction mixture is worked up by size exclusion chromatography through a Sephadex G25 M column (Amersham-Pharmacia Biotech AB, Uppsala, Sweden).

When this is done, the yields of oligonucleotide are as a rule between 70 and 90%. The ferroceneacetic acid-labeled oligonucleotides are synthesized by activating the ferroceneacetic acid with TBTU (Fluka) and subsequently coupling it to the free amino group of the oligonucleotide. For this, 4.9 μ g of the ferroceneacetic acid (Strem Chemicals, Kehl, Germany) are dissolved in 100 μ l of DMF in the first step. 75 μ l of this solution are then added to a solution of 6 mg of TBTU in 75 μ l of DMF and activated at room temperature for 1 hour. 10 OD of the oligonucleotide to be modified (as a 1 mM aqueous solution) are treated with 3.2 μ l of TEC buffer. 25 μ l of the activated ferroceneacetic acid and 95 μ l of DMF are subsequently added. After a reaction time of from 20 to 24 hours at room temperature, the oligonucleotide is purified as described above.

30

The electrodes are modified with the redox-labeled oligonucleotides in accordance with the directions given for the unmodified 5'-thiol-amino-modified oligonucleotides.

35 Example 3: Electrochemical experiments

The redox-modified oligonucleotide monolayers are characterized electrochemically by means of cyclovoltametry (CV) and difference pulse

voltametry (DPV) carried out in 20 mM phosphate buffer, pH 7. In the case of PQQ-modified nucleic acid oligomers bound to a gold electrode, a signal of the reversible redox reaction occurs at a potential of approx. -100 mV (against Ag/AgCl). This shows that the PQQ redox units are bonded covalently, by way of the nucleic acid oligomers, on the modified electrode. The redox signals for ferroceneacetic acid-modified oligonucleotide monolayers occur at potentials of approx. +200 mV (against Ag/AgCl) and are as a rule broader and less intensive than the corresponding PQQ signals.

10

Example 4: Experiments with intercalators:

Incubating PQQ-oligoelectrodes (prepared as described in example 2, method A) in intercalator solutions:

15 The incubation, for a PQQ-Zi6-oligonucleotide electrode **Seq.ID No. 4** (table 1), was carried out in intercalator solution (10 μ M Hoechst 33258 (bisbenzimidazole) (Fluka) in 1M phosphate buffer (pH 6.6)) at room temperature for 20 minutes.

20 Curve (51) in Fig. 5 shows the DPV measurement result provided by a Zi6-PQQ electrode in 20 mM phosphate buffer at pH 6.6 (reductive scan, 10 mV/s, step size 500 ms, pulse width 20 ms, pulse height 25 mV) before the incubation with Hoechst 33258.

Curve (52) shows the DPV measurement result obtained after incubating in 10 μ M Hoechst 33258 in 1M phosphate buffer at RT for 20 min.

The electrochemical comparison of the electrode shows a marked decrease in the PQQ oxidation signal, down to at most 20% (ratio of the peak heights) of the starting value, after incubating in the intercalator solution (Fig. 5). The structure of the oligonucleotide is consequently altered by intercalators such that the electron transfer is strongly inhibited.

30

Example 5: Experiments using DNase I:

35 When subsequently subjected to electrochemical characterization by CV and DPV, incubating a PQQ-modified oligonucleotide electrode (prepared as described in example 2, method A) in a DNase I solution (2U in 100 μ l of 1x T7 transcription buffer, as a kit supplied by Promega, Madison, USA)

for 15 (Fig. 6 curve (62)) and 30 minutes (Fig. 6 curve (63)), respectively, at 37°C is seen to result in almost complete suppression of the PQQ redox signals. The comparison measurement, shown in Fig. 6 curve (61), was performed prior to incubating with DNase I.

- 5 The effect shows clearly that the PQQ-modified oligonucleotide strands are cut by the protein, with it being possible to monitor the chronological course of the reaction: after 30 minutes, the redox signal has declined to 15-20% of the reference signal while it has declined to 5-7% of the signal after 60 minutes.

10

Example 6: Experiments using transcription factors:

- While examples 4 and 5 demonstrate interactions between proteins and DNA which are not sequence-specific, sequence-specific interactions
15 between redox-labeled DNA oligonucleotides and transcription factors are investigated in the present example 6. The oligonucleotide sequences employed are listed in table 1 together with their transcription factors (TCF) which bind to them specifically.

- The SP1 transcription factor (as an extract prepared from E. coli) was
20 kindly provided by Prof. Suske (Marburg University), while the Oct2a TCF was purchased as a kit containing buffer solutions (DIG Gelshift kit) from Roche. HeLa-Scribe Nuclear Extract (Gel Shift Assay Grade, containing SP1 TCF as a constituent), likewise in the form of a kit (Gel Shift Assay Systems), and TATA binding protein were purchased from Promega.

- 25 Incubating a PQQ-Zi-3-oligonucleotide electrode, containing a **Seq. ID No. 1** oligomer, in an Oct2a TCF solution (containing 5 µl of Oct2a/90 µl of buffer; composition as stated by the manufacturer) still gave no change in the DPV redox signal after 60 minutes at room temperature (Fig. 7). Curve (71) in Fig. 7 depicts the measurement before adding Oct-II, while curve
30 (72) shows a measurement 30 minutes after adding Oct-II, and curve (73) shows a measurement 60 minutes after adding Oct-II; in no case was there any significant change in the height of the signal. Consequently, nonspecific interactions can be excluded. Incubating the same electrode in an SP1 TCF solution (10% Suske SP1 extract (10 µl) in Roche kit binding
35 buffer (90 µl)) resulted, after an incubation time of only 30 minutes, in a significant decrease in the redox signal (Fig. 8 curve (81), oxidation signal before incubating with SP1, curve (82), oxidation signal after incubating the electrode with SP1 solution for 30 minutes), which redox signal decreased

markedly still further after a further 90 minutes of incubation (Fig. 8 curve (83)). Quantitative assessment of the peak height ratios and area ratios indicated that the signal decreased down to 50% after the first incubation and decrease still further down to 30-35% after the second incubation phase.

Whereas Oct2a does not elicit any change in the redox behavior of the SP1-specific oligonucleotide monolayer electrode, the current flow is markedly decreased when incubating with the SP1 transcription factor. This constantly demonstrates the specific interaction between the SP1 TCF and the oligonucleotide strands (Zi-3 **Seq. ID No. 1** has an SP1-specific recognition sequence; Zi-5 **Seq. ID No. 3** was used as an alternative).

In addition, experiments were carried out using ferroceneacetic acid-modified DNA probes. The DNA electrodes were prepared as described in method B in example 2 and applied to the gold electrode. The SP1-specific probe (Zi-3 **Seq. ID No. 1**) was incubated for 90 minutes with HeLa-Scribe Nuclear Extract (5.5 mg/ml, Gel Shift Assay Grade, Promega) in analogy with the direction supplied by Promega (60 µl of water, 20 µl of 5x Gel Shift Binding Buffer, 20 µl of nuclear extract). This resulted in a marked decrease (of the order of size of 45-50%) in the redox signal (Fig. 9 curve (92)) being detected when compared to the reference measurement made prior to the incubation (Fig. 9, curve (91)).

The experiment using TATA-binding protein (10 fpu, Promega (10 µl)) was carried out in an analogous manner. A small nonspecific decrease in the redox peak (Fig. 10, curve (102)) was observed, as compared with the reference measurement made at incubation (Fig. 10, curve (101)), when double-stranded nucleic acid probes were used which contained the Zi-3 sequence (table 1, **Seq. ID No. 1**). When a TATA-specific sequence (Zi-4 **Seq. ID No. 2**, table 1) was incubated with the TATA-binding protein (10 fpu) for 40 and 70 minutes, it was possible to observe a marked decrease down to 75% and 60% of the starting signal, respectively (Fig. 11 curve (112)) after 40 minutes of incubation, curve (113) after 70 minutes of incubation, curve (111) reference measurement prior to incubating with TATA-binding protein). The control experiment using nuclear extract and a TATA-specific DNA probe (Zi-4 **Seq. ID No. 2** (table 1)) only led to a change in the shape of the peak (Fig. 12, curve (121) shows the redox signal prior to the incubation, while curve (122) shows the redox signal after

incubating with a HeLa cell extract solution for 30 minutes) and not to any decrease in the redox signal.

5 In a further experiment, the influence of TATA-binding protein (TFIID) on electron transfer in immobilized oligonucleotides containing a specific recognition sequence (Zi-4 **Seq. ID No. 2**) was investigated.

For this, a cleaned gold electrode was treated with 100 μ M Zi-4-ferroceneacetic acid in 0.9M phosphate buffer (+ 1 mM DTT); incubation
10 time: 4 hours at 4°C. The monolayer gaps were passivated with mercaptohexanol (1 mM solution in H₂O) at RT for 1 hour.

The measurement is carried out in an electrolyte solution (20 mM potassium phosphate buffer, pH 7.0) by means of DPV (pulse height 25 mV, rate of advance 10 mV/s, pulse duration 20 ms, step size 500 ms,
15 potential against Ag/AgCl (3M NaCl)).

Incubating the electrode in pure GelShift Binding Buffer (Promega) is used for performing control measurements, with the proportion of TFIID protein in the incubation solution being varied (from 1 μ l of TFIID + 90 μ l of binding
20 buffer to 10 μ l of TFIID + 90 μ l of binding buffer) and the corresponding change in the electrochemical signal being determined.

The pure binding buffer only has a negligible influence on the height of the DPV redox peak. (Fig. 13, curve **(131)** shows the ferrocene reduction signal from the reference measurement (after incubating with binding buffer for 30
25 minutes) and curve **(132)** shows the redox signal of the electrode after incubating the binding buffer for a further 20 minutes); there is only a very slight decrease in the signal heights. After incubating the electrode in TFIID protein solution (1 μ l of TFIID + 90 μ l of binding buffer) for 20 minutes, there is a significant decrease in the DPV signal height (Fig. 13, curve
30 **(133)**), which decrease is to be attributed to specific interactions between the restriction enzyme and the binding site in the oligonucleotide strand.

When the incubation time is the same, increasing the enzyme concentration results in a greater decrease in the signal. Fig. 13, curve
35 **(137)** shows the effect on the reduction signal of incubating the electrode for 20 minutes in 5 μ l of TFIID + 90 μ l of binding buffer, while curve **(138)** shows the corresponding signal obtained after incubating for 20 minutes in 10 μ l of TFIID + 90 μ l of binding buffer).

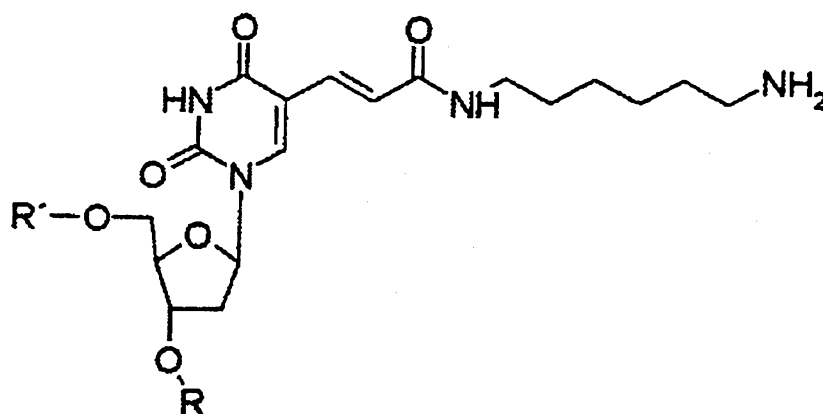
In addition to measuring the effect of the concentration of the transcription factor, it was also possible to measure the chronological dependence of the binding of the transcription factor TFIID to the double-stranded nucleic acid probes. Thus, the reduction signal produced by a solution consisting of 1 μ l of TFIID + 90 μ l of binding buffer declines still further after 40 minutes of incubation (Fig. 13, curve (134)); after that, an equilibrium becomes established under the given reaction conditions, with the height of the signal not changing any further over time (Fig. 13, curve (136) shows the reduction signal produced by a solution consisting of 1 μ l of TFIID + 90 μ l of binding buffer after 70 minutes of incubation, while curve (135) shows the reduced signal after 160 minutes of incubation). Fig. 13, curve (139) shows the increase in binding events resulting from a solution consisting of 10 μ l of TFIID + 90 μ l of binding buffer after a total of 40 minutes of incubation as compared with incubating a solution of the same concentration for only 20 minutes (curve (138)).

Table 1 Sequences of the oligonucleotides (Interactiva, Ulm, Germany) employed

Oligo	Sequence (5' $\rightarrow$ 3')	specific for
Zi-3	ATT CGA TCG GGG CGG GGC GAG CTT XTT GCT CGC CCC GCC CCG ATC GAA T (Seq.ID No. 1)	Sp1
Zi-4	GCA GAG CAT ATA AGG TGA GGT AGG ATT TXT TCC TAC CTC ACC TTA TAT GCT CTG C (Seq. ID No. 2)	TATA
Zi-5	ATT CGA TCG GGG CGG GGC GAG CTT TTX GCT CGC CCC GCC CCG ATC GAA T (seq. ID No. 3)	Sp1
Zi-6	GCA GAG CAT ATA AGG TGA GGT AGG AXT TTT TCC TAC CTC ACC TTA TAT GCT CTG C (Seq. ID No. 4)	TATA

In the oligomer sequences in table I, X represents a commercially available aminomodifier dT building block (formula I); the nucleotide building block which is located at the 5' end of the oligomer is thiol-modified on C6 (obtainable from Interactiva).

5



Formula I

Patent claims:

1. A nucleic acid probe which comprises a nucleic acid sequence which is bound to a conductive surface, which is at least partially double-stranded and to which at least one electron donor unit or at least one electron acceptor unit is bound.
5
2. A nucleic acid probe as claimed in claim 1, wherein the bound-on double-stranded nucleic acid probes are composed of two complementary nucleic acid molecules.
10
3. A nucleic acid as claimed in claim 1, wherein the bound-on double-stranded nucleic acid probes are composed of nucleic acid sequences which contain self-recognizing domains.
15
4. A nucleic acid probe as claimed in one of claims 1 to 3, wherein the double-stranded nucleic acid probes possess the same nucleic acid sequence.
- 20 5. A nucleic acid probe as claimed in one of claims 1 to 3, wherein the double-stranded nucleic acid probes possess different nucleic acid sequences.
- 25 6. A nucleic acid probe as claimed in one of claims 1 to 5, wherein the double-stranded nucleic acid probes are applied to a conductive array surface.
7. A nucleic acid probe as claimed in one of claims 1 to 5, wherein the double-stranded nucleic acid probes are applied to an unstructured conductive surface.
30
8. A nucleic acid probe as claimed in one of the preceding claims, wherein the double-stranded nucleic acid probes are stabilized by bridge bonds.
35
9. A nucleic acid probe as claimed in one of the preceding claims, wherein the double-stranded nucleic acid probes are stabilized by disulfide bridges.

10. A nucleic acid probe as claimed in one of the preceding claims, wherein the double-stranded nucleic acid probes contain double-stranded DNAs, double-stranded RNAs or DNA-RNA hybrids.
- 5 11. A nucleic acid probe as claimed in one of the preceding claims, wherein regeneratable and/or inducible electron donor units or electron acceptor units are covalently bonded on the nucleic acid.
- 10 12. An aggregate which comprises double-stranded nucleic acid probes as claimed in one of the preceding claims 1 to 11 and substances which are selected from the group of substances which interact directly with double-stranded nucleic acids.
- 15 13. An aggregate as claimed in claim 12, wherein the substance which interacts directly is a protein and/or a peptide and/or a single-stranded and/or a double-stranded nucleic acid and/or a mimetic and/or an intercalating and/or a nucleic acid-damaging substance and/or a cytostatic agent.
- 20 14. An aggregate as claimed in claim 12 or 13, wherein the aggregate comprises additional substances which interact indirectly.
- 25 15. A method for detecting interactions between double-stranded nucleic acids and substances which interact with them, which comprises
a) probes as claimed in claims 1 to 11 are exposed to a reaction medium, and
b) the generated change in electron flow through the probes is measured.
- 30 16. The method as claimed in claim 15, wherein the reaction medium is a solution which contains at least one interacting substance.
- 35 17. The method as claimed in claim 16, wherein the reaction medium is a solution which contains at least one interacting polymerase, transcription factor, repressor, enzyme and/or its inhibitor and/or activator and/or mimetic and/or intercalating and/or nucleic acid-damaging substance and/or cytostatic agent.

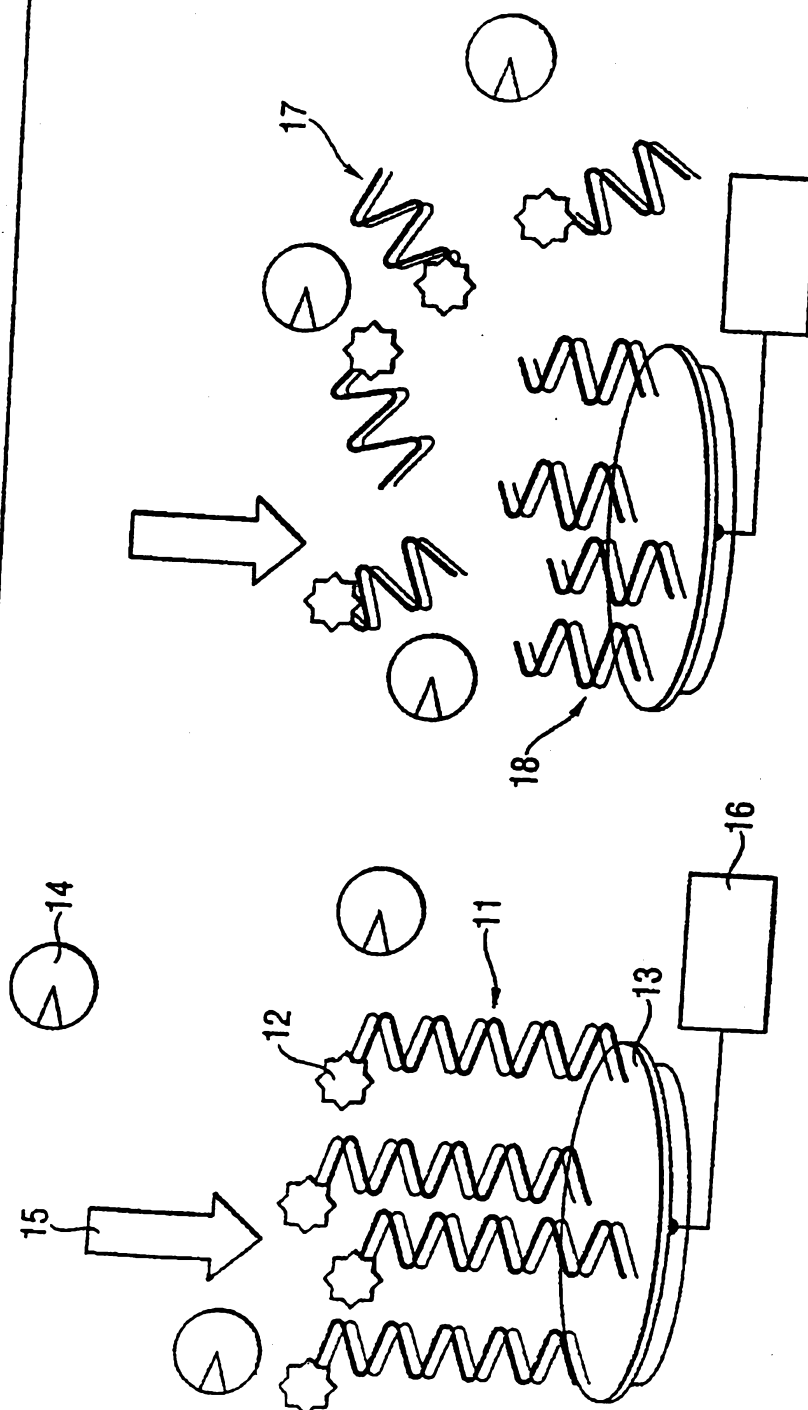
18. The method as claimed in claims 15 to 17, wherein the reaction medium contains single-stranded and/or double-stranded nucleic acids.
- 5 19. The method as claimed in claim 15, wherein the reaction medium contains DNA-damaging or carcinogenic substances.
20. The method as claimed in one of claims 15 to 19, wherein the reaction medium is an aqueous, salt-containing solution.
- 10 21. The method as claimed in one of claims 15 to 20, wherein the reaction solution is buffered.
22. The method as claimed in one of claims 15 to 21, wherein the detection takes place at a temperature between 4 and 40°C.
- 15 23. The use of the double-stranded nucleic acid probes as claimed in one of claims 1 to 11 for detecting interactions, equilibrium constants, competitive effects or reaction rates between double-stranded nucleic acids and substances which interact with them directly and/or indirectly.
- 20 24. An analytic agent which comprises double-stranded nucleic acid probes as claimed in claims 1 to 11 for identifying factors which exert an indirect or direct effect on the double-stranded nucleic acid probe.
- 25 25. An analytic agent which comprises double-stranded nucleic acid probes as claimed in claims 1 to 11 for identifying nucleic acid sequences which interact with substances which interact directly or indirectly.
- 30 26. A method for preparing double-stranded nucleic acid detectors which comprises:
- 35 a) synthesis of a single-stranded nucleic acid,
b) intermolecular hybridization with a nucleic acid which contains a complementary domain,
c) covalent bonding of the electron donor units or electron

- acceptor units to the nucleic acid, and
- d) covalent bonding of the nucleic acid to a conductive surface.

27. A method for preparing double-stranded nucleic acid detectors
5 which comprises:
- e) synthesis of a single-stranded nucleic acid which contains self-recognizing domains which are separated by way of a bridge,
- f) intramolecular hybridization of the self-recognizing domains of the single-stranded nucleic acid,
- 10 g) covalent bonding of the electron donor units or electron acceptor units to the nucleic acid, and
- h) covalent bonding of the nucleic acid to a conductive surface.
28. The method for preparing double-stranded nucleic acid detectors as
15 claimed in claim 27, wherein the bridge is composed of at least one nucleotide.
29. The method for preparing double-stranded nucleic acids as claimed
20 in claim 27, wherein an artificial, at least monoatomic bridge, a disulfide bridge, a stilbenedicarboxyamide bridge, a ruthenium complex bridge, a hexaethylene glycol bridge, a terephthalimide bridge, a branched or unbranched diol bridge, a 3'-aminomodifier-CPG bridge or a branched phosphoramidite bridge is introduced.

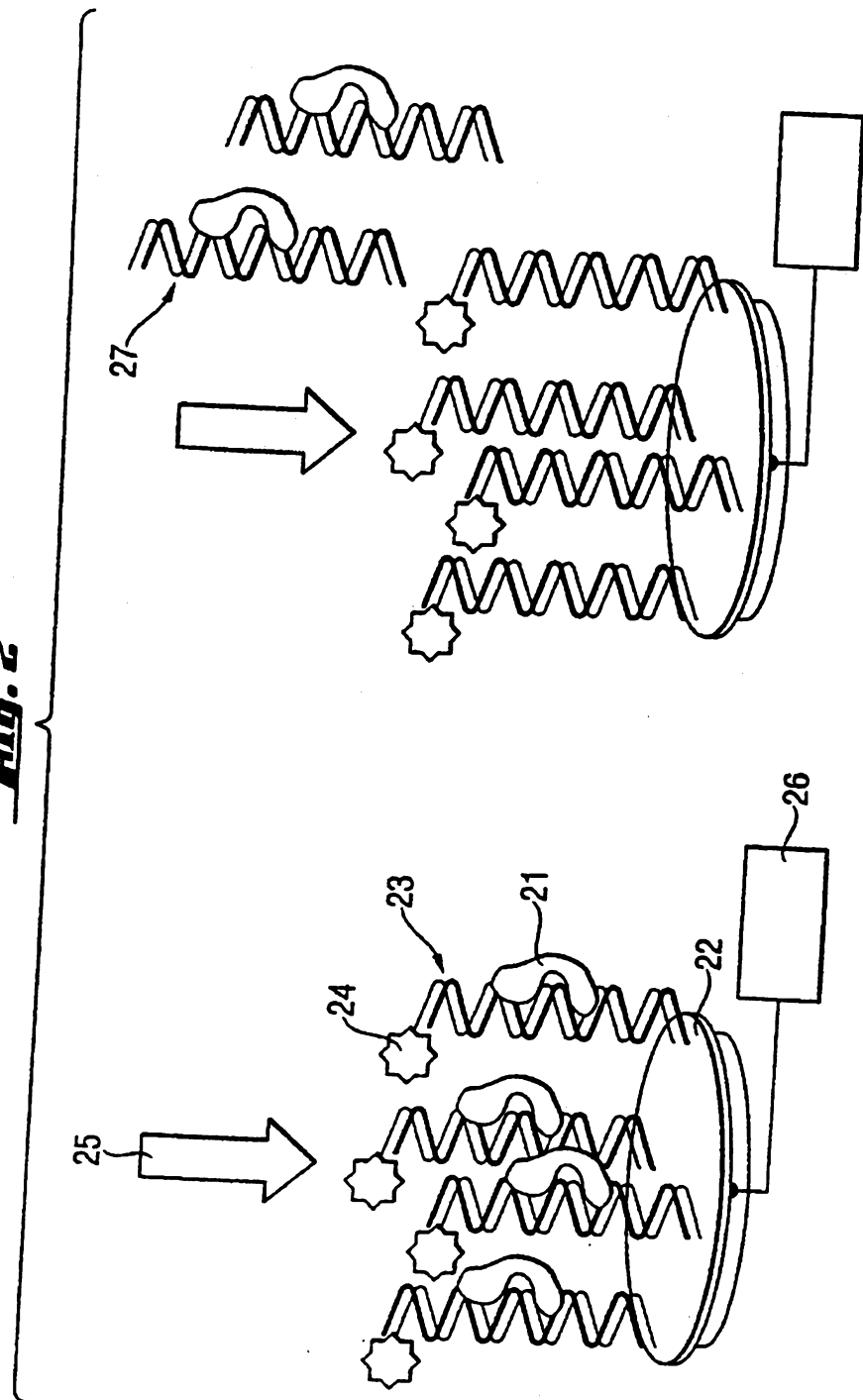
1/13

Fig. 1



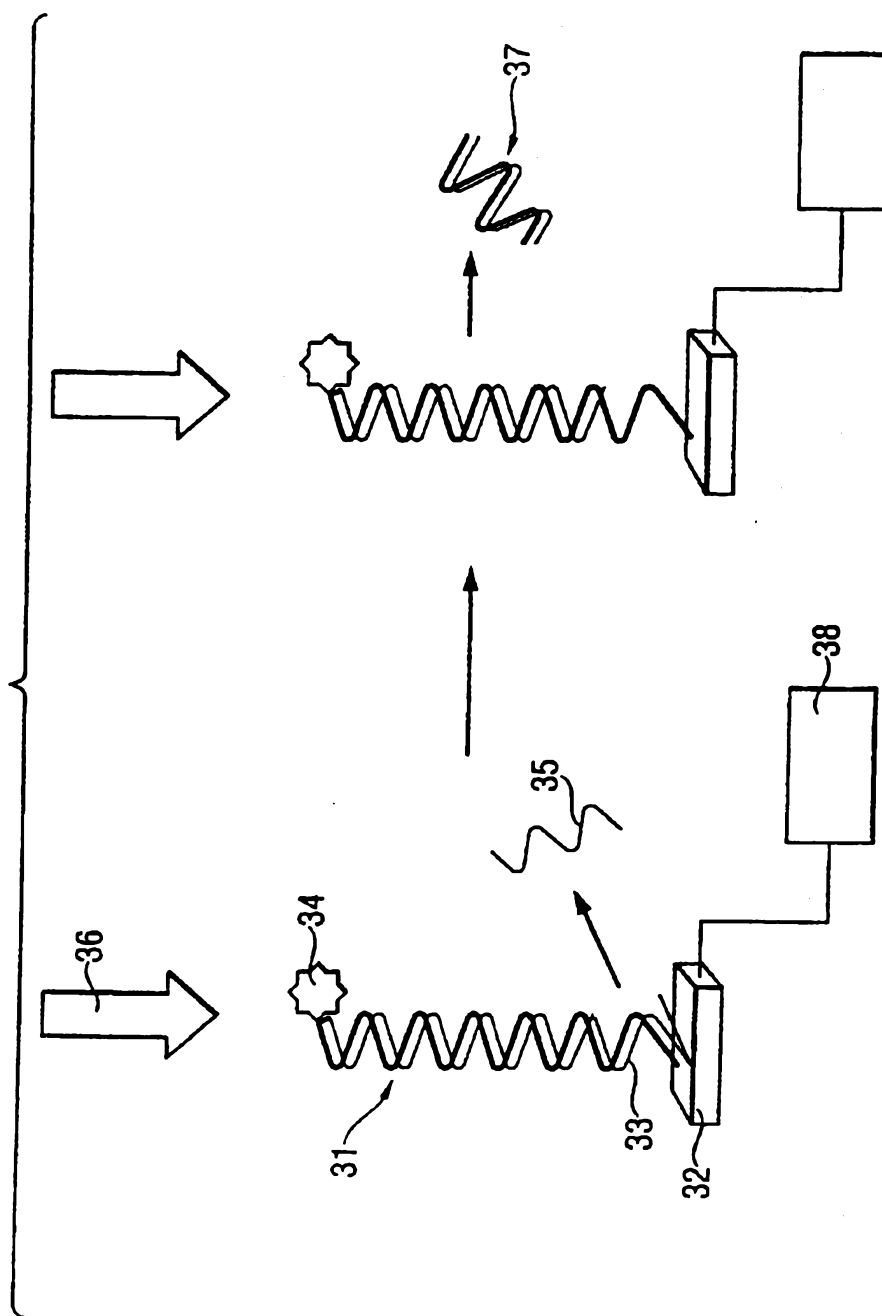
2/13

Fig. 2



3/13

Fig. 3



4/13

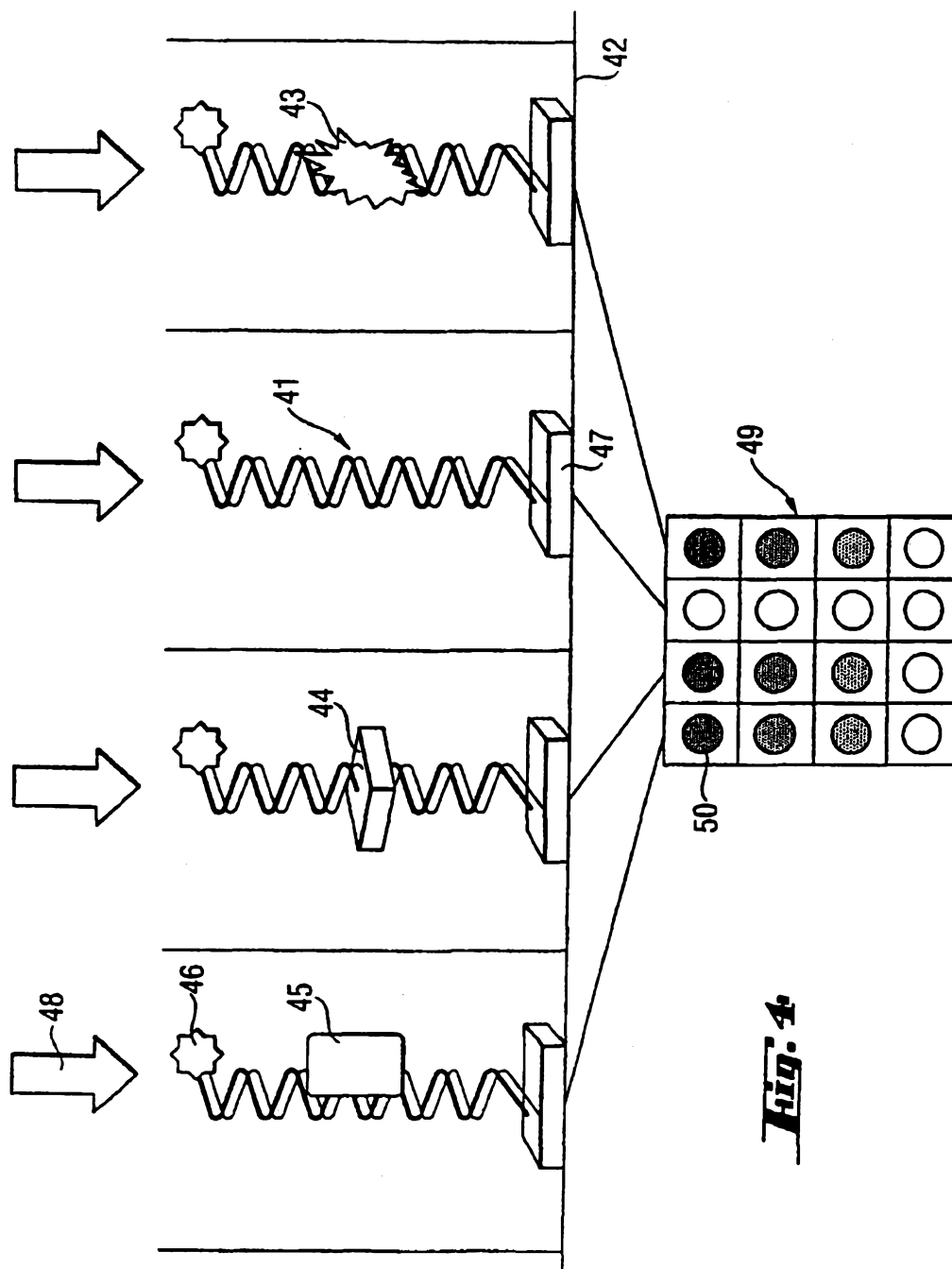


Fig. 4

5/13

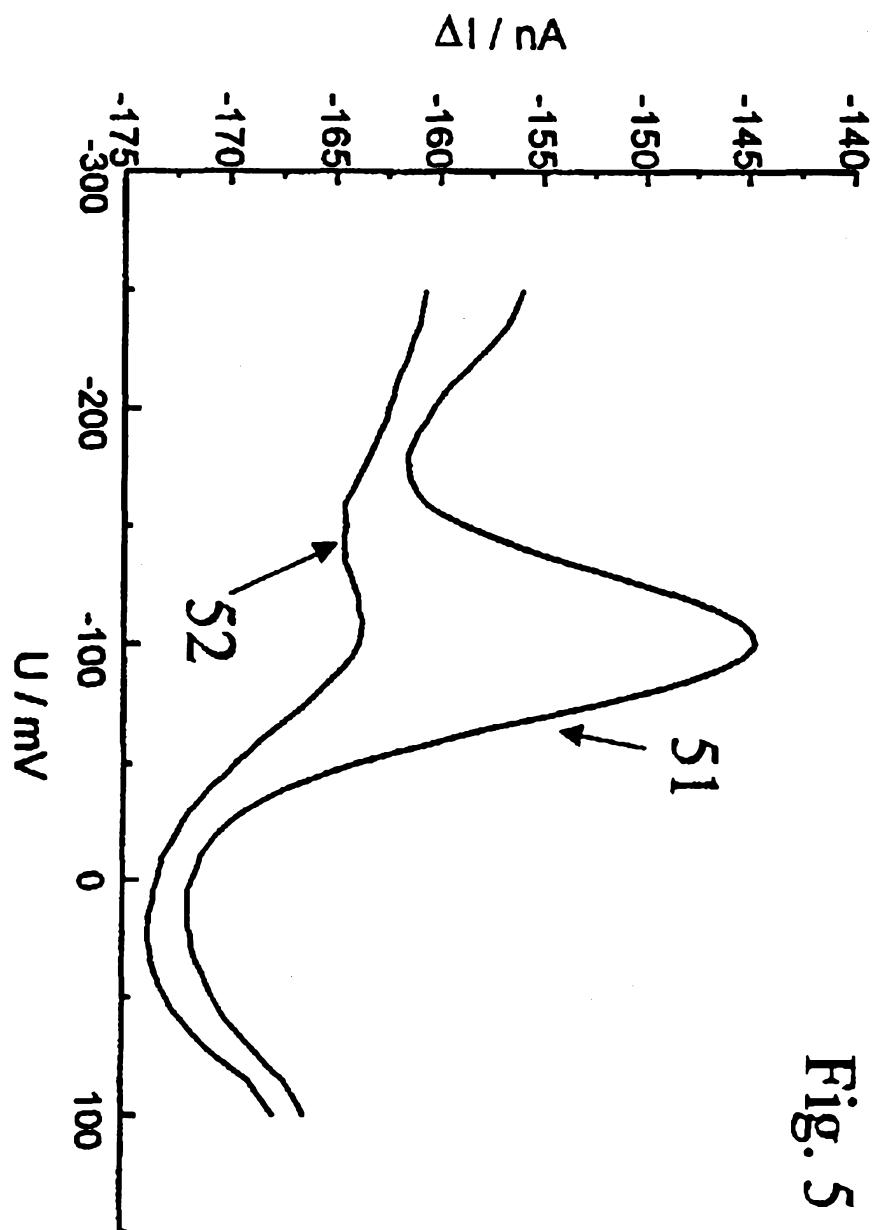


Fig. 5

6/13

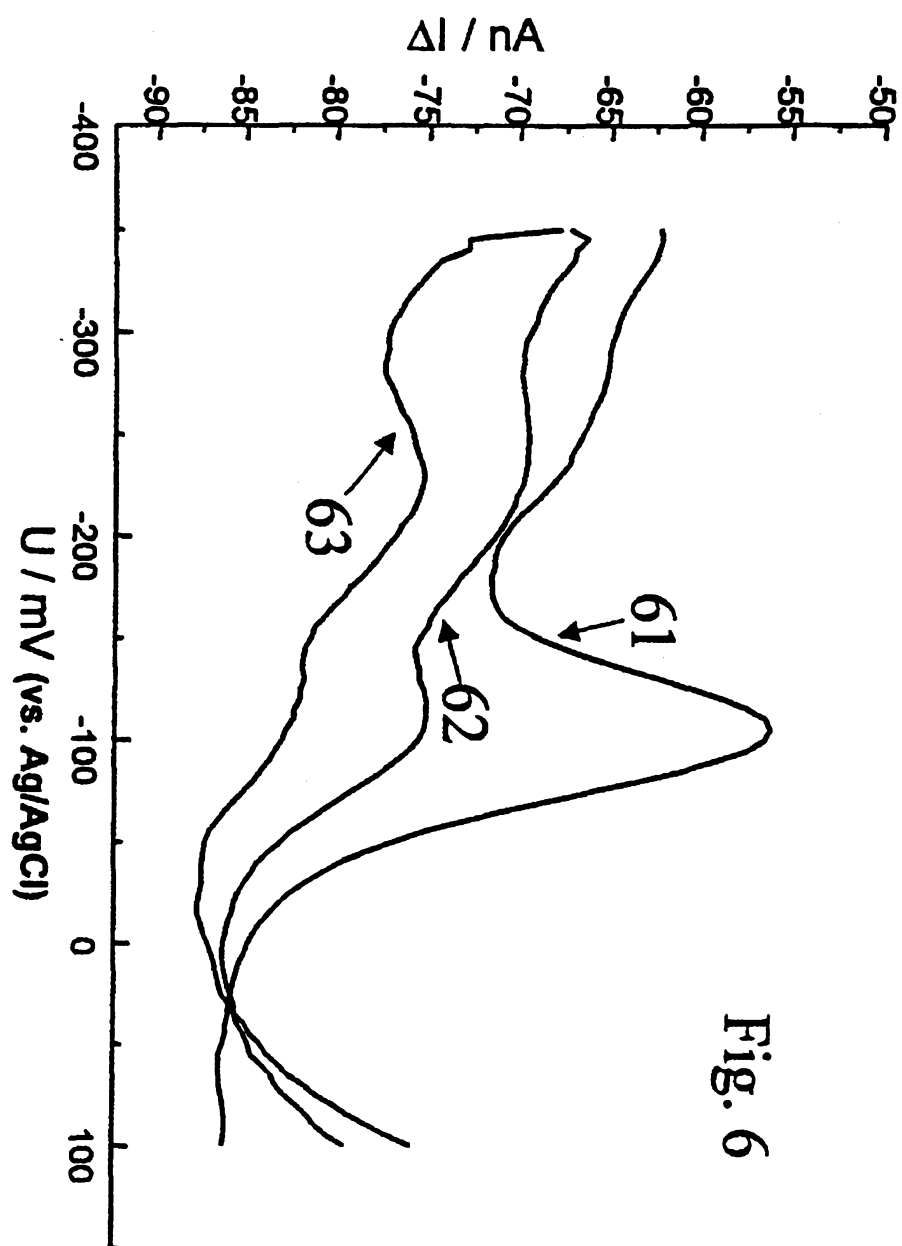


Fig. 6

7/13

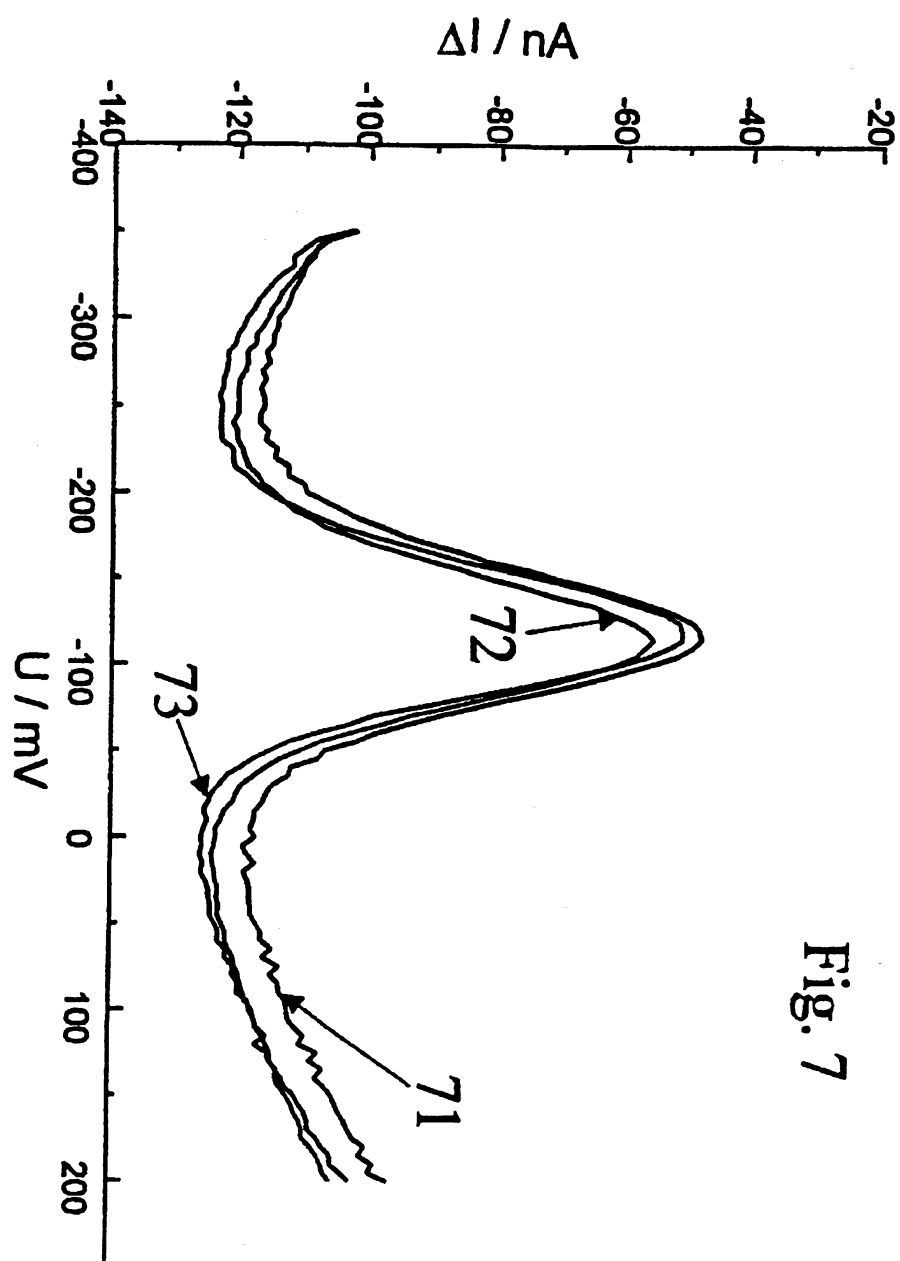


Fig. 7

8/13

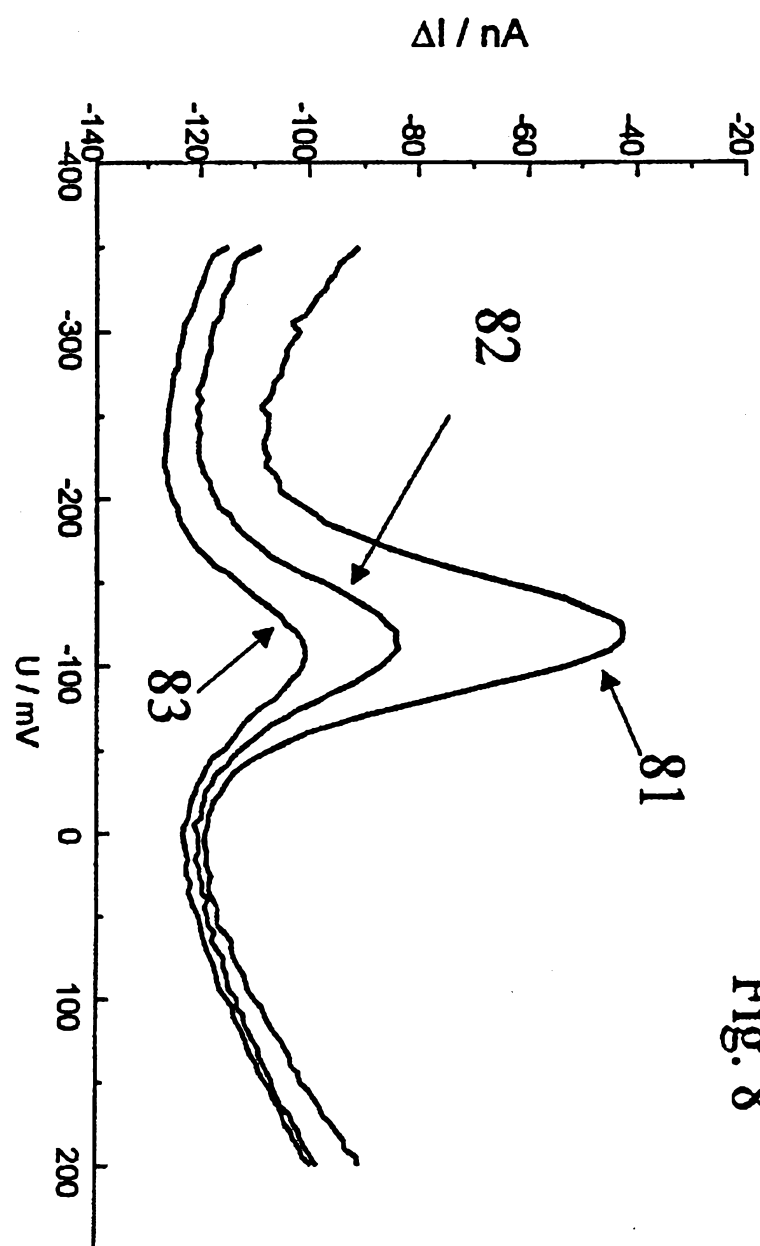
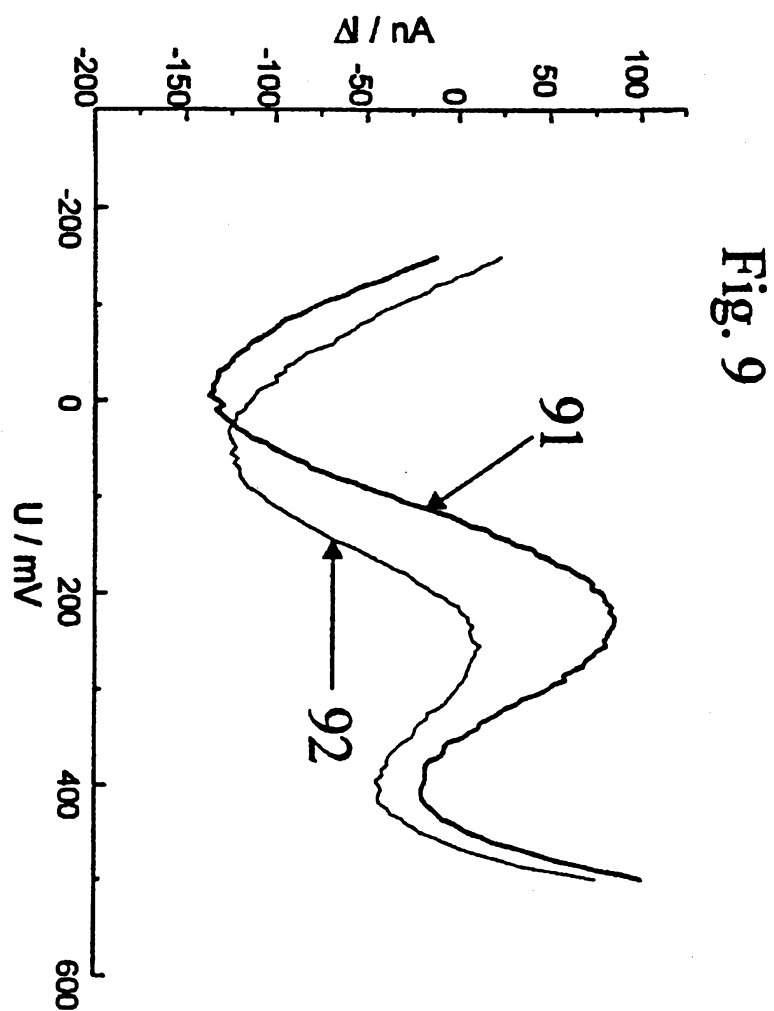


Fig. 8

9/13



10/13

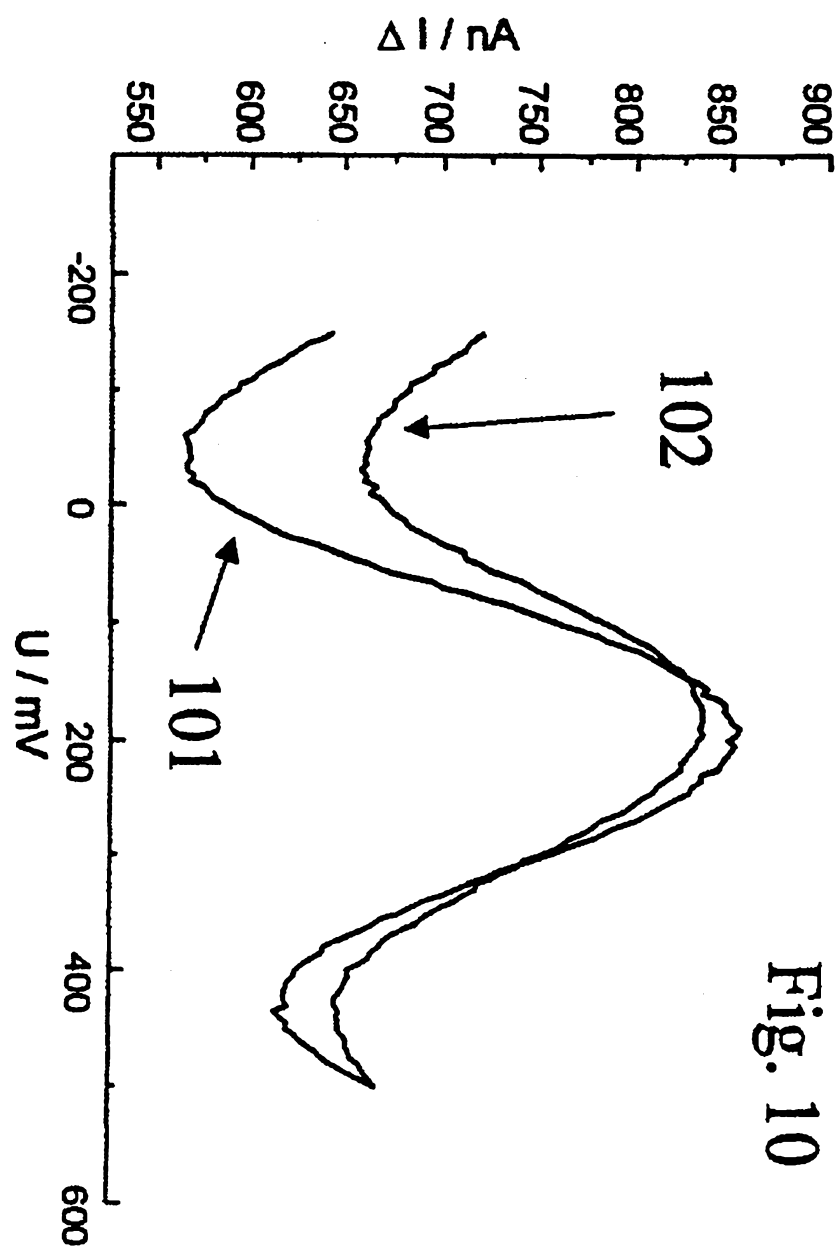
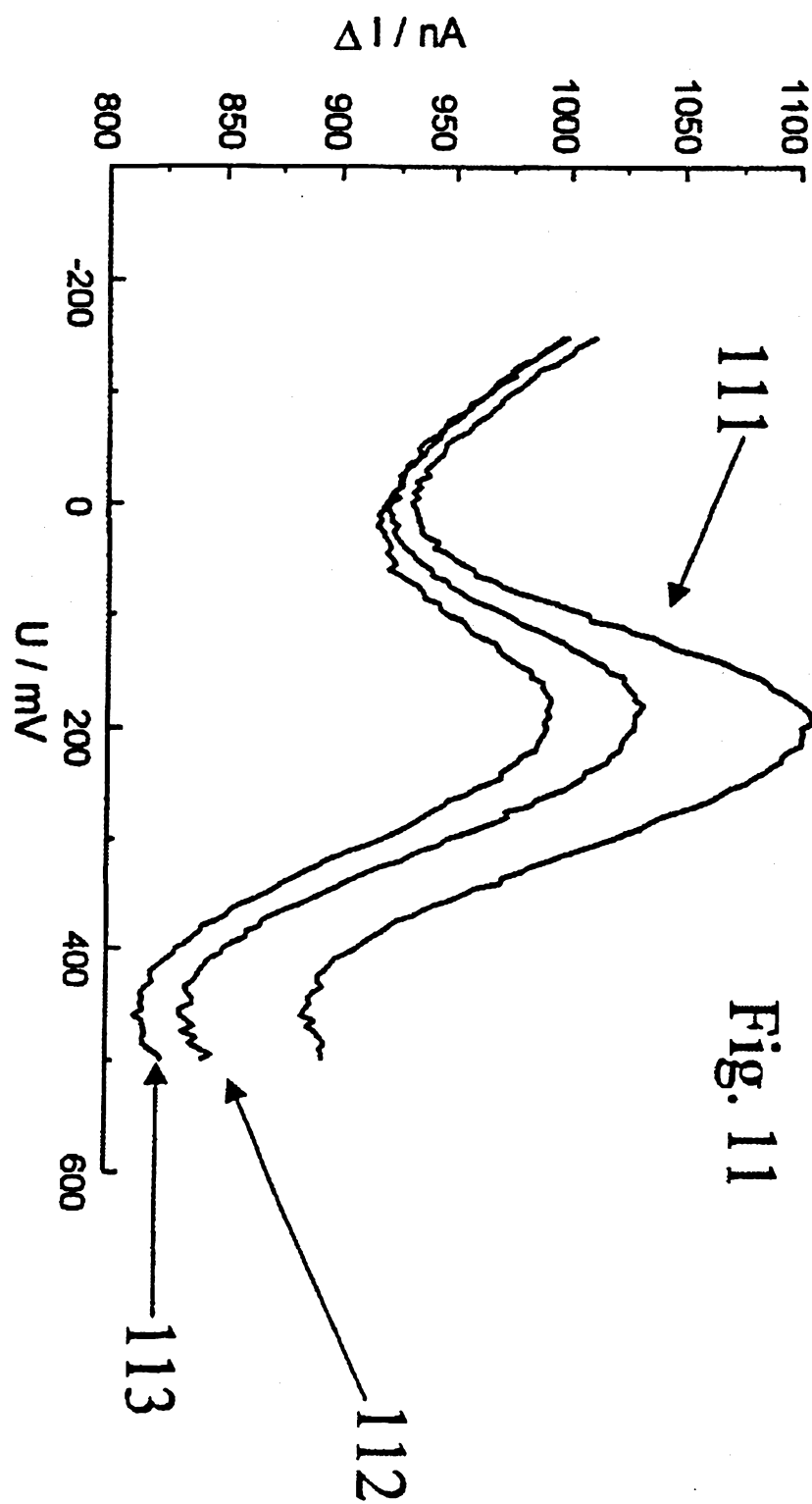


Fig. 10

11/13



12/13

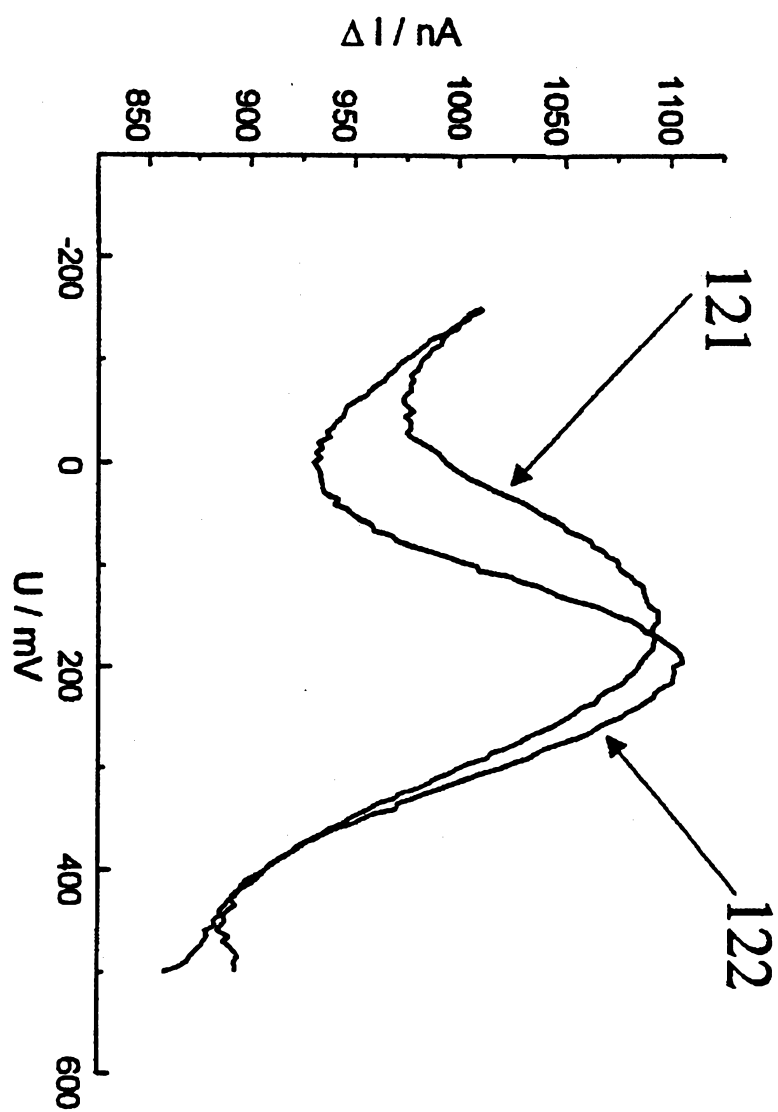
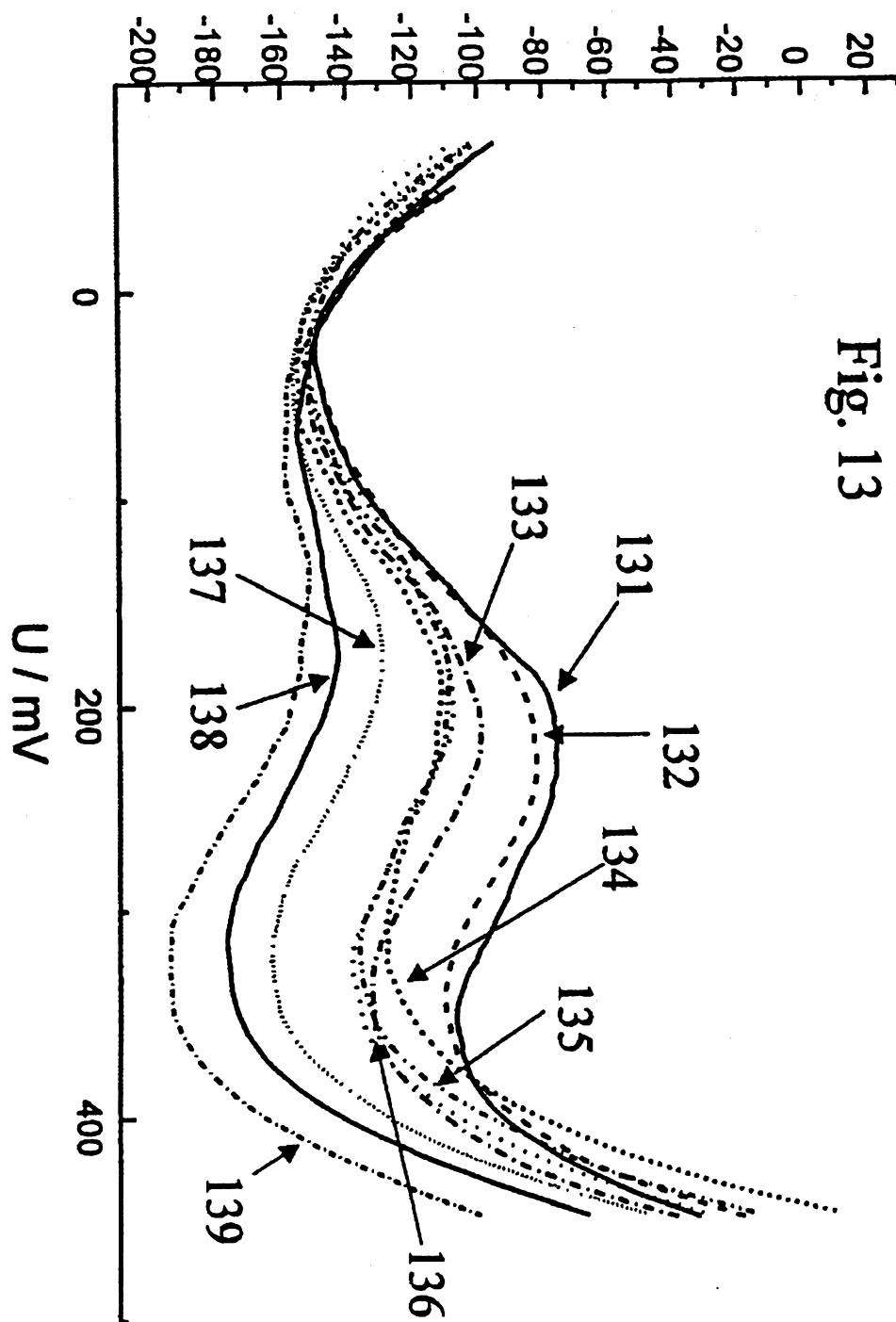


Fig. 12

13/13

Fig. 13



SEQUENCE LISTING

<110> Aventis Research & Technologies GmbH & CO KG

5 <120> Double-stranded nucleic acid probes and their use

<130> HOE 1999/F 050

<140> 19950969.7-41

10 <141> 1999-10-22

<160> 4

<170> PatentIn Ver. 2.1

15

<210> 1

<211> 49

<212> DNA

<213> Artificial sequence

20

<220>

<223> Description of the artificial sequence:

Detector sequence containing an aminomodifier
dT building block at position 25

25

<400> 1

attcgatcgg ggcggggcgga gcttttgcg cgcggcgccc cgatcgaat 49

<210> 2

30 <211> 55

<212> DNA

<213> Artificial sequence

<220>

35 <223> Description of the artificial sequence:

Detector sequence containing an aminomodifier
dT building block at position 29

<400> 2

40 gcagagcata taaggtgagg taggattttt tcctacctca ccttatatgc tctgc 55

<210> 3

<211> 49

<212> DNA

45 <213> Artificial sequence

<220>

<223> Description of the artificial sequence:

Detector sequence containing an aminomodifier
dT building block at position 27

5

<400> 3

attcgatcgg ggcggggcga gcttttgct cgccccgccc cgategaat 49

<210> 4

10 <211> 55

<212> DNA

<213> Artificial sequence

<220>

15 <223> Description of the artificial sequence:

Detector sequence containing an aminomodifier
T building block at position 26

<400> 4

20 gcagagcata taaggtagg taggattttt tcctacctca cttatatgc tctgc 55

Abstract:

Double-stranded nucleic acid probes and their use

- 5 The present invention relates to electronically readable double-stranded nucleic acid probes and their use for rapidly and simply detecting interactions between double-stranded nucleic acids and factors which interact with them directly or indirectly, and to methods for preparing the double-stranded nucleic acid probes.