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(54) **Coating of graphene.**

(57) The present invention is in the field of highly crystalline graphene and coating said graphene with a layer. Said graphene may have further structures, such as nanopores, nanogaps, and nanoribbons. The coated graphene can be used for biomolecular analysis and modification, such as DNA-sequencing, as a sensor, etc. The invention therefor also relates to use of coated graphene.

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Coating of graphene

FIELD OF THE INVENTION

The present invention is in the field of graphene and coating said graphene with a layer. Said graphene may have further structures, such as nanopores, nanogaps, and nanoribbons. The coated graphene can be used for biomolecular analysis and modification, such as DNA-sequencing, as a sensor, etc. The invention therefor also relates to use of coated graphene.

BACKGROUND OF THE INVENTION

Graphene is carbon comprising material. Its structure relates to one-atom-thick planar sheets of sp2-bonded carbon atoms that are crystallographically densely packed in a honeycomb crystal lattice. The crystalline or "flake" form of graphite consists of many graphene sheets stacked together.
It can be a basic building block for graphitic materials of all other dimensionalities. It can be wrapped up into fullerene, rolled into 1D carbon nanotubes or stacked into 3D graphite.

Graphene has attracted a lot of research interest because of its promising electronic applications related to its theoretical superior electron mobility, mechanical strength and thermal conductivity. It may have wide range of applications, for instance, field-effect transistors, photonic or optoelectronic device, as a gas or liquid membrane, sequencing DNA through nano-holes in graphene etc. Graphene macroscopic samples have unusual properties such as a bipolar-transistor effect, ballistic transport of charges, large quantum oscillations, etc.

Most of these applications demand modification of a graphene sheet into specific nano-patterns. In general production methods of graphene do not provide a monolayer thereof; at the best islands of a monolayer are obtained.

Nanopores are heavily studied for single-molecule screening and DNA sequencing. Because graphene may be in the form of layers of only one atom thin and may have excellent electrical properties, it is regarded as a potential successor to biological and silicon-based nanopores.

For nanopore-based DNA single-molecule analysis and sequencing a nanopore, a tiny hole in a membrane, can in principle be used as a nanoscale recorder that scans a DNA molecule

from head to tail to ultimately read off the genetic information, for example using the ion current passing through the pore to probe the identity of the base. In the last decade, many groups have developed strategies to detect DNA molecules using nanopores to understand the biophysics of DNA translocation. Only very recently, it was demonstrated that biological nanopores can be used to obtain sequence information if a DNA polymerase is used to slowly ratchet the DNA through the pore. Recently, graphene nanopores were introduced. In principle crystalline graphene forms an ultimate nanopore membrane since it would be a hexagonal carbon sheet with a thickness of only a single atom, yet it is fully preventing ion transport across the membrane. Furthermore, it is electrically conductive, which opens up new modalities of directly probing the chemical nature of the bases, for example by running a tunneling current through the DNA molecule that is traversing a graphene gap.

A problem with nanostructures such as nanopores is, especially when analyzing biomolecules such as DNA, that pores tend to clog and biomolecules may stick either to membranes or in pores. As a result an analysis is at best incomplete and more likely impossible. A further problem with nanopores and the like is that dimensions thereof are poorly defined. Further also at an edge thereof the graphene is no longer a monolayer, but typically a multilayer (5-10 layers), e.g. due to processing. The edges themselves also are irregular, for instance not crystalline any more. As a consequence no reliable results, e.g. in terms of conductance, electrical current, etc. can be obtained. The effect of the above is that the nanostructures made are worthless.

Various patent documents and scientific documents recite coating of nanotubes, such as with enzymes. It is a purpose of the coating to functionalize the nanotubes. As a consequence characteristics of the nanotubes themselves changes.

Various patent documents and scientific documents recite coating of graphene oxide, which oxide is of a different nature. An objective may be to provide dispersions of graphene (oxide) in polar solvents. It is noted that one of the characteristics of an oxide is that it is not conducting or at the most semi-conducting (electrically).

Various documents focus on self-assembled layers, specifically on metallic surfaces. Typically organosilane functionalization is used, which is in principle a method that is only applicable with reduced graphene oxide where graphene defects allow bond formation with the silane.

Various documents recite interaction of a coating (molecule) which relies on presence of defects in a structure of a surface to be coated. As a consequence characteristics of the structure are altered, typically adversely.

The present invention therefore relates to a graphene layer and coating said graphene with a layer, which overcomes one or more of the above disadvantages, without jeopardizing functionality and advantages.

SUMMARY OF THE INVENTION

The present invention relates in a first aspect to a method according to claim 1, in a second aspect to a graphene layer comprising at least one mono-layer of molecules, in a third aspect to a device comprising said graphene layer, in a fourth aspect to use of said graphene layer comprising at least one mono-layer of molecules, in a fifth aspect to a functionalized graphene layer comprising a mono-layer of molecules, and in a sixth aspect to a method of translocating single strand DNA using said graphene layer.

For a present application, it has been found crucial to understand and block strong interactions between DNA and graphene. Inventors here demonstrate a novel scheme to prevent DNA-graphene interactions, based on a tailored self-assembled monolayer. For bare graphene, inventors have identified a remarkable phenomenon: it has been found that the better the crystallographic quality, the stronger DNA clogging of the pore is induced. Inventors developed a general strategy to tailor an in principle hydrophobic surface of e.g. graphene nanopores by designing a dedicated self-assembled monolayer of molecules, such as pyrene ethylene glycol, rendering its surface hydrophilic. Inventors demonstrate that this prevents DNA from clogging graphene nanopores and show that single-stranded DNA can now be detected and analyzed while at the same time maintaining excellent nanopore durability and reproducibility.

It has been identified that to pursue the present approach in some aspects thereof, it is important to maintain the crystallinity of graphene *right up to* (and preferably including) the edges of a nanostructure such as a nanopore and the like. The edges themselves may be considered as irregular structures, in that by absence of carbon full crystallinity is lost. The present invention relates to nanostructures that, apart from a (one carbon atom) wide edge (region), the crystallinity of the graphene is largely without defects. The present invention in particular relates to nanostructures that are crystalline in an area of 0.3-10 nm from the edge. As such well-defined, highly crystalline, monolayers of graphene are provided, having no hilly structures (e.g. multi layers) near and/or at the edge. As a consequence the present graphene is fully covered up to the edge of a nanostructure with the present monolayer. In fact, future graphene devices - those for instance involving nanogaps or nanoribbons - were theoretically predicted to have sequencing capabilities if, and only if, graphene remains unaltered electrically and this is realized by conserving its crystallinity up to the edges. It is noted that the edge is considered to be a critical part in such devices, e.g. in terms of crystallinity. If the nanostructures are not provided in a controllable, reproducible and reliable manner, a device will have varying and not very predictable characteristics, which is undesired in many instances. Such devices could to some extent function, but would have to be calibrated individually. Present inventors identified that DNA translocation of single-stranded and double-stranded DNA is in fact much more difficult when graphene nanopores are clean and crystalline, due to severe clogging and sticking of DNA. A general approach is developed to modify functionality of graphene with (indirect) binding of functional groups. Especially binding is targeted, rather than a direct functionalization of graphene, in order to prevent irreversible electrical damage to the graphene. First of all a monolayer on graphene with present first molecules is formed, and then the first molecules are reacted with second molecules, the second molecules optionally functionalizing the monolayer. As mentioned, the graphene remains unaltered. As such the

graphene can be functionalized, or likewise tailored, with respect to e.g. a solvent, such as an aqueous solvent, an oily solvent, an organic solvent, and with respect to molecules present in such a solvent, such as biomolecules, and with respect to a further optional layer, such as e.g. in a semiconductor, in a membrane, etc. The functionalized graphene is therefor suited for many applications.

In prior art construction of graphene nanopores, pores were fabricated at room temperature by locally bombarding a monolayer locally with a 300 keV electron beam, as is shown in Figure 1A. However these conditions yield the deterioration of the graphene lattice with increasing beam exposure time (as evidenced by the loss of the characteristic hexagonal diffraction pattern of graphene; Figure 1A, situations 1-3). Present inventors have overcome this problem by exposing graphene at temperatures above 500 °C in a STEM mode of a TEM. As such it is now possible to preserve the graphene lattice neighboring e.g. a nanopore (Figure 1B). In the case of pure graphene (including graphene nanostructures) obtained from the above methodology, no (or virtually no) defects are present.

Inventors fabricated graphene nanopores using the above approach, in an example with diameters from 3 to 20 nm, which were probed ionically in a buffer containing 1M KCl and 10mM Tris (pH 8.1). Figure 1C plots the conductance values of these nanopores versus the pore diameter. As expected, the conductance of the nanopore increases for increasing pore diameter. The conductance can be modeled, e.g. by describing the total conductance G of a pore with diameter d in a buffer of conductivity σ as the inverse sum of the access resistance contribution and the resistance of a cylinder with a length L. A fit to the model shows that the present conductance values are distributed between L = 0 nm and L = 3 nm, with a best fit at L = 1.2 nm; in other words close to a length for a monolayer of graphene. Such further evidences the fact that taking prior art data the structures disclosed therein relate to pores having a cylinder length of about 9 nm; that is the pores do not relate to monolayers of graphene, but typically to 5-10 layers, contrary to

claims made. More precise, the pores relate to distorted graphene structures with a certain thickness, the thickness being a factor thicker than that of a monolayer graphene, whereas surrounding graphene could be one monolayer thick, e.g. visualized as a hilly structure around an opening. Such is also fully in line with observations of present inventors, in that highly crystalline and atomically flat nanopores and the like could not be made at e.g. room temperature. Surprisingly, if the crystalline nanopores reported in Figure 1C are used to detect DNA molecules, severe clogging is experienced (Figure 2A), as seen by the stepwise decrease in ionic current of the nanopore yielding irreversible pore closure. For a couple of seconds of incubation in the presence of DNA, a few translocation events are observed. Then, however, the pore gets clogged. The open pore current dropped to nearly zero, signifying a closed, irreversibly clogged, pore. Even short 1V pulses (Figure 2A) were not sufficient to unclog the pore. Present inventors imaged this particular pore before and after clogging (Figure 2B and 2C, respectively). After use, the DNA material is clearly visible on the STEM micrographs, as a white blob-like aggregate in the pore, along with the fibril-like structures around the pore.

Inventors hypothised that the clogging is due to DNA that sticks to the graphene. To investigate this, inventors studied single-stranded DNA on graphite with atomic force microscopy (AFM). When DNA is incubated on the surface of graphite (Figure 2D) it is found to adsorb on the surface, as seen by the appearance of higher height patches on AFM images (Figure 2E). Presumably, the interaction of DNA molecules relates to irreversible adsorption on the surface of graphene. To counteract these adsorption phenomena, it was proposed earlier that a very high salt concentration (namely buffers containing 3M KCl) might hinder DNA (single-stranded and double-stranded) from adsorbing on graphene. This is however contradicted by the present observations with single-stranded DNA (Figure 2D at 3M KCl) and double-stranded DNA (at KCl concentrations of 1M and 3M, and various pH's ranging from 8.1 to 12; Figure 2E). While such in-

teractions are desirable in some sensor devices, they are preferably prevented in nanopore translocation where each nucleobase should slide through the nanopore (as opposed to stick irreversibly to the graphene surface).

To address the issue of clean crystalline graphene nanopores getting clogged in the presence of DNA, inventors designed a dedicated self-assembled monolayer. It is noted that often a monolayer will be provided at any freely accessible side of graphene, i.e. on one side or on two sides. Preferably the monolayer is orthogonal, that is that the present second molecule (or second group), or at least a part thereof, is directed away from the graphene surface, protruding in e.g. a solvent. In the present application the term orthogonal is to be understood as being under an angle with respect to the graphene surface, the angle being large enough for the part of the second molecule to protrude in a solvent, such as an angle of 30-90 degrees. In an example it is based on the combination of two chemicals, namely an aminopyrene molecule and a N-hydroxysuccinimide derivative of a 4-mer ethylene glycol molecule (Figure 3A i) and ii) respectively). While the pyrene moiety will stick to the graphene, the ethylene glycol will stick out into a solution, and render the graphene surface hydrophilic. Note that, importantly, this self-assembled passivation scheme keeps the graphene material intact from chemical and electrical degradation that would otherwise easily result from oxidation or covalent passivation methods. In an example the coating is applied in two consecutive steps from a 10 mg/mL solution of both molecules each in methanol. It has been found that solutions having a concentration of 0.1-10 mg/ml are preferred, even more preferred 1-10 mg/ml. If a higher concentration is chosen a shorter reaction/interaction time is sufficient, and vice versa. With a higher concentration a better coverage is obtained, and second molecules protrude somewhat more into e.g. a solvent. In a first step, interactions drive the adsorption of a monolayer of aminopyrene on graphene. In the example this is followed by the aminolysis of the N-hydroxysuccinimide ester on the carbonyl group (blue, Figure 3Aii) by the primary amine on the pyrene mole-

cule (red, Figure 3Ai), forming a chemically stable peptide NHCO bond between the two molecules (Figure 3A iii).

Inventors characterized DNA passivation properties of the self-assembled monolayer using AFM. Importantly, inventors found that DNA did not adsorb on graphite coated with the present self-assembled monolayer, even at concentrations of DNA as high as 10 ng/μL. This is evidenced by the similarities between the control AFM image (Figure 3B, self-assembled monolayer on HOPG incubated with 10mM Tris, 1M KCl, 8M urea, pH 8.1 for 10 minutes) and the same self-assembled monolayer incubated with the same buffer containing 10 ng/μL of single-stranded DNA (Figure 3C). The self-assembled monolayer thus appears to act as an effective hydrophilic barrier that prevents the hydrophobic interaction between nucleobases in DNA and aromatic hexagons in graphene.

Most importantly, using this strategy, inventors were able to reproducibly translocate single-stranded DNA without pore clogging, with a total experiment time easily approaching hours, as evidenced by the stable conductance levels over the experimental time (Figure 3D and inset).

In order to estimate the added thickness of the self-assembled monolayer on the graphene, inventors probed a change in pore conductance upon applying the present coating for three pores with diameters of 5, 10, and 15 nm, respectively (Figure 3E). Fitting the data inventors find that the length L of the assumed cylinder (self-assembled monolayer, graphene layer, self-assembled monolayer) increased from 1.5 nm to an apparent thickness L* of 5.5 nm upon forming the present self-assembled monolayer on the three nanopores mentioned above(Figure 3E, blue curve). This suggests an added thickness of about 4 nm. Since the self-assembled monolayer in principle forms on both sides of the graphene membrane (on the top and bottom of the nanopore), the thickness per layer is about 2 nm. This value is in agreement with the expected head-to-tail length of the example Pyr-NHCO-EG4 molecule (0.4 nm for aminopyrene and 1.5 nm for the aminolyzed 4-mer ethylene glycol).

However, it is assumed that ethylene glycol chains

are presumably also protruding into the nanopore area; hence the pore will effectively have a smaller diameter d*. Results of fitting are summarized in Table S1 and show a coating thickness and protrusion distance x of 0.7 ± 0.1 nm. A similar value is obtained by fitting to the data presented in Figure 3E (i.e., lowest reduced χ^2 for x = 0.6 ± 0.2 nm). A value of 0.6 - 0.7 nm is in good agreement with an estimated persistence length of ethylene glycol molecules in water (i.e., 0.3-0.5 nm).

The three coated pores studied above were used for translocation experiments with single-stranded DNA. In an example first the nanopore with a diameter of 10 nm was studied (Figure 4). Single-stranded DNA can be driven electrophoretically through the present nanopore and detected by monitoring the ion current. Upon addition of the circular M13 single-stranded DNA molecule on one side of the pore and applying a voltage of 200 mV across the graphene membrane, a series of spikes is observed in the conductance traces (Figure 4A). Each temporary drop in the measured conductance, ΔG, arises from a single DNA molecule that translocates through the pore. Two characteristic signals are observed, corresponding to two types of translocation events: type 1 events (where the circular molecule translocates in an non-folded conformation) and type 21 events (where the circular DNA molecule is in a folded conformation. Examples events are shown in Figure 4B. From a large number (n = 545) of such events, inventors obtain a histogram of conductance blockade levels ΔG, as presented in Figure 4B. Three peaks are visible, the first being the open-pore current at 0 nS (i.e., the baseline); the peak at 3.8 ± 0.5 nS which corresponds to one circular M13 molecule in the pore (i.e., two parallel single strands); and the peak at 7.5 ± 0.6 nS due to two parts of same DNA molecule in the pore (i.e., four single stands). A scatter plot of ΔG versus the time duration of the events is shown in Figure 4C. Each dot in this diagram represents a single M13 DNA translocation event. In addition to the event amplitude, inventors studied the translocation times of the events. The average translocation time is found to be 180 ± 30 μs.

A similar analysis was carried for the two other nanopores (i.e., 5 and 15 nm), and conductance and dwell time histograms are shown in Figure 5A and 5B respectively. As for the 10 nm pore, type 1 translocations are the most represented with conductance blockade amplitudes of ΔG_{5nm} = 5.8 ± 0.1 nS and ΔG_{15nm} = 3.4 ± 0.1 nS. The most probable translocation times in the distribution of the events are 250 ± 50 µs and 135 ± 20 µs for the 5 and 15 nm pore respectively. As represented in Figure 5C, both these conductance blockade and dwell times increase when the diameter of the pores decreases, a trend that was also found for silicon-nitride pores. Figure 5C does capture the trend in $\Delta G(d)$ qualitatively but quantitatively does not describe very well the values of the conductance blockade expected from the models developed for solid-state nanopores.

From present experiments, inventors conclude that if e.g. hydrophobicity of graphene is tailored with short hydrophilic ethylene glycol chains, then graphene nanopores can be used to reproducibly detect single DNA molecules without major sticking and clogging of the pore as it is observed when no self-assembled monolayer are used. Inventors identified a coating procedure using very short chains, such as 2-10 monomeric units, ideally 4, of e.g. ethylene glycol, to prevent any biomolecules from interacting with graphene (more specifically graphene nanopores). In an example it is preferred to have a relatively thin layer of graphene covered with the present monolayer. Inventors showed that pores are reproducible and stable and do not get clogged upon adsorption of double-stranded and single-stranded DNA, while the graphene remains unaltered chemically, which is a prerequisite to the design of future hydrophilic graphene nanopore, nanoribbon and nanogap devices. Inventors thus demonstrated a general approach to tailor the hydrophobicity of graphene.

Thereby the present invention provides a solution to one or more of the above mentioned problems.

Advantages of the present description are detailed throughout the description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates in a first aspect to a method of forming a functionally modified graphene surface, according to claim 1, wherein the graphene is preferably highly crystalline. The method may be performed in one reactor.

In principle the first and second molecule may be reacted first forming a combined molecule, as an alternative, and then an aromatic part of the combined molecule may be interacted with the graphene surface. In other words, the present method may be performed in any sequence of steps identified.

In the present application, with terms as "molecule", "group", "moiety", "solvent", and examples given thereof, also substituted variants thereof are included, as well as mixtures thereof.

In the method a suitable solvent is provided, capable of dissolving the first molecules and graphene and preferably also the second molecules such that both present interaction and reaction can take place. It is noted that in an example the present method comprises two sequential steps; one for interaction of the first molecule with the graphene, a second for reacting the first and second molecules, such as by a condensation reaction, thereby forming a chemical bond. Thereto the first molecules have a binding group comprising at least one aromatic hydrocarbon group. It has been found that the at least one aromatic hydrocarbon group and the graphene have a sufficient strong interaction. Further, the first molecules have a chemically active first moiety, capable of reacting with a chemically active second moiety of second molecules, or vice versa. The reaction provides a strong chemical bond between the first and second molecule. In principle first the first and second molecule may react forming a reaction product, and then the reaction product may be interacted with the graphene; however, it has been found that in the latter case a good coverage of the graphene is difficult to achieve. It is noted that the first molecule and graphene interact; as a consequence especially the physical nature of graphene remains still sufficient to provide reliable results, e.g. when measuring conductance, applying an electrical current, etc.

In an example of the present method the aromatic hy-

drocarbon group has 1-20 aromatic groups, such as 2-10 aromatic groups, preferably being a poly aromatic hydrocarbon group, selected from naphthalene, phenanthrene, anthracene, tetracene, chrysene, triphenylene, pyrene, pentacene, corannulene, hexacene, coronene, benzo(a)pyrene, heptacene, octacene, ovalene, undecacene, decacene, and combinations thereof. It has been found experimentally that somewhat larger first molecules comprising at least a few aromatic groups provide a good interaction with graphene. It is noted that in principle also a mixture of first molecules may be provided. As such functionality can be tailored in more detail. It has been found that naphthalene, anthracene, and pyrene are very suitable binding groups, i.e. provide good interaction.

Further aromatic groups, such as nucleotides, amino acids, may also be used in the present method and graphene.

In an example of the present method the first moiety is selected from one or more of alcohols, carboxylic acids, ethers, esters, amino acids, amines, amides, and derivatives thereof, such as salts thereof. Amides, alcohols and carboxylic acids are preferred, e.g. because these molecules can be reacted in the present solvent without further measures. It is possible to make use of more than one moiety per first molecule, thereby forming "dimers", oligomers", etc. It is preferred to have 1-4 moieties per first molecule, and to form 1-4 bonds with the present second molecule.

In an example of the present method the second moiety is selected from one or more of alcohols, carboxylic acids, ethers, esters, amino acids, amines, amides, and derivatives thereof, such as salts thereof. Amides, alcohols and carboxylic acids are preferred, e.g. because these molecules can be reacted in the present solvent without further measures. It is possible to make use of more than one moiety per first molecule, thereby forming "dimers", oligomers", etc.

In an example of the present method the step d) reaction is a condensation reaction, preferably forming one or more of a peptide, an ester, and an ether. If required boundary conditions may be adjusted to achieve a desired result, such as temperature, pH, buffer, activator, time and catalyst. For the present method the boundary conditions as present are typically

sufficient. As a consequence a chemical bond is provided that is suited for a specific purpose, e.g. being stable, relatively strong, applicable in a variety of environments and not interfering with intended use of the present graphene monolayer.

In an example of the present method the first molecule further comprises one or more of an alkane group, such as a cycloalkane group, and derivatives thereof, such as having 1-12 carbon atoms, preferably having 5-6 carbon atoms.

In an example of the present method the second molecule comprises a tail, the tail being selected from alcohols, such as mono-alcohols, alkanediols, alkanetriols, carboxylic acids, ethers, esters, amino acids, amines, amides, alkanes, alkenes, sugars, and combinations thereof, and derivatives thereof. In an example the tail is designed to prevent interaction of solutes and graphene. In an example the tail is designed to improve solubility of the graphene in the solvent. It is preferred to use relatively short second molecules, such as having less than 10 monomeric units.

In an example of the present method the solvent is an alcohol, such as a C_1-C_{12}-alcohol, such as methanol, ethanol, and propanol, preferably methanol. The solvent may be in its pure form, a mixture of alcohols, alcohol comprising water, etc. Methanol is preferred as it supports the intended reaction between first and second molecule sufficiently, and it provides good solubility towards graphene. Preferably a non-toxic (or slightly toxic) solvent is used.

In an example of the present method the second molecule has a length smaller than 20 nm, preferably smaller than 10 nm. For various applications a relatively short second molecules is preferred. It has been found that the length of the second molecule is important in order to maintain the present atomically thin electrode design, especially when uncontrolled variations in current or conductivity of graphene are best avoided. In some applications the second molecule preferably does not interfere with e.g. a molecule to be analyzed or sequenced.

In a second aspect the present invention relates to a graphene comprising at least one mono-layer of molecules according to claim 7, preferably a highly crystalline graphene

layer. The molecules comprise a binding group which comprises at least one aromatic hydrocarbon group, a second group, the second group being connected to the binding group.

In an example of the present graphene preferably at least two aromatic hydrocarbon groups are present in the molecules, as is indicated above.

In an example of the present graphene the second group is bounded to the binding group by one or more of an ester, an ether, and a peptide.

In an example of the present graphene the second group is selected from one or more of alcohols, such as monoalcohols, alkanediols, alkanetriols, carboxylic acids, ethers, esters, amino acids, amines, amides, alkanes, alkenes, sugars, and derivatives thereof.

In principle the present graphene layer is obtainable by the above present method. Therefor, details of the present method in principle apply one to one to the present graphene.

In an example of the present graphene the aromatic hydrocarbon group has 1-20 aromatic groups, such as 2-10 aromatic groups, preferably selected from naphthalene, phenanthrene, anthracene, tetracene, chrysene, triphenylene, pyrene, pentacene, corannulene, hexacene, coronene, benzo(a)pyrene, heptacene, octacene, ovalene, undecacene, decacene, and combinations thereof.

In an example of the present graphene the graphene comprises a structure with at least one edge selected form one or more of a nanopore, a nanoribbon, a nanogap, preferably having a width of 3-20 nm. It has been found that for some application it is important to have a very precisely defined structure, in terms of shape, size, diameter etc. The better the definition of the structure the better results e.g. in terms of accuracy, reproducibility, analysis, etc. of a structure in use are obtained. For various applications the present structure is defined with an accuracy of 0.1 nm or better, which is in the order of one atom (C). The width of the present structure can be tailored to its intended use. For instance, a nanogap is envisaged for analyzing and for sequencing DNA, having a width of some 3 nm. Also characteristics of solvent, analytes, etc. may be taken into account when designing the present structure. The

present graphene may have more than one structures. Also in this respect it is noted that various documents claim to provide similar structures; however using the prior art techniques mentioned in those documents such is effectively not possible.

In an example of the present graphene the edge of the structure is a monolayer and has a defect density of less than 1 defects/10 nm^2. The defect density is for some applications relatively important. As mentioned it has been found that for instance accuracy and reproducibility of conductivity and electrical current rely heavily on the crystallinity of the graphene used. The present graphene therefor preferably has a defect density of less than a few defects per unit area. It is noted that the present defect density is extremely low. Defects typically relate to impurities, distortion of crystal lattice, etc. As such also a method of forming nanostructures in combination with the present method is important, in order to keep a defect density as low as possible. Such is in particular important for sequencing of biomolecules. In order to obtain high speed of electrons (in the graphene) and ballistic transport the present example of coated highly crystalline graphene layer has found to be very suited.

In an example of the present graphene the graphene monolayer has a length of 1 mm - 5 cm, whereas the width is 1 mm-2cm. Such a graphene layer is large enough to handle, to process, and provides the present advantages.

Preferably the graphene layer comprises a number of nanostructures, such as an array of nanopores, such as an array of 1-10 by 1-100 nanopores (e.g. 10 x 10), allowing parallel measurements. For such structures the crystallinity of the graphene and the exact dimensions of the structure are even more important to provide reliable and reproducible results.

In a further aspect the present invention relates to a device comprising the present graphene layer.

In a further aspect the present invention relates to a use of a graphene layer according to claim 12, preferably a highly crystalline graphene layer. Examples of such uses are given in the description and in the examples.

In a further aspect the present invention relates to graphene layer for use in one or more sequencing, analyzing,

and sensing, especially of biomolecules, such as for DNA-sequencing, for RNA-sequencing, for analyzing biomolecules, and for reproducing biomolecules, preferably a highly crystalline graphene layer. It is noted that prior art graphene is of insufficient crystalline quality, especially close to edges of a nanostructure, to perform reliable, reproducible and controllable measurements. Further with the present quality fast recording is possible, contrary to prior art devices.

It is noted that some prior art devices may start with relatively crystalline graphene (relatively far away from an edge thereof), but upon applying a coating or the like inherently the crystallinity is destroyed.

In a further aspect the present invention relates to a functionalized graphene or graphene layer.

In a further aspect the present invention relates to a method of translocating single strand DNA using a graphene layer according to the invention, preferably a highly crystalline graphene layer.

The invention is further detailed by the accompanying figures and examples, which are exemplary and explanatory of nature and are not limiting the scope of the invention. To the person skilled in the art it may be clear that many variants, being obvious or not, may be conceivable falling within the scope of protection, defined by the present claims.

SUMMARY OF FIGURES

Figure 1 A-C show crystalline nanopore in monolayer graphene and ion transport characteristics.

Figure 2 A-E show DNA molecules clog crystalline graphene nanopores.

Figure 3 A-E show Non-covalent functionalization of graphene with hydrophilic groups to prevent DNA from interacting with graphene.

Figure 4 A-D show Translocation characteristics for a crystalline 10 nm graphene nanopore functionalized with a self-assembled monolayer.

Figure 5 A-C show Translocation characteristics for 5, 10 and 15 nm coated graphene nanopores.

DETAILED DESCRIPTION OF FIGURES

Figure 1 shows a crystalline nanopore in monolayer graphene and ion transport characteristics. A) Contamination and amorphization induced by a focused electron beam on graphene at room temperature during nano-pore drilling in HREM mode. HREM nanopore drilling was carried at 300kV, spot size 4 and C2 aperture 20nm using an FEI Titan, equipped with Cs image corrector. Electron beam, focused into 10-nano size probe, was exposed in situations 1-4 on graphene with increased residual time, namely 10, 20, 30 and 40 seconds respectively. After the electron beam exposure nano-electron diffraction were taken and the results are shown in the bottom panel of 1A. B) 80kV HREM image of a 3 nm pore with clean and crystalline edge drilled in STEM mode at 600 °C using an FEI Titan 60-300 PICO TEM equipped with a high brightness electron gun, an electron gun monochromator, a probe aberration corrector and a CS-CC achro-aplanat image corrector. C) Dependence of the conductance of crystalline nanopores on pore diameter. Black lines represent a model of conductance (see Eq. 1) and is plotted for L= 0 nm, 3 nm, and 10 nm, where L represents the thickness of the nanopore membrane. The solid red line represent the best fit (lowest reduced χ^2) for L = 1.2 nm.

Figure 2 shows DNA molecules clog crystalline graphene nanopores. A) Ionic current versus time of a 5 nm diameter graphene nanopore incubated with single stranded DNA M13 at a concentration of 2.5 ng/uL in 1M KCl and 8M urea. At time 0.7 s (*), the voltage is switched from 0 mV to 200 mV, resulting in a baseline current of -5.2 nA and upward peaks corresponding to DNA translocation events. After 2 seconds at 200 mV, the current baseline starts to decrease to zero in discrete steps, corresponding to a clogged pore. Large 1V pulses are subsequently applied across the nanopore in order to try to restore the stable current baseline, but this was unsuccessful. B-C) The 5 nm nanopore discussed in panel A before the translocation of DNA (B) and the same nanopore after the experiment that showed pore clogging (C), both imaged in the STEM mode of the TEM. D) Atomic force micrographs (AFM) of highly oriented pyrolytic graphite (HOPG) incubated 5 minutes with a solution of 3M KCl and 8M urea and rinsed with ultrapure water. E) HOPG incu-

bated 5 minutes with single-stranded M13 DNA (10 ng/μL) in the same buffer.

Figure 3 shows non-covalent functionalization of graphene with hydrophilic groups to prevent DNA from interacting with graphene. A) Chemical structures of 1-aminopyrene (i), a N-hydroxysuccinimide ester derivative of a 4-mer ethylene glycol molecule (ii), and (iii) the product of the chemical reaction between i) and ii). B-C) HOPG coated with a self-assembled monolayer made of iii) and incubated with 1M KCl and 8M urea in absence (B), and after 10 minutes of subsequent incubation with the same buffer containing 10 ng/μL of single-stranded M13 (C). D) Representative raw trace of the ionic current versus time for a 14 nm diameter graphene nanopore coated with the SAM and incubated with single stranded DNA M13 at a concentration of 10 ng/μL in 1M KCl and 8M urea and plotted against the experimental time. The inset represents the variation in the conductance of the nanopore plotted against the experimental time. E) Conductance of three graphene pores with diameters respectively of 5, 10 and 15 nm before (red squares) and after applying the self-assembled monolayer consisting of molecule iii) (blue circles). The red solid line correspond to a fit of Eq. 1, yielding L = 1.5 nm. The blue solid line is a fit of Eq. 2 for L=1.5 nm yielding x = 0.7 nm.

Figure 4 shows translocation characteristics for a crystalline 10 nm graphene nanopore functionalized with a self-assembled monolayer. A) Translocation of circular M13 single stranded DNA across a 10 nm nanopore in a graphene monolayer. DNA molecules were dissolved in 10mM Tris (pH 8.1), 1M KCl and 8M urea. B) Examples of translocation events of non-folded (type 1, top panel) and partially folded DNA (type 21, bottom panel) molecules recorded at 200 mV in this 10 nm pore. C) Conductance histogram collected from 545 translocation events, including the open-pore conductance before and after the event. D) Scatter diagram of the amplitude of the conductance blockade versus translocation time for DNA translocation through a 10 nm diameter nanopore in a graphene monolayer. The accompanying histograms for all the event types are included at the top and the right. Each point in this scatter diagram corresponds to a single translocation event. Applied voltage is 200 mV.

Figure 5 shows translocation characteristics for 5, 10 and 15 nm coated graphene nanopores. A) Conductance blockade histograms. B) Dwell-time histograms obtained from analyzing the scatter diagrams for a 5 nm (gray) and 15 nm (black) graphene nanopore. C) Conductance blockades and dwell times (inset) versus pore diameter plotted for the three graphene nanopores. Black solid line represent the best fit of $\Delta G(d)$ at dssDNA = 2.2 ± 0.3 nm.

EXAMPLES

The invention although described in detailed explanatory context may be best understood in conjunction with the accompanying examples and figures.

Diameter d (nm)	G after coating (nS)	x (L =0 nm)	x (L=1.5 nm)
5.1	22.1	0.90 ± 0.01	0.70 ± 0.01
10.2	65.2	1.01 ± 0.01	0.76 ± 0.01
14.9	109	1.10 ± 0.02	0.82 ± 0.02

Table S1. Numerical solutions

It should be appreciated that for commercial application it may be preferable to use one or more variations of the present system, which would similar be to the ones disclosed in the present application and are within the spirit of the invention.

CONCLUSIES

1. Werkwijze voor het vormen van een functioneel gemodificeerd grafeenoppervlak, omvattende de stappen van:

a) het verschaffen van een grafeen monolaag,

b) in een geschikt oplosmiddel het verschaffen van eerste moleculen omvattend een bindingsgroep,

b1) waarbij de bindingsgroep ten minste één aromatische koolwaterstofgroep omvat, bij voorkeur ten minste twee aromatische koolwaterstofgroepen, waarin de eerste moleculen verder een chemisch actieve eerste eenheid omvatten,

c) het interacteren van de eerste moleculen en het grafeen waardoor ten minste één (mono) laag van de eerste moleculen wordt gevormd op het grafeenoppervlak, en

d) het laten reageren van de chemisch actieve eerste eenheid van de eerste moleculen met een chemisch actieve tweede eenheid van tweede moleculen.

2. Werkwijze volgens conclusie 1,

b1) waarbij de aromatische koolwaterstof groep 1-20 aromatische groepen heeft, zoals 2-10 aromatische groepen, bij voorkeur gekozen uit naftaleen, fenantreen, antraceen, tetraceen, chryseen, trifenyleen, pyreen, pentaceen, corannulene, hexaceen, coroneen, benzo(a)pyreen, heptaceen, octaceen, ovaleen, undecaceen, decaceen, en combinaties daarvan,

waarbij de eerste eenheid is gekozen uit één of meer van alcoholen, carbonzuren, ethers, esters, aminozuren, aminen, amiden, en derivaten daarvan, en

d2) waarbij het tweede deel van het tweede molecuul is gekozen uit één of meer alcoholen, carbonzuren, ethers, esters, amino-zuren, aminen, amiden, en derivaten daarvan.

3. Werkwijze volgens één of meer der voorgaande conclusies, waarbij in stap d) het reageren een condensatiereactie is, die bij voorkeur één of meer van een peptide, een ester en een ether vormt.

4. Werkwijze volgens één of meer der voorgaande conclusies,

b1) waarbij de eerste molecuul verder omvat één of meer van een alkaangroep, zoals een cycloalkaangroep, en derivaten daarvan,

waarbij d2) het tweede molecuul een staart omvat, waarbij de staart wordt gekozen uit alcoholen, zoals mono-alcoholen, alkaandiolen, alkanetriolen, carbonzuren, ethers, esters, amino-zuren, aminen, amiden, alkanen, alkenen, suikers, en combinaties daarvan, en derivaten daarvan

5. Werkwijze volgens één of meer der voorgaande conclusies, waarbij het oplosmiddel een alcohol is, zoals een C_1-C_{12}-alcohol, zoals methanol, ethanol en propanol, bij voorkeur methanol.

6. Werkwijze volgens één of meer der voorgaande conclusies, waarbij het tweede molecuul een lengte heeft van kleiner dan 20 nm, bij voorkeur kleiner dan 10 nm.

7. Grafeenlaag omvattende ten minste één monolaag van moleculen, waarbij de moleculen omvatten een bindingsgroep met ten minste één aromatische koolwaterstofgroep, bij voorkeur ten minste twee aromatische koolwaterstofgroepen, een tweede groep, waarbij de tweede groep is verbonden met de bindingsgroep zoals door een ester, een ether, een peptide, waarbij de tweede groep wordt gekozen uit één of meer alcoholen, zoals mono-alcoholen, alkaandiolen, alkaantriolen, carbonzuren, ethers , esters, aminozuren, aminen, amiden, alkanen, alkenen, suikers, en derivaten daarvan.

8. Grafeenlaag volgens conclusie 7, waarbij de aromatische koolwaterstof groep 1-20 aromatische groepen heeft, zoals 2-10 aromatische groepen, bij voorkeur gekozen uit naftaleen, fenantreen, antraceen, tetraceen, chryseen, trifenyleen, pyreen, pentaceen, corannuleen, hexaceen, coroneen, benzo(a)pyreen, heptaceen, octaceen, ovaleen, undecaceen, decaceen, en combinaties daarvan.

9. Grafeenlaag volgens één of meer der conclusies 7-8, waarbij het grafeen een structuur omvat met ten minste één rand gekozen uit één of meer van een nanoporie, een nanokoord, een nanogat, bij voorkeur een breedte van 3-20 nm,
waarbij het grafeen bij voorkeur hogelijk kristallijn is en waarbij de rand van de structuur een monolaag is een defectdichtheid van minder dan 1 defect/10 nm^2 heeft.

10. Grafeenlaag volgens één of meer der conclusies 7-9, waarbij het grafeen een array van nanostructuren omvat, zoals een reeks nanoporiën.

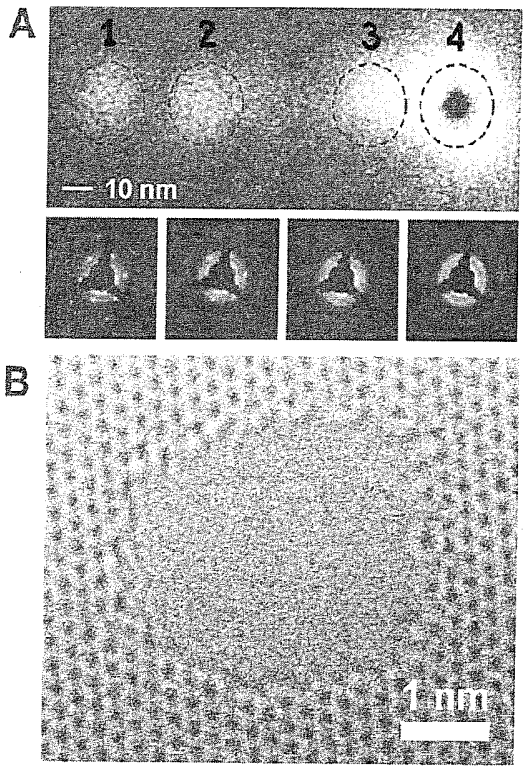
11. Inrichting omvattende een grafeenlaag volgens één of meer der conclusies 7-10, zoals een NEMS, een MEMS, een schakeling, een membraan, een energie-opslaginrichting, elektronica, een coating, een kleefstof, een sensor, optica, fotonica, een lasertoepassing, een touchscreen, een nanochemisch apparaat, en combinaties daarvan.

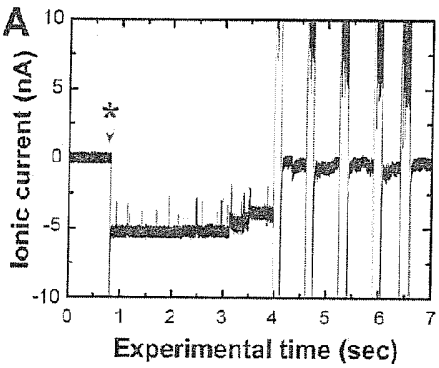
12. Gebruik van grafeenlaag volgens één of meer der conclusies 7-10, in een biologische toepassingen, een biomedische toepassing, voor moleculaire diagnostiek, voor de analyse van monsters, zoals bloedmonsters, als een sensor voor osmose, als een membraan, voor specifieke adsorptie, voor biomoleculaire analyse, in een dispersie, als een smeermiddel, en combinaties daarvan.

13. Grafeenlaag volgens één of meer der conclusies 7-10 voor toepassing in één of meer van sequencing, analyse, en detectie, vooral van biomoleculen, zoals voor DNA-sequencing, voor RNA-sequencing, voor het analyseren van biomoleculen, en voor reproductie van biomoleculen.

14. Gefunctionaliseerd grafeen volgens één of meer der conclusies 7-10, zoals gefunctionaliseerd met ten minste één monolaag van moleculen, waarbij de moleculen omvatten een bindingsgroep met ten minste één aromatische koolwaterstofgroep, bij voorkeur ten minste twee aromatische koolwaterstofgroepen, een tweede groep, waarbij de tweede groep is verbonden met de bindingsgroep zoals door een ester, een ether, een peptide, waarbij de tweede groep wordt gekozen uit één of meer alcoholen, zoals mono-alcoholen, alkaandiolen, alkaantriolen, carbonzuren, ethers , esters, aminozuren, aminen, amiden, alkanen, alkenen, suikers, en derivaten daarvan.

15. Werkwijze voor translocatie van enkelstrengs DNA met ten minste één grafeenlaag volgens één of meer der conclusies 7-10.





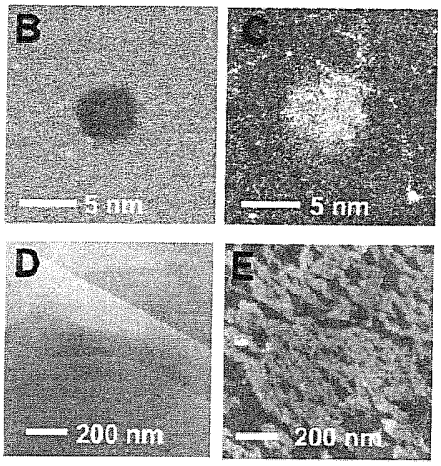


Fig. 2A-E

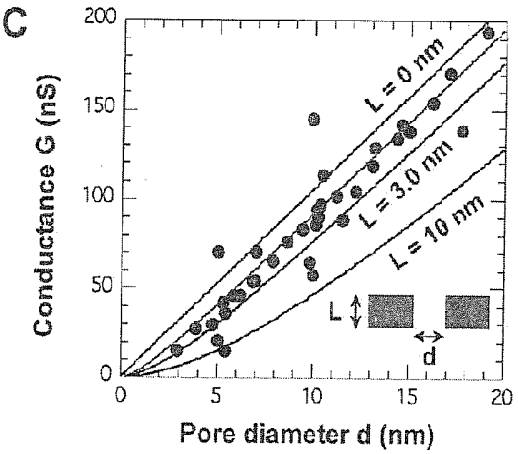
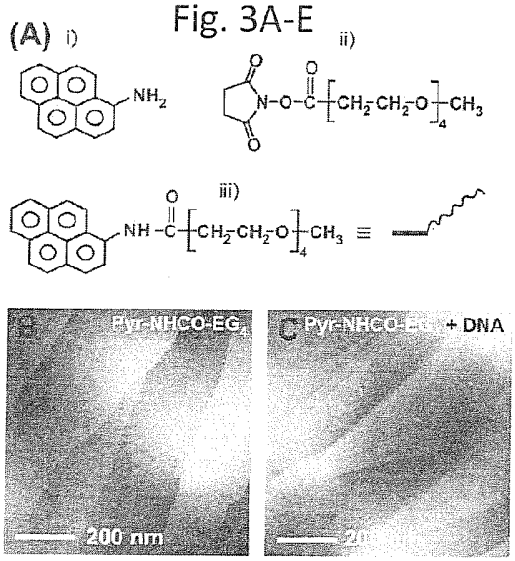
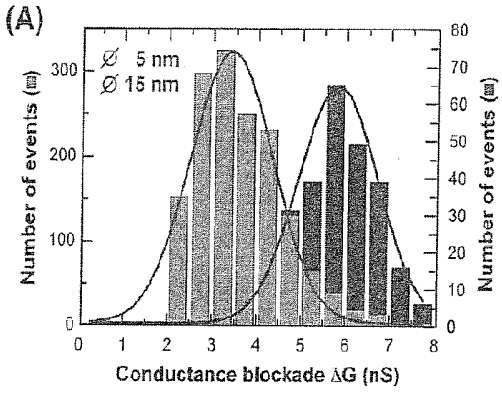
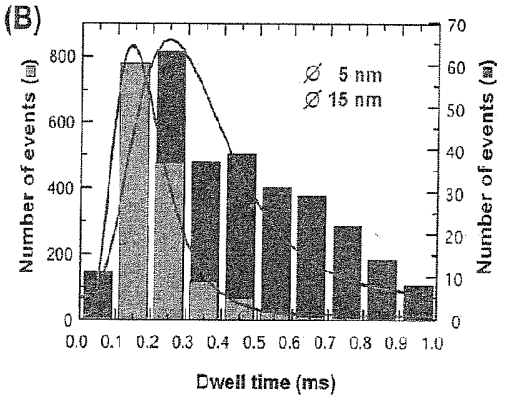


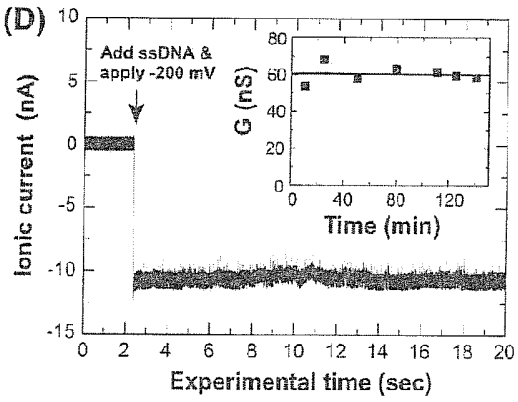
Fig. 1A-C

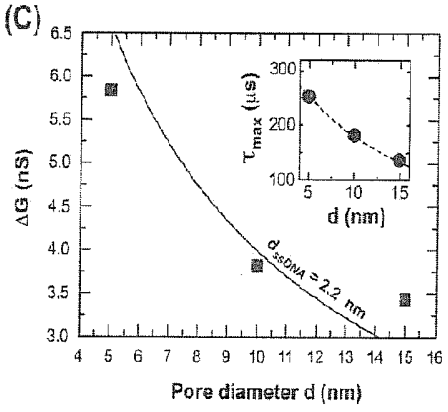
Fig. 3A-E











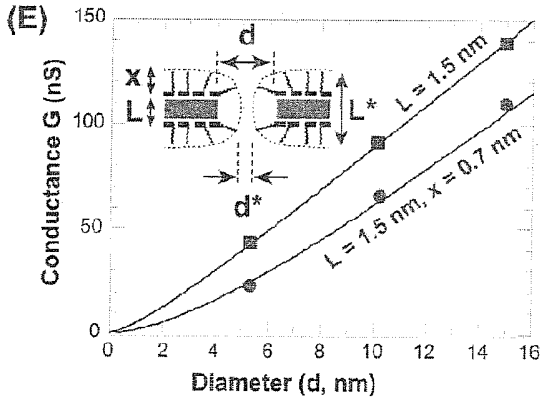
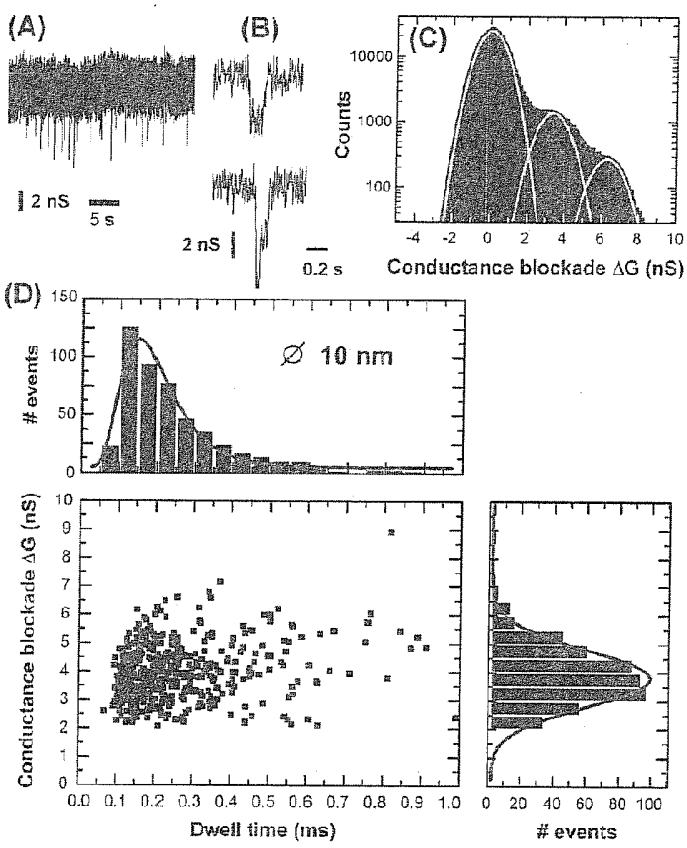


Fig. 5A-C

Fig. 4A-D


(A)
(B)
(C)
2 nS
5 s
2 nS
0.2 s
Counts
10000
1000
100
-4 -2 0 2 4 6 8 10
Conductance blockade ΔG (nS)
(D)
events
150
100
50
0
∅ 10 nm
Conductance blockade ΔG (nS)
10
9
8
7
6
5
4
3
2
1
0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Dwell time (ms)
0 20 40 60 80 100
events

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	NL 48970
Nederlands aanvraag nr. **2010776**	Indieningsdatum **08-05-2013**
	Ingeroepen voorrangsdatum
Aanvrager (Naam) **Technische Universiteit Delft**	
Datum van het verzoek voor een onderzoek van internationaal type **13-07-2013**	Door de Instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr. **SN 60388**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

C01B31/04 **C12Q1/68**

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC	**C01B** **C12Q**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	[ ]	**GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	[X]	**GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2010776

A. CLASSIFICATIE VAN HET ONDERWERP
INV. C01B31/04 C12Q1/68
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte miminum documentatie (classificatie gevolgd door classificatiesymbolen)
C01B C12Q

Onderzochte andere documentatie dan de mimimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data, INSPEC, COMPENDEX, CHEM ABS Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
	EENHEID VAN UITVINDING ONTBREEKT zie aanvullingsblad B -----	
X	WEILI WEI ET AL: "Chiral detection using reusable fluorescent amylose-functionalized graphene", CHEMICAL SCIENCE, deel 2, nr. 10, 4 augustus 2011 (2011-08-04), bladzijde 2050, XP055098154, ISSN: 2041-6520, DOI: 10.1039/c1sc00308a * bladzijde 2050, rechter kolom, alinea 2 - bladzijde 2051, rechter kolom, alinea 3 * * bladzijde 2055, linker kolom, alinea 3 - rechter kolom, alinea 1 * -----	1-9, 11-14
	-/--	

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[ ] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

° Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
29 januari 2014	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sevillano Rodriguez

2

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2010776

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	JASON A. MANN ET AL: "Preservation of Antibody Selectivity on Graphene by Conjugation to a Tripod Monolayer", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, deel 52, nr. 11, 11 maart 2013 (2013-03-11), bladzijden 3177-3180, XP055098219, ISSN: 1433-7851, DOI: 10.1002/anie.201209149 * bladzijde 3177, linker kolom, alinea 2 - bladzijde 3178, linker kolom, alinea 1 * * bladzijde 3180, linker kolom, alinea 2 * * Scheme 1 *	1-14

X	CHIH-CHUN TENG ET AL: "Thermal conductivity and structure of non-covalent functionalized graphene/epoxy composites", CARBON, ELSEVIER, OXFORD, GB, deel 49, nr. 15, 24 juni 2011 (2011-06-24) , bladzijden 5107-5116, XP028390292, ISSN: 0008-6223, DOI: 10.1016/J.CARBON.2011.06.095 [gevonden op 2011-07-23] * bladzijde 5107, linker kolom, alinea 1 * * bladzijde 5108, rechter kolom, alinea 3 - bladzijde 5110, linker kolom, alinea 1 * * figuren 1, 2 *	1-14

X	JINGQUAN LIU ET AL: "Synthesis, Characterization, and Multilayer Assembly of pH Sensitive Graphene-Polymer Nanocomposites", LANGMUIR, deel 26, nr. 12, 15 juni 2010 (2010-06-15) , bladzijden 10068-10075, XP055098180, ISSN: 0743-7463, DOI: 10.1021/la1001978 * bladzijde 10069, rechter kolom, alinea 1 - bladzijde 10070, linker kolom, alinea 3 * * Scheme 1 *	1-10, 12-14

X	JINGQUAN LIU ET AL: "Thermosensitive graphene nanocomposites formed using pyrene-terminal polymers made by RAFT polymerization", JOURNAL OF POLYMER SCIENCE PART A: POLYMER CHEMISTRY, deel 48, nr. 2, 15 januari 2010 (2010-01-15), bladzijden 425-433, XP055098188, ISSN: 0887-624X, DOI: 10.1002/pola.23802 * bladzijde 426, rechter kolom, alinea 1 - bladzijde 428, linker kolom, alinea 2 * * Scheme 1 *	1-10, 12-14

	-/--	

2

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2010776

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie °	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	JASON A. MANN ET AL: "Multivalent Binding Motifs for the Noncovalent Functionalization of Graphene", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, deel 133, nr. 44, 9 november 2011 (2011-11-09), bladzijden 17614-17617, XP055098198, ISSN: 0002-7863, DOI: 10.1021/ja208239v * bladzijde 17614, rechter kolom, alinea 2 - bladzijde 17615, rechter kolom, alinea 1 * * Scheme 1 * -----	1-10,14

2

GEBREK AAN EENHEID VAN UITVINDING

Octrooiaanvrage Nr.:
SN 60388
NL 2010776

AANVULLINGSBLAD B

De Instantie belast met het uitvoeren van het onderzoek naar de stand van de techniek heeft vastgesteld dat deze aanvrage meerdere uitvindingen bevat, te weten:

1. conclusies: 1-14

Process for making a functionalized graphene layer, graphene layer and uses thereof

---

2. conclusie: 15

Process for the translocation of DNA strands

Het vooronderzoek werd tot het eerste onderwerp beperkt.

It is considered that there are two inventions covered by the claims indicated as follows:
1. claims:
1-14
Process for making a functionalized graphene layer, graphene layer and uses thereof
2. claims:
15
Process for the translocation of DNA strands

The single general inventive concept linking this two inventions is a graphene layer comprising at least a monolayer of molecules, wherein the molecules comprise a binding group having at least one aromatic hydrocarbon group, a second group, wherein the second group is attached to the bonding group is selected from one or more alcohols.
Document D1 discloses a functionalized graphene layer with Anthracene-labeled amylose, consisting of the compound formed by the condensation reaction of the amylose reducing end with the amino group of the 2-amino-anthracene (page 2050, right-side column, paragraph 2 - page 2051, right-side column, paragraph 3; page 2055, left-side column, paragraph 3 - right-side column, paragraph 1).
Therefore, the single general inventive concept is not new.
In conclusion, the groups of claims define two different inventions not linked by a single general inventive concept.
The application, hence does not meet the requirements of unity of invention.

OCTROOICENTRUM NEDERLAND

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN60388	08.05.2013		NL2010776

International Patent Classification (IPC)
INV. C01B31/04 C12Q1/68

Applicant
Technische Universiteit Delft

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the application
- ☒ Box No. VIII Certain observations on the application

Examiner
Sevillano Rodriguez

Application number

WRITTEN OPINION

NL2010776

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable have not been examined in respect of

☐ the entire application

☒ claims Nos. 15

because:

☐ the said application, or the said claims Nos. relate to the following subject matter which does not require a search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no search report has been established for the whole application or for said claims Nos. 15

☐ a meaningful opinion could not be formed as the sequence listing was either not available, or was not furnished in the international format (WIPO ST25).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; or such tables were not available in electronic form.

☐ See Supplemental Box for further details.

Box No. IV Lack of unity of invention

1. The requirement of unity of invention is not complied with for the following reasons:

   see separate sheet

2. This report has been established in respect of the following parts of the application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. (see Search Report)

WRITTEN OPINION

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	5, 6, 9, 10
	No:	Claims	1-4, 7, 8, 11-14
Inventive step	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the application

see separate sheet

Re Item IV

Lack of unity of invention

It is considered that there are two inventions covered by the claims indicated as follows:

1. claims: 1-14

   Process for making a functionalized graphene layer, graphene layer and uses thereof

2. claims: 15

   Process for the translocation of DNA strands

The single general inventive concept linking this two inventions is a graphene layer comprising at least a monolayer of molecules, wherein the molecules comprise a binding group having at least one aromatic hydrocarbon group, a second group, wherein the second group is attached to the bonding group is selected from one or more alcohols.

Document D1 discloses a functionalized graphene layer with Anthracene-labeled amylose, consisting of the compound formed by the condensation reaction of the amylose reducing end with the amino group of the 2-amino-anthracene (page 2050, right-side column, paragraph 2 - page 2051, right-side column, paragraph 3; page 2055, left-side column, paragraph 3 - right-side column, paragraph 1).

Therefore, the single general inventive concept is not new.

In conclusion, the groups of claims define two different inventions not linked by a single general inventive concept.

The application, hence does not meet the requirements of unity of invention.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 WEILI WEI ET AL: "Chiral detection using reusable fluorescent amylose-functionalized graphene",
CHEMICAL SCIENCE,
deel 2, nr. 10, 1 januari 2011 (2011-01-01), bladzijde 2050,
XP055098154,
ISSN: 2041-6520, DOI: 10.1039/c1sc00308a

D2 JASON A. MANN ET AL: "Preservation of Antibody Selectivity on Graphene by Conjugation to a Tripod Monolayer",
ANGEWANDTE CHEMIE INTERNATIONAL EDITION,
deel 52, nr. 11, 11 maart 2013 (2013-03-11), bladzijden 3177-3180,
XP055098219,
ISSN: 1433-7851, DOI: 10.1002/anie.201209149

1 The present application does not meet the criteria of patentability, because the subject-matter of claims 1-4, 7, 8 and 11-14 is not new.

1.1 Document D1 discloses a process for the preparation of a functionalized graphene comprising the steps of reacting amylose with 2-amino-anthracene, further reacting the resulting compound with graphene oxide and reducing the graphene oxide to obtain a graphene layer functionalized with an anthracene molecule bound to amylose.

Therefore, the subject-matter of claims 1-4 is not new.

1.2 Document D1 discloses a functionalized graphene layer with Anthracene-labeled amylose, consisting of the compound formed by the condensation reaction of the amylose reducing end with the amino group of the 2-amino-anthracene. The functionalized graphene is used as a sensor for amino acids (page 2050, right-side column, paragraph 2 - page 2051, right-side column, paragraph 3; page 2055, left-side column, paragraph 3 - right-side column, paragraph 1).

Therefore, the subject-matter of claims 7, 8 and 11-14 is not novel.

2 The present application does not meet the criteria of patentability, because the subject-matter of claims 5 and 6 does not involve an inventive step.

2.1 Claim 5 refers to the solvent used for the graphene functionalization. It is considered that the skilled person would determine the optimal solvent to carry out the functionalization reaction just by mere routine experimentation, without the exercise of inventive skill.

Thus, the subject-matter of claim 5 does not involve an inventive step.

2.2 Claim 6 refers to the length of the molecule attached to the aromatic hydrocarbon. It is considered that the skilled person would determine the optimal length of the molecules used for the graphene functionalization just by mere routine experimentation, without the exercise of inventive skill.

Therefore, the subject-matter of claim 6 is not inventive.

Re Item VIII

Certain observations on the application

1 Claims 1, 9 and 10 are not clear.

1.1 The matter for which protection is sought in claim 1 is not clearly defined. The functional features "the interaction of the first molecule and the graphene is formed" and "to let react the first chemical active moiety of the first molecule and the second active moiety of the second molecule" does not enable the skilled person to determine which technical features are necessary to perform the stated function.

1.2 Furthermore, claim 1 relates to a "molecule containing an aromatic hydrocarbon" and to a "second molecule containing a chemical active moiety". Plausible support has, however, so far only been provided for "amino pyrene" and "the N-hydroxysuccinimide derivative of a 4-mer ethylene glycol molecule". The wording of claim 1 encompasses products which represent an unreasonable generalization from the specific disclosure.

1.3 In addition, given the lack of technical information, it would require an unreasonable amount of experimentation to carry out the invention across the whole scope of the claim, thus imposing a severe and undue burden to the person trying to carry out the process.

1.4 Moreover, a claim is inventive if it is inventive over the whole scope of the claim. -This means that a technical effect must be obtained over the whole range-. In the present case, however, it would be clear to the skilled person that the use of "any molecule containing an aromatic hydrocarbon" or " any molecule containing a chemical active moiety" would not achieve the intended effect.

1.5 Claims 9 and 10 do not meet the requirement of clarity because the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.