(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
1 June 2006 (01.06.2006)

PCT

(10) International Publication Number
WO 2006/056948 A1

(51) International Patent Classification:
B01J 19/00 (2006.01) *C12Q 1/68* (2006.01)

(21) International Application Number:
PCT/IB2005/053878

(22) International Filing Date:
23 November 2005 (23.11.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
04106088.0 25 November 2004 (25.11.2004) EP

(71) Applicant *(for all designated States except US)*: **KONINKLIJKE PHILIPS ELECTRONICS N.V.** [NL/NL]; Groenewoudseweg 1, NL-5621 BA Eindhoven (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **DUINEVELD, Paulus, C.** [NL/NL]; c/o Prof. Holstlaan 6, NL-5656 AA Eindhoven (NL). **STAPERT, Hendrik, R.** [NL/NL]; c/o Prof. Holstlaan 6, NL-5656 AA Eindhoven (NL). **DECRE, Michel, M., J.** [BE/NL]; c/o Prof. Holstlaan 6, NL-5656 AA Eindhoven (NL). **VAN BOMMEL, Ties** [NL/NL]; c/o Prof. Holstlaan 6, NL-5656 AA Eindhoven (NL).

(74) Agents: **VAN WERMESKERKEN, Stephanie, C.** et al.; Prof. Holstlaan 6, NL-5656 AA Eindhoven (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

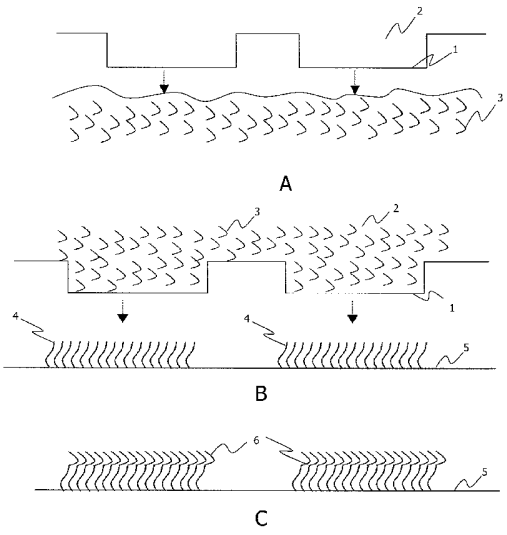
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** METHOD FOR CHEMICALLY ACTIVATING MOLECULES OR PROTECTING ACTIVE MOLECULES



(57) **Abstract:** The invention pertains to a method for chemically activating activatable molecules oh a 'substrate comprising the steps: providing an activation or protective agent to a stamp; bringing the stamp containing the activation agent into contact with at least part of the activatable molecules or the stamp comprising the protective agent into contact with part of the active molecules; and activating the at least partly contacted group of activatable molecules with the activation agent to obtain activated molecules or protecting the at least partly contacted active molecule with the protective agent to obtain protected molecules. The invention further relates to a method for obtaining more than one molecular layer on a substrate .

WO 2006/056948 A1

Method for chemically activating molecules or protecting active molecules

The present invention relates to a method for chemically activating activatable molecules or protecting active molecules on a substrate. The invention further relates to a method for obtaining molecular layers on a substrate.

In molecular diagnostics use is made of arrays, also called biochips. Arrays are substrates that contain a high number of probes on a relatively small area, currently in the order of 1x3 inch x inch, which is the size of a microscope plate that is often used in array-based genomics and proteomics. Gene sequences can be placed and synthesized on the substrate using lithographic techniques or inkjet printing techniques. These production techniques as well as the biological samples are expensive. Proteins (protein arrays) are usually placed using spotting robots. Circular spots can be formed with a typical diameter of about 150 μm. A minimum spot diameter of about 60 μm was reported so far on relative hydrophobic surfaces (contact angle above 20 degrees). Lithographic techniques such as used in the production of DNA arrays may be suitable for small peptide arrays (up to 20 mers) but cannot be used for protein arrays as protein peptide sequences are usually largely exceeding 20.

Diagnostic cartridges will, apart from a detection part also contain channels for transportation of fluids and most likely also separation, heating, cooling, mixing and filtering modules/chambers. These cartridges are of about the same size as the bio-arrays currently available. Because diagnostic cartridges require a much higher level of integration, the detection part will be much smaller than the total cartridge. The area where the sensor bioprobes should be placed is often typically below 1x1 cm^2, which is much smaller than a microscope slide.

Moreover, miniaturization of the detection part is also driven by cost reduction. First, because the detection part of the cartridge may well be made in silicon, the size of this part should be as small as possible. Second, the bioprobes are highly expensive molecules and the quantities used should be minimized as much as possible. Multi-analyte detection protocols for molecular diagnostics will need the ability of measuring typically between 3 – 10000 and preferably 10-1000 biological compounds (DNA/RNA, proteins, sugars, metabolites, cells) on a single cartridge.

An important aspect of the fabrication of biosensors is the immobilization of bio-molecules (the bioprobes) on inorganic (glass), organic, polymeric, metallic or silicon surfaces. Especially, when covalent immobilization of a bio-molecule is desired, activation of the surface is needed before the bio-molecule can efficiently be attached. For example, for covalent immobilization of bioprobes to carboxylic acid groups a standard method is to dip the substrate in an aqueous solution of activation agent, such as (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, or a mixture thereof with N-hydroxysuccinimide, and to wash and optionally dry the substrate thereafter. This method has the drawback that it takes a considerable amount of time to achieve maximum activation. Further the whole activatable substrate will react with the same activation molecules, thus patterning of the substrate cannot be done in this way. Finally, this is a wet process wherein a considerable volume of activation solution is used. After activation, the substrate must be washed and dried to remove (excess) activation molecules from positions of the substrate.

It is an object of present invention to provide a method that is devoid of the disadvantages of the activation methods of the prior art, i.e. to provide a method, which reduces the amount of time which is necessary for obtaining activated molecules, which allows activating a smaller area than is possible using the methods of the prior art, and which reduces the required amount of activation agent solution.

To this end the invention relates to a method for chemically activating activatable molecules or protecting active molecules on a substrate comprising the steps:

- providing an activation or protective agent to a stamp;
- bringing the stamp containing the activation agent into contact with at least part of the activatable molecules or the stamp comprising the protective agent into contact with part of the active molecules; and
- activating the at least partly contacted activatable molecules with the activation agent to obtain activated molecules or protecting the at least partly contacted active molecule with the protective agent to obtain protected molecules.

Using this method, only the activatable molecules that are contacted, will be activated and only the contacted active molecules will be protected. Furthermore, this method permits activating or protecting areas with a surface area smaller than 100 μm^2.

It is to be stressed that the term 'activating' refers to chemical reactions between the activatable molecules on the substrate and the molecule(s), the activation agent, on the stamp to make an inreactive group reactive, or to make a less reactive group more reactive towards the substrate.

'Protecting' refers to chemical reactions between the active molecules on the substrate and the protective agent on the stamp providing a protective group onto the active moiety. The result of the chemical reaction is that protective groups are incorporated to the substrate. Protective groups, such as frequently used in peptide chemistry, serve the purpose of making active groups temporarily inreactive towards a second (incubation) step. The unprotected active molecules can be attached to a second molecule.

Deprotection, i.e. the cleavage of protective groups is encompassed in the definition of the expression 'activating'.

A suitable name for this method of micro-contact printing is micro-contact chemistry as the activation or protective agent reacts only locally, i.e. on the contact interface between the stamp and the molecules, with the activatable molecules, or with the active molecules, respectively. It is a 'dry' method: viz. dipping of the substrate in a solution is not necessary. Therefore, micro-contact chemistry is much faster because washing and drying steps to remove the activation agent or the protective agent from undesired positions on the substrate are not necessary.

Preferably, the activatable molecules or the active molecules are contained in a molecular monolayer. Virtually all molecules in such a monolayer can be accessed easily by the activation or the protective agent.

According to a preferred embodiment, a stamp having a patterned surface containing an activation or a protective agent is contacted with activatable or active molecules, respectively, on a substrate. This embodiment has the advantage that the activatable molecules can be activated, or the active molecules can be protected in a patterned manner after which bio-molecules can be patterned and immobilized on the surface. It is stressed that protection and deprotection of molecules is an alternative for activating activatable molecules. By activating part of the activatable molecules, a part of the molecules is prone to further reaction, and another part not. By protection-deprotection the same situation can be obtained, viz. a part of the molecules is protected, after which a part of the molecules is (still) prone to further reaction, and another part not.

When successively two or more stamps are used having different patterned surfaces, the activatable molecules can be activated successively at different positions. An advantage thereof is that to a layer of the same activatable molecules different bio-molecules can be attached. For example, a first stamp containing an activation agent can be used to activate a part of the layer having activatable molecules to obtain a certain activated pattern, after which bio-molecules can be attached to the activated molecules. Then a stamp having

another pattern containing an activation agent, which may be different or the same as the activation agent contained in the first stamp, is used to activate another part of layer containing the activatable molecules, followed by attaching other bio-molecules to the activated part.

Activatable molecules are molecules having a chemical group that is able to react with an activation agent. The reaction between the chemical group and the activation agent results in molecules having the chemical group activated and apt to further reaction or having temporarily protective group cleaved to obtain a de-protected molecule, which is active.

Active molecules are molecules having an active group, which can be protected by a protective agent.

Examples of activatable groups are: carboxylic acid, sulfurhydryl, hydroxyl, aldehyde, carbohydrate, primary and secondary amine, methylketon, epoxide, lactone (such as caprolactone and lactide), cyclic carbonate, unsaturated compounds (such as in (meth)acrylate, vinylether, ketene, etc.).

Carboxylic acids are preferred as activatable groups. The advantage of a carboxylic acid group-containing molecule is their easy conversion to an activated ester group-containing molecule or to a hydrazide by reaction with a variety of activation agents. An activated ester can easily react with primary or secondary amine containing molecules. This coupling procedure is often used in bio-conjugate chemistry. Hydrazides can react with aldehyde containing molecules to give a hydrazone.

The materials to make the stamp are preferably suitable to permit the activation or the protective agent diffusing into the stamp. The choice of stamp material depends on the solvent, in which the activation agent is dissolved.

Stamps as used in this method can be made of common thermoplastics, (hydro)gels, or thermosets.

Examples of materials suitable for stamps that allow an activation agent soluble in water to diffuse into the stamp include, but are not restricted to: hydrophilic thermoplastic elastomers (TPE's) and hydrogels. Examples of TPE's are poly(ether-block-ester)s, such as multiblock polymers comprising blocks of poly(ethylene glycol) (PEG) or poly(tetramethylene glycol) (PMTG) and poly(butylene terephthalate) (PBT).

Examples of materials suitable for stamps, for applying an activation agent soluble in organic solvents such as ethanol or dichloromethane, are polysiloxane, like

polydimethylsiloxane, which is for instance commercially available as Sylgard® 184 from DOW Corning (UK).

The substrate on which the activatable or active molecules are provided, is not critical and can be made of silicon, polymer, glass, gold, aluminium-oxide or any other suitable material, provided that there is good adhesion between the substrate and the activatable molecules. Substrate preference depends on the chosen application. For example, when a biosensor that optically detects captured bio-molecules is being developed, one wants to use transparent or reflective substrates. Or, when electrochemical or impedimetric detection is preferred the substrate preferably consists of (semi-) conducting material, such as gold, platinum, copper, alumina, indium tin oxide, (doped) silicon, etc. Substrates that have excellent adhesion between activatable molecules and the substrate's surface are gold or silver, especially when the activatable molecules contain a sulfurhydryl group.

The activatable molecules are activated by applying a stamp provided with activation agent. The activation agent can be provided to the stamp by soaking the stamp in a liquid containing the activation agent, followed by removing adhered liquid from the stamp.

The same applies for protecting active molecules. Active molecules can be protected by applying a stamp provided with protective agent. The protective agent can be provided to the stamp by soaking the stamp in a liquid containing the protective agent, followed by removing adhered liquid from the stamp.

The liquid containing the activation or protective agent preferably is an aqueous solution comprising the activation or protective agent.

For some activation procedures it is necessary to make use of a combination of different molecules in order to activate the activatable molecules. One of the molecules may act as a catalyst or may be consumed during the activation procedure, where the other molecule is bonded to the activatable molecule to form the activated molecule. This has been illustrated in the example.

Optionally, a stimulus-sensitive activation agent is used and the activating step c) is triggered by a stimulus, such as heat, pH, UV, and visible light.

After being activated with a stimulus-sensitive activation agent, the activated molecules may be stimulus-sensitive (photoactive, etc.) themselves. The stimuli can be used to either further activate or de-active the activated molecules.

Examples of conventional activation agents are: carbodiimides, such as 1,3-dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, mixtures of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide with N-hydroxysuccinimide or with

sulfo-N-hydroxysuccinimide, mixtures of a base or acid with iodine (I_2) or with bromine (preferably acidic conditions to stop the reaction after one activation step), hydrazine, periodates, nitric acid, hydroxypentafluorobenzene, 1,1'-carbonyldiimidazol (CDI), tert-butyl esters, maleimide derivatives, anhydrides, pyridylthio-erythritol, tosylates (such as p-toluenesulfonylchloride and sulfonylchloride SO_2Cl), acylchlorides, isocyanates, isothiocyanates, (epi-)chlorohydrins, azides, psoralen, epoxide, lactone (such as caprolactone and lactide), cyclic carbonate, unsaturated compounds (such as in (meth)acrylate, vinylether, ketene, etc.), dithioesters, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and derivatives thereof.

Preferably, (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, or a mixture of (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and N-hydroxysuccinimide, dissolved in water is used. These activation agents are particularly useful because they are frequently used for the conjugation of bio-molecules and cells. Bio-molecules include, but are not restricted to: peptides, proteins (including antibodies, F_{ab} fragments, single chain antibodies, enzymes), oligonucleotides (DNA and RNA), aptamers, amino acids, dATP, dCTP, UTP, dGTP, and dTTP.

Protective agents are well known in the art and commonly used for protecting active groups such as hydroxy, thiol, amine, carboxylic groups, and the like.

The present invention also relates to method for obtaining n molecular layers on a substrate, wherein n is an integer of at least 2, by applying one of the previously mentioned methods, followed by a step d) comprising bonding a next molecule to the activated molecules or the active molecules to obtain a further layer, which molecules may be activatable and activated or which molecules are active and may be partially protected, step d) being repeated (n-2) times to obtain n molecular layers.

The advantage of this method for obtaining a specific number (n) of molecular layers on a substrate is that the method is very flexible. If desired, only the method using a stamp (called micro-contact chemistry) is used for activating the activatable molecules or protecting active molecules on the surface as well as for applying other molecular layers onto the activated molecules or the active molecules, which are not protected. The method also permits applying molecular layers using other deposition techniques such as inkjet printing or electro spraying in combination with micro-contact chemistry. The inkjet printing technique, for example, implies the use of a printer head for dispensing a liquid comprising molecules, which will form a molecular monolayer.

In following two paragraphs, as a non-limitive example, application of a stack of four molecular layers is described via activation of an activatable molecule using an activation agent. It will be clear that a similar or the same stack can be obtained via protection of part of active molecules on a substrate using a protective agent, and subsequently applying a molecular layer on the active molecules, which were not protected.

To obtain a stack of, for instance, four molecular layers on a substrate, n is 4. According to steps a, b and c of the previously mentioned methods activated molecules on the substrate are obtained. In step d, a next (2^{nd}) molecule is bonded to the activated molecules to obtain a 2^{nd} molecular layer. The 2^{nd} molecules should be activatable, otherwise activation of the 2^{nd} molecular layer and bonding of a 3^{rd} molecular layer to the 2^{nd} molecular layer is impossible. The application and activation of the 2^{nd} molecular layer can be performed using a stamp, but also other deposition methods (such as inkjet, electro-spraying, etc.) can be used. Step d is repeated n-2 times, thus (n is 4) 2 times more. The first time, a 3^{rd} molecule is bonded to the activated 2^{nd} molecules to obtain a 3^{rd} molecular layer, which is activated. The second time, a 4^{th} molecule is bonded to the 3^{rd} molecular layer. Optionally, a washing step and/or drying step are performed between the applications of two molecular layers.

Thus each layer, to which a following layer of molecules should be bonded, must be activatable. The final layer may be activatable, but this is no longer a requirement.

This method can be used to obtain any desired number n molecular layers on the either activated or active molecules. It is possible that two or more molecular layers contain the same type of molecules. It is also possible that the layer of molecules is a layer of activatable or active bio-molecules.

Preferably at least one, more preferably each of the layers, is a molecular monolayer. Monolayers have the advantage that virtually all activatable molecules are easily accessible by the activation agent and that all active molecules are easily accessible by the protective agent.

The invention is illustrated by the Figs. 1A-1C and by the example. The embodiments of the figures and example are illustrative only, and should not be considered as limitative.

The figures and the example illustrate the activation of activatable molecules using an activation agent. The person skilled in the art will immediately understand that active molecules can be protected using a protective agent.

Figs. 1A-C show a schematic scheme of a procedure for chemically activating a group of activatable molecules on a substrate using a stamp, i.e. micro-contact chemistry.

Fig. 1A shows a patterned surface 1 of a stamp 2 and a solvent, in which an activation agent is dissolved (3). The stamp 2 is preferably made of a hydrophilic gel when the solution is aqueous. In this example, the solvent is water and the activation agent is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC).

To provide the hydrophilic gel of the stamp 2 with activation agent, the patterned surface 1 is dipped into the aqueous solution. EDC 3 diffuses through the patterned surface 1 into the hydrophilic gel 2. When the gel 2 contains sufficient EDC, the gel 2 is removed from the solution and the wetted surface 1 of the stamp 2 is blotted to remove the adhered solution.

Fig. 1B shows a stamp made of hydrophilic gel 2 containing EDC 3 and a group of activatable molecules 4 provided onto a substrate 5. The activatable molecules 4 are preferably carboxylic group-containing compounds. Substrate 5 is preferably made of gold. The carboxylic group-containing activatable molecules 4 are patterned (for illustration purposes; the activatable molecules do not have to be patterned) and arranged in a monolayer. The activation agent EDC can easily access virtually all group-containing activatable molecules due to their arrangement in the monolayer.

The patterned surface 1 of the hydrophilic gel 2 is brought into contact with the patterned monolayer of carboxylic group-containing activatable molecules 4 provided on the gold substrate 5. When brought in contact with EDC-containing hydrophilic gel, the carboxylic group-containing activatable molecules on the gold substrate react with EDC to give activated molecules.

Fig. 1C shows the group of activated molecules 6. The activated molecules 6 are the result of the reaction between the EDC and the carboxylic group-containing activatable molecules.

The invention is further illustrated by the following non-limitative example.

Example

A molecular monolayer of mercapto undecanoic succinimide ester was provided onto a gold substrate as follows.

An aqueous solution of activation agent was prepared by mixing 400 mM of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 200 mM of N-hydroxysuccinimide (NHS) in 1 ml of demineralized water.

Stamps were made of a polymer hydrogel prepared by polymerizing 7.20 g of hydroxyethylacrylate, 1.80 g of polyethyleneglycoldiacrylate, 1.00 g of water and 0.5 % by weight of photo-initiator Darocure® 1173 (2-hydroxy-2-methyl-1-phenyl-1-propanone). The preparation of these stamps are described in more detail in earlier International patent application IB2004/052528 of the Applicant, titled 'Molecular stamp for printing biomolecules onto a substrate'. The surface of the stamp and the molecular monolayer were either patterned or flat.

To provide the stamps with activation agent, the stamps were dipped in the aqueous solution of EDC and NHS for 15 minutes, and EDC and NHS diffused into the stamps.

The stamps were subsequently blotted with a tissue to remove adhered solution.

Then the stamps were brought into contact with the mercapto undecanoic acid monolayers. The period of contact was set at 5 minutes or 30 minutes.

The contacted monolayers were analyzed by grazing angle Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrometry. Resonance peaks were found at 1744 cm^{-1}, 1785 cm^{-1}, and 1817 cm^{-1}, which are characteristic for succinimide ester, which was formed by reaction of NHS and mercapto undecanoic acid. These peaks are not present in spectra of untreated monolayers, and show that the activation reaction with NHS was successful. The activated monolayers had a surface area smaller than 100 μm^2.

CLAIMS:

1. A method for chemically activating activatable molecules or protecting active molecules on a substrate comprising the steps:

- providing an activation or protective agent to a stamp;
- bringing the stamp containing the activation agent into contact with at least part of the activatable molecules or the stamp comprising the protective agent into contact with part of the active molecules; and
- activating the at least partly contacted activatable molecules with the activation agent to obtain activated molecules or protecting the at least partly contacted active molecule with the protective agent to obtain protected molecules.

2. The method according to claim 1 wherein the activatable molecules or the active molecules are contained in a molecular monolayer or as a part of a (structured) monolayer.

3. The method according to claim 1 or 2, wherein the stamp has a patterned surface.

4. The method according to any one of claims 1-3, wherein at least two stamps are used each having another pattern.

5. The method according to any one of claims 1-4 wherein the activatable molecule is a carboxylic acid group-containing molecule.

6. The method according to any one of claims 1-5 wherein the stamp is a hydrophylic gel.

7. The method according to any one of claims 1-6 wherein step a) is performed by soaking the stamp in a liquid containing the activation agent or the protective agent, followed by removing adhered liquid from the stamp.

8. The method according to any one of claims 1-7 wherein 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide or a mixture thereof with N-hydroxysuccinimide is used as the activation agent.

9. The method according to claim 1-7 wherein the activation agent is stimulus-sensitive and activating step c) is triggered by a stimulus.

10. The method according to claim 9, wherein the obtained activated molecules are stimulus-sensitive.

11. A method for obtaining n molecular layers on a substrate, wherein n is an integer of at least 2, by applying the method of any one of claims 1-10, followed by a step d) comprising bonding a next molecule to the activated molecules or active molecules to obtain a further layer, which molecules may be activatable and activated or which molecules are active and may be partially protected, step d) being repeated (n-2) times to obtain n molecular layers.

12. The method according to claim 11 wherein at least one of the molecular layers is a monolayer.

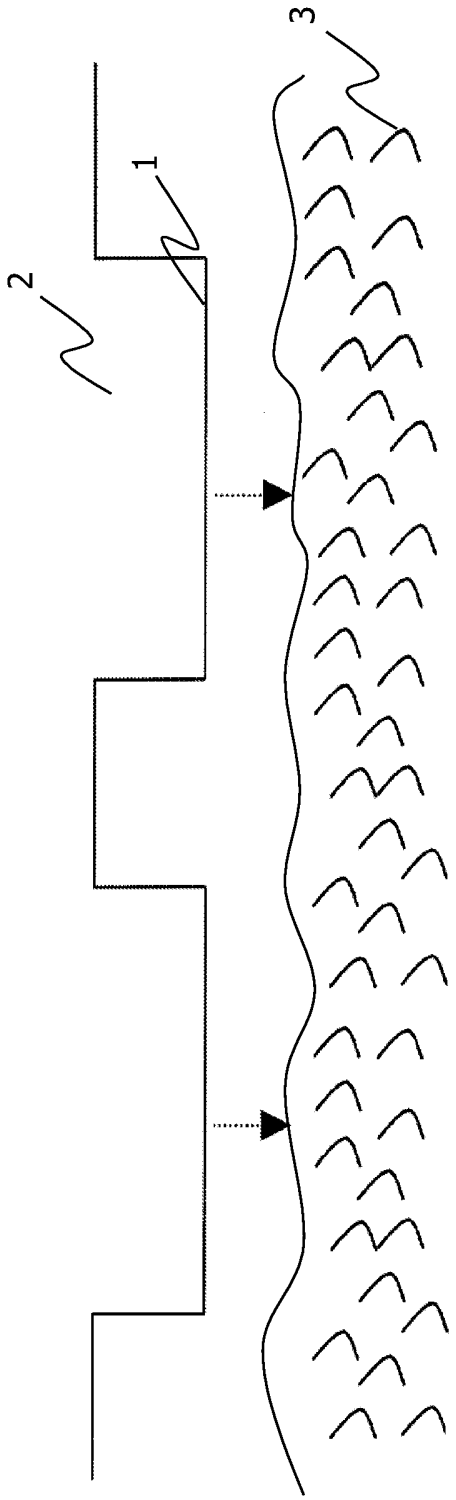


FIG.1A

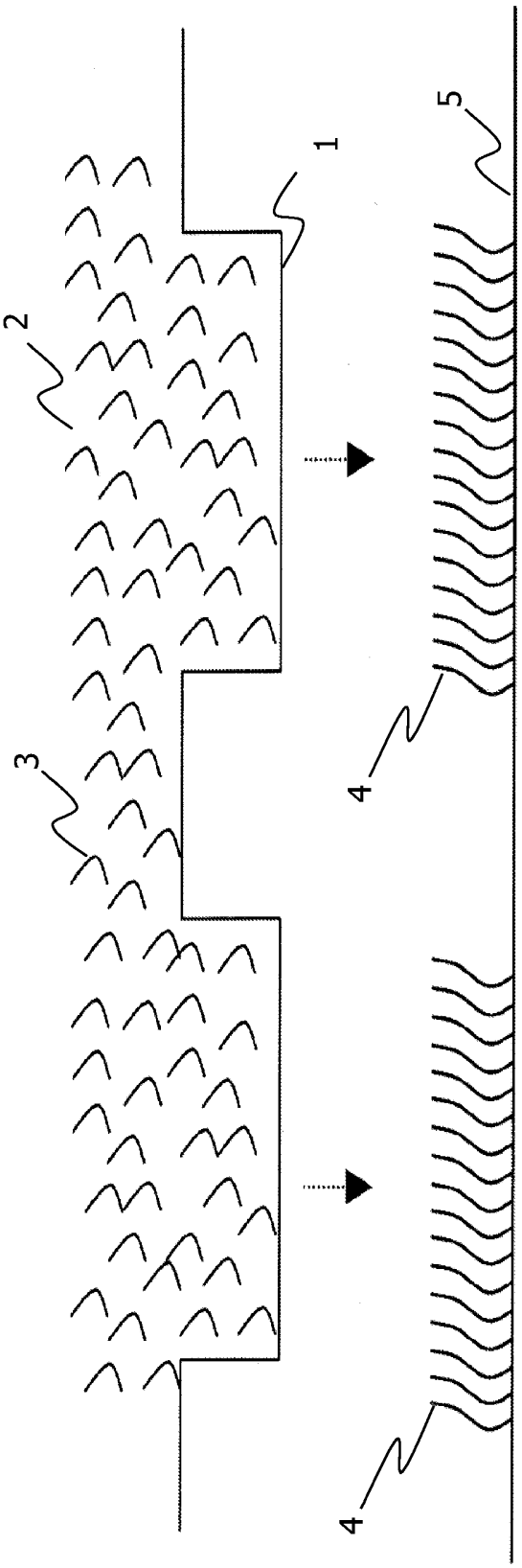


FIG.1B

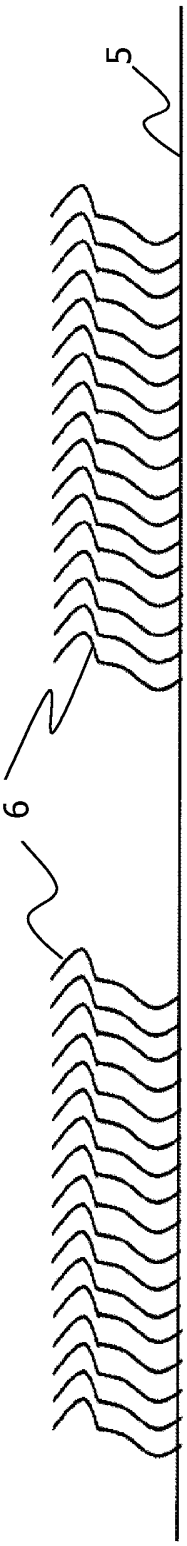


FIG.1C

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2005/053878

A. CLASSIFICATION OF SUBJECT MATTER
B01J19/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01J C12Q

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 195 43 232 A1 (HANS-KNOELL-INSTITUT FUER NATURSTOFF-FORSCHUNG E.V., 07745 JENA, DE) 15 May 1997 (1997-05-15) claims 1-3; figures 1-3	1-12
X	US 6 423 552 B1 (LU ZUHONG ET AL) 23 July 2002 (2002-07-23) paragraphs [0044], [0045]; claims 1-14; example 1	1-12

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2006	29/03/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Veefkind, V

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2005/053878

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIAO P F ET AL: "Soft lithography for oligonucleotide arrays fabrication" PROCEEDINGS OF THE 23RD. ANNUAL INTERNATIONAL CONFERENCE OF THE IEEE ENGINEERING IN MEDICINE AND BIOLOGY SOCIETY. 2001 CONFERENCE PROCEEDINGS. (EMBS). INSTANBUL, TURKEY, OCT. 25 - 28, 2001, ANNUAL INTERNATIONAL CONFERENCE OF THE IEEE ENGINEERING IN M, vol. VOL. 1 OF 4. CONF. 23, 25 October 2001 (2001-10-25), pages 3104-3107, XP010592334 ISBN: 0-7803-7211-5 the whole document	1-12
X	WO 02/099423 A (NANOTYPE GMBH; BLANK, KERSTIN; SPINNLER, KATRIN; MAI, THAO; OESTERHELT) 12 December 2002 (2002-12-12) claim 1	1
A	page 11, line 16 - line 19	8
A	US 2003/059537 A1 (CHILKOTI ASHUTOSH ET AL) 27 March 2003 (2003-03-27) paragraphs [0108], [0153], [0154]	8
A	US 5 948 621 A (TURNER ET AL) 7 September 1999 (1999-09-07) claims, figures	1-12
A	MARTIN B D ET AL: "Direct Protein Microarray Fabrication Using a Hydrogel Stamper" LANGMUIR, AMERICAN CHEMICAL SOCIETY, NEW YORK, NY, US, vol. 14, no. 15, 21 July 1998 (1998-07-21), pages 3971-3975, XP002200398 ISSN: 0743-7463 the whole document	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2005/053878

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 19543232 A1	15-05-1997	NONE	
US 6423552 B1	23-07-2002	AU 2148099 A	25-10-1999
		WO 9951770 A1	14-10-1999
		CN 1193049 A	16-09-1998
WO 02099423 A	12-12-2002	CA 2449224 A1	12-12-2002
		EP 1558930 A2	03-08-2005
US 2003059537 A1	27-03-2003	AU 4537801 A	17-09-2001
		CA 2400814 A1	13-09-2001
		EP 1269189 A2	02-01-2003
		WO 0167104 A2	13-09-2001
		US 6444254 B1	03-09-2002
US 5948621 A	07-09-1999	NONE	