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Datasheet for the decision of 2 December 2014

Case Number: T 2586/11 - 3.4.02

Application Number: 05824471.6

Publication Number: 1841883

IPC: C12Q1/68, G01N13/12, G01N33/53, G01N33/566

Language of the proceedings: EN

Title of invention:
A METHOD FOR ANALYZING NUCLEOBASES ON A SINGLE MOLECULAR BASIS

Applicant:
Japan Science and Technology Agency

Relevant legal provisions:
EPC 1973 Art. 56

Keyword:
Inventive step (yes)

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Case Number: T 2586/11 - 3.4.02

D E C I S I O N
of Technical Board of Appeal 3.4.02
of 2 December 2014

Appellant: (Applicant)	Japan Science and Technology Agency 1-8, Honcho 4-chome Kawaguchi-shi, Saitama 332-0012 (JP)
Representative:	Calamita, Roberto Dehns St Bride's House 10 Salisbury Square London EC4Y 8JD (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 16 June 2011 refusing European patent application No. 05824471.6 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	A. G. Klein
Members:	F. J. Narganes-Quijano
	B. Müller

Summary of Facts and Submissions

I. The appellant (applicant) has lodged an appeal against the decision of the examining division refusing European patent application No. 05824471.6 based on International application No. PCT/JP2005/024285 (International publication No. WO 2006/070946).

In its decision the examining division referred to documents

D1: GB-A-2235049

D9: "Electrochemical responses and surface structure of gold electrodes modified with nucleic acid base derivatives", Y. Sato *et al.*, The Electrochemical Society Inc. (US), 205th Meeting, Abstract 647 (2004),

and held that the subject-matter of claims 1 to 4 then on file did not involve an inventive step over the closest state of the art represented by document D1, in combination with the teaching of document D9 (Article 56 EPC 1973).

II. With the statement setting out the grounds of appeal the appellant submitted sets of claims amended according to a main and a series of auxiliary requests and requested that the decision under appeal be set aside and a patent be granted.

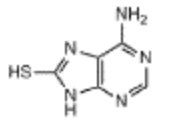
III. In reply to a telephone consultation with the rapporteur of the Board the appellant, with the letter dated 15 October 2014, filed an amended set of claims 1 to 4 replacing the set of claims of the main request, and pages 2, 5, 6, 10 and 12 of the description and drawing

sheets 1/7 to 7/7 replacing the corresponding application documents on file.

IV. Claims 1 to 4 of the present main request are all independent claims and are worded as follows:

"1. A method for pinpointing a target nucleobase in a nucleic acid, which comprises:

scanning a nucleobase molecular tip on the nucleic acid, wherein the nucleobase molecular tip is a gold tip chemically modified with a nucleobase complementary to the target nucleobase, and the complementary nucleobase is selected from the following thiol derivatives of adenine (I), guanine (II), cytosine (III) and uracil (IV):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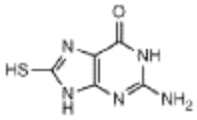
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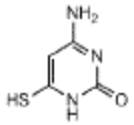
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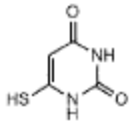
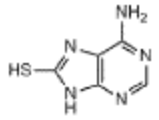

(IV)

measuring the tunnelling current between each nucleobase in the nucleic acid and the nucleobase molecular tip with scanning tunnelling microscopy; and

pinpointing the target nucleobase as the nucleobase from which the tunnelling current is facilitated upon scanning the nucleobase molecular tip."

"2. A method for typing a target nucleobase in a nucleic acid, which comprises:

scanning four nucleobase molecular tips on the nucleic acid, wherein the four nucleobase molecular tips are gold tips chemically modified with the following thiol derivatives of adenine (I), guanine (II), cytosine (III) and uracil (IV), respectively:



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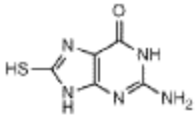

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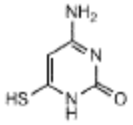

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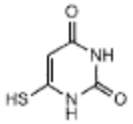


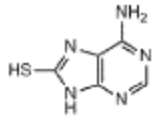
(IV)

measuring the tunnelling currents between the target nucleobase in the nucleic acid and each nucleobase molecular tip with scanning tunnelling microscopy; and

determining the type of the target nucleobase that is complementary to the nucleobase on the nucleobase molecular tip by which the largest tunnelling current is measured."

"3. A method for sequencing a nucleic acid, which comprises:

scanning sequentially four nucleobase molecular tips on the nucleic acid, wherein the four nucleobase molecular tips are gold tips chemically modified with the following thiol derivatives of adenine (I), guanine (II), cytosine (III) and uracil (IV), respectively: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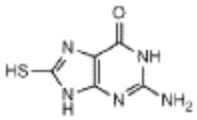
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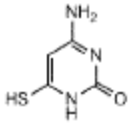
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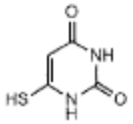
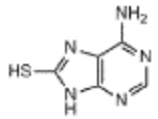
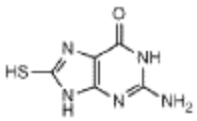

(IV)

measuring the tunnelling currents between each nucleobase in the nucleic acid and each nucleobase molecular tip with scanning tunnelling microscopy; and

determining the type of each nucleobase that is complementary to the nucleobase on the nucleobase molecular tip by which the largest tunnelling current is measured, thereby sequencing the nucleic acid."

"4. A set of four nucleobase molecular tips for scanning tunnelling microscopy, which consists of four gold tips chemically modified with the following thiol derivatives of adenine (I), guanine (II), cytosine (III) and uracil (IV), respectively:


(I)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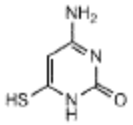
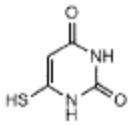

(III)


(IV)

."

Reasons for the Decision

1. The appeal is admissible.

2. *Amendments*

The Board is satisfied that the application documents amended according to the present main request of the appellant comply with the formal requirements of the EPC. In particular, independent claims 1 to 3 are based on the combination of claims 1 and 2, claims 1 and 3, and claims 1 and 4 of the application as published, respectively, together with the passage on page 10, lines 9 to 14 of the description and the thiol derivatives 1 to 4 shown in Fig. 1 (b) of the application as published; and independent claim 4 is based on independent claim 6 of the application as published, together with the aforementioned disclosure of the description and Fig. 1 (b) of the application as published. The description has been amended in order to comply with the requirements of the EPC, and in particular with those set forth in Article 84, second sentence and Rule 27(1) (b) and (c) EPC 1973.

3. *Claim 1 - Inventive step*

3.1 The Board concurs with the examining division's finding that document D1 represents the closest state of the art. The document pertains to the field of scanning tunnelling microscopy (in the following "STM") and proposes a STM technique (abstract and Figure 2) consisting in scanning a nucleobase molecular tip on a

DNA strand (page 1, lines 29 to 32, and page 2, lines 12 to 16 and lines 19 to 28). The nucleobase molecular tip is a gold tip chemically modified with one of the four nucleobases (page 2, lines 19 to 23, together with page 3, lines 13 to 17 and the paragraph bridging pages 3 and 4). The tunnelling current between the nucleobases in the DNA strand and the nucleobase molecular tip is then measured (claim 2 together with page 2, lines 28 to 34) and, after repeating the measurements with different nucleobases in the tip (page 1, lines 38 to 40, and page 4, second paragraph), the tunnelling current measurements are used for mapping the structure of the DNA strand (page 1, first paragraph and lines 29 to 32) by determining which of the four nucleobases constituting the DNA strand is at a particular location (page 1, lines 22 to 24, and lines 38 to 40). The determination of the location of the nucleobases is based on the fact that each nucleobase binds stronger to its complementary nucleobase than to a non-complementary nucleobase, and this is reflected in the measured tunnelling current (page 1, lines 28 and 29 and lines 32 to 34, together with paragraph bridging pages 2 and 3).

3.2 Claim 1 is directed to a method for pinpointing a target nucleobase in a nucleic acid using a STM technique of the type disclosed in document D1. However, as already held by the examining division in its decision, while in document D1 the molecular tip is a gold tip chemically modified with one of the four nucleobases, i.e. adenine, guanine, cytosine and uracil (page 2, lines 19 and 20, page 3, third paragraph, and page 4, second paragraph), claim 1 requires that the nucleobase is selected from the thiol derivatives of adenine, guanine, cytosine and uracil defined in the claim (see point IV above).

The Board therefore concurs with the examining division that the subject-matter of claim 1 is novel over the disclosure of document D1 by virtue of the use of the mentioned thiolated forms of the nucleobases.

The question arises whether the fact that claim 1 is directed to a method for pinpointing a target nucleobase in a nucleic acid constitutes a further distinguishing feature over the disclosure of document D1. However, since, as shown below, the claimed method, in the Board's view, involves an inventive step by virtue of the use of the thiolated derivatives, there is no need to address the question of whether the method of mapping the structure of a DNA strand disclosed in document D1 implicitly anticipates, or at least renders obvious, its application for the purposes of pinpointing a particular one of the four nucleobases in the DNA strand as claimed.

3.3 In its decision the examining division held that the distinguishing feature identified in point 3.2 above involved no special technical effect, that consequently the objective problem solved by the claimed method over document D1 could only be seen in an alternative method of attaching the nucleobases to the gold tip, and that the claimed solution was rendered obvious by the disclosure of document D9.

The Board, however, does not find the line of argument followed by the examining division persuasive. The description of the application emphasizes the role played by the features of the claimed tip in obtaining a coplanar orientation between the nucleobase in the tip and the complementary nucleobase in the nucleic acid, the coplanar configuration having a clear impact on the formation of the hydrogen-bonds between the

complementary nucleobases and therefore on the generation of the electron tunnelling current to be measured (page 19, lines 13 to 22). Furthermore, while document D1 acknowledges that the nucleobases "have some rotational freedom" (page 3, lines 4 to 7), the description of the application discloses that in the case of the claimed invention "the base-base coplanarity is attained probably by the rotation of a carbon-sulfur bond in the thio-base on a tip" (page 19, lines 22 to 24), and in the Board's view this physical mechanism, though explained in a rather speculative manner, supports an improved effect of the use of the claimed thiolated derivatives on the electron tunnelling current between pairs of complementary nucleobases and therefore on the selective identification of the location in the DNA strand of the nucleobases complementary to the nucleobase present in the tip (see the electron tunnelling measurements and the STM image profiles disclosed in Figures 2 to 6 together with the corresponding description).

In addition, contrary to the examining division's view, document D9 fails to disclose or suggest thiolated derivatives of the nucleobases in a gold tip for use in STM techniques. Indeed, document D9 reports on the electrochemical properties of planar gold electrodes modified with thiolated derivatives of nucleobases such as 6-amino-8- and 2-amino-6-purinethiol, and 2-thiouracil, and discloses that these derivatives can be used as a "good promoter for electrochemistry of cytochrome *c*" and that they "associate with complementary nucleic bases in solution followed by the change in current response of cytochrome *c*" (title, and first column, penultimate paragraph; see also second column, first and second paragraphs). The Board is unable to find in this disclosure a hint towards the use

of thiolated derivatives of the nucleobases in molecular gold tips for use in STM. In particular, the reference to the association of the thiolated derivatives with the corresponding complementary nucleobases is only made in the context of the thiolated derivatives constituting a layer formed on the planar electrode and the complementary nucleobases being suspended in solution, and this disclosure would dissuade the skilled person from considering any application of the teaching of the document to a tip-like shaped electrode of the type used in STM and to the mapping of nucleobases immobilized in a DNA strand. Furthermore, document D9 makes reference to STM, but only as one of the techniques used in confirming the formation of hydrogen bond complexes between the thiolated derivatives in the gold planar electrodes and the complementary nucleobases present in the solution (first column, penultimate paragraph, last sentence), and there is no suggestion towards the use of thiolated derivatives in the STM tip. Finally, even assuming that the skilled person would understand the disclosure of document D9 as suggesting some beneficial effect of the use of thiolated derivatives on the formation of hydrogen bonding complexes between complementary nucleobases, the achievement of such an effect would, according to the disclosure of document D9, be confined to the context of an "immobilized base and the complementary base in solution" (document D9, second column, second paragraph, penultimate sentence), and there is no hint in the document that the thiol groups may play a beneficial role in the relative orientation of the complementary bases in the STM technique under consideration in which both the nucleobase in the tip and the nucleobases in the DNA strand are in an immobilized state.

In view of all these considerations, the Board concludes that documents D1 and D9 do not render obvious the method defined in claim 1.

3.4 The remaining documents on file are less pertinent for the issues under consideration.

3.5 The Board concludes that the method defined in claim 1 involves an inventive step over the available prior art (Article 56 EPC 1973).

4. *Independent claims 2, 3 and 4 - Inventive step*

When compared with claim 1, independent claims 2 and 3 are essentially directed to the application of the STM technique defined in claim 1 and discussed in point 3 above to a method of typing a target nucleobase in a nucleic acid and to a method of sequencing a nucleic acid, respectively. Since, as already concluded in point 3.3 above, the mentioned STM technique is not rendered obvious by the available prior art, the subject-matter of independent claims 2 and 3 also involves an inventive step (Article 56 EPC 1973).

Independent claim 4 is directed to a set of four nucleobase molecular tips for STM, each of the four tips consisting of a gold tip chemically modified with a respective one of the same four thiolated nucleobases referred to in claim 1. Document D1 already discloses a set of four gold tips each with a corresponding one of the four nucleobases (page 1, lines 38 to 40), but, as already concluded in point 3.3 above, none of the documents on file discloses or suggests replacing the nucleobases by the corresponding thiolated nucleobases as required by claim 4. Therefore, also the subject-

matter of this claim involves an inventive step within the meaning of Article 56 EPC 1973.

5. The Board is also satisfied that the application documents amended according to the present main request and the invention to which they relate meet the remaining requirements of the EPC within the meaning of Article 97(1) EPC. The Board concludes that the decision under appeal is to be set aside and a patent to be granted on the basis of the application documents amended according to the present main request of the appellant.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to grant a patent in the following version:
   - claims: claims 1 to 4 filed with the letter dated 15 October 2014,
   - description: pages 1, 3, 4, 7 to 9, 11 and 13 to 22 filed with the letter dated 20 July 2007 and pages 2, 5, 6, 10 and 12 filed with the letter dated 15 October 2014, and
   - drawings: sheets 1/7 to 7/7 filed with the letter dated 15 October 2014.

The Registrar: The Chairman:

M. Kiehl A. G. Klein

Decision electronically authenticated