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Datasheet for the decision of 22 November 2006

Case Number: T 0110/04 - 3.4.03

Application Number: 96916490.4

Publication Number: 827628

IPC: H01J 49/04

Language of the proceedings: EN

Title of invention:
Methods and apparatus for sequencing polymers with a statistical certainty using mass spectrometry

Applicant:
PERSEPTIVE BIOSYSTEMS, INC.

Opponent:
-

Headword:
Polymer sequencing using mass spectrometry

Relevant legal provisions:
EPC Art. 54(2), 56, 123(2)

Keyword:
"Amendments (permitted)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0110/04 **-** 3.4.03

D E C I S I O N
of the Technical Board of Appeal 3.4.03
of 22 November 2006

Appellant: PERSEPTIVE BIOSYSTEMS, INC.
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 31 July 2003 refusing European application No. 96916490.4 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chair: R. G. O'Connell
Members: E. Wolff
P. Mühlens

Summary of Facts and Submissions

I. European patent application 96 916 490.4 was refused on the ground that claim 1 of each of the applicant's requests lacked an inventive step over a combination of document

D6: US 5 288 644 A

and any one of the following documents

D1: Nuclear Instruments & Methods in Physics Research, vol. 218, 1983, Amsterdam NL, Pages 276-286, R.J. Colton: 'Secondary Ion Mass Spectrometry : High-Mass Molecular and Cluster Ions'

D2: International Journal of Mass Spectrometry and Ion Physics, vol. 31, 1979, Amsterdam NL, Pages 65-69, J.G. Van Raaphorst et al: 'The Evaluation Of Measurement Data In Thermal Ionisation Mass Spectrometry'

D3: Trac: Trends In Analytical Chemistry, vol. 12, no. 10, Nov./Dec. 1993, Pages 413-421, Roepstorff P: 'Mass Spectrometry Of Proteins'

D7: Chemical Physics Letters, Vol. 181, No. 5, 5 July 1991, Pages 479-484

II. During the appeal procedure, the board also considered the relevance of the following further documents which are cited as pertinent background in the introductory part of the description

C1: Rapid Protein Sequencing by the Enzyme-Thermospray LC/MS Method; Stachowiak et al., J. Am. Chem. Soc, pp 1758-1765, vol. 110, No. 6, 1988

C2: Carboxy-terminal Protein Sequence Analysis using Carboxypeptidase P and Electrospray Mass Spectrometry; Smith et al, Techniques in Protein Chemistry IV, pp 463-470, 1993

C3: Simultaneous Analysis of C-Terminal Amino Acid Residues of Peptide Mixtures by Combination of Filed Desorption Mass Spectrometry and Carboxypeptidase Digestion; Hong et al., Biomedical Mass Spectrometry, pp 450-457, vol. 10, No. 9, 1983

III. Oral proceedings were held on 29 March 2006 at which the appellant submitted a new main request and three new auxiliary requests.

IV. Claim 1 of the main request reads as follows:

1. A method of obtaining sequence information about a polymer comprising a plurality of monomers of known mass, said method comprising the steps of:

(a) providing a mass spectrometer sample plate comprising a reaction surface, wherein said reaction surface comprises a hydrolyzing agent;

(b) hydrolyzing on said reaction surface said polymer to break inter-monomer bonds and provide a set of polymer fragments, each polymer fragment differing

from each other polymer fragment by one or more of said monomers of known mass;

(c) measuring the difference *x* between the mass-to-charge ratios of a pair of polymer fragments;

(d) asserting as a mean difference between the mass-to-charge ratios of the pair of fragments measured at step c) a value μ which corresponds to a known mass-to-charge ratio of one of said monomers of known mass;

(e) selecting a confidence level for μ;

(f) analysing x to determine if x is statistically different from μ at the selected confidence level; and

(g) determining if μ is assignable to x at the selected confidence level based upon the analysis in step f).

Claims 2 to 12 are dependent on claim 1. Claim 13 is worded as follows:

13. A kit for obtaining sequence information by mass spectrometry about a polymer comprising one or more monomers of known mass, wherein said kit comprises:

(a) a mass spectrometer;

(b) a sample plate for use in conjunction with the mass spectrometer, the sample plate comprising a reaction surface, wherein said reaction surface

comprises a hydrolyzing agent arranged to provide hydrolysing on said reaction surface a polymer comprising a plurality of monomers of known mass to break inter-monomer bonds and provide a set of polymer fragments, each polymer fragment differing from each other polymer fragment by one or more of said monomers of known mass, the sample plate being arranged to hold said set of polymer fragments; and

(c) a computer program stored on a computer readable disc, the program being loadable onto a computer so as to render the computer able to acquire data from the mass spectrometer and to obtain sequence information about a polymer according to the method steps of claim 1.

V. In addition to the amended claims, the appellant also submitted consequentially amended pages of the description during the oral proceedings.

VI. The central argument put forward by the appellant on the issue of inventive step was that the prior art neither disclosed nor suggested obtaining sequence information in the manner of the invention by statistical analysis of a mixture of polymer fragments without the fragments having been separated into different groups beforehand.

VII. At the end of the oral proceedings the board decided that the procedure should be continued in writing.

Reasons for the Decision

1. The appeal is admissible.

The main request

2. *Amendments (Article 123(2) EPC)*

2.1 Claim 1 of the main request, as compared to claim 1 in its originally filed form,

(i) now specifies that, as described (, e.g., page 13, lines 19 to 29; page 21, lines 3 to 24; page 26, line 26 to page 27, line 10), the polymer is hydrolysed on the reaction surface of a sample plate to provide the polymer fragments (paragraph (a) and the first part of paragraph (b)), and

(ii) clarifies that it is, as described (e.g., page 4, lines 26 to 29; page 14, lines 1 to 12), the difference x between the mass-to-charge ratios of a pair of polymer fragments which is measured, and

(iii) clarifies that the asserted value μ corresponds, as described (e.g. page 4, line 29 to page 5, line 1; page 25, lines 13 to 17)), to a known mass-to-charge ratio of one of said monomers of known mass.

2.2 Claim 13 concerns a kit of parts. Paragraphs a) and b) mirror the corresponding text of claim 1, and paragraph c) of the claim now refers expressly to a

computer program stored on a computer readable disc which serves to obtain sequence information by the method of claim 1.

2.3 The amendments made to the description provide for consistency of wording with the amended claims and remove references to apparatus that is no longer claimed.

2.4 The board is satisfied that the amendments are based on corresponding disclosures in the application as originally filed and are therefore permitted by Article 123(2) EPC.

3. *Novelty*

3.1 The invention as claimed in claim 1 provides a method of obtaining sequence information from a polymer using sets of polymer fragments and mass spectrometry. The method relies on a statistical correlation between a measured difference x and an asserted difference μ of the mass-to-charge ratios of pairs of polymer fragments, without having to separate out the polymer fragments beforehand. The polymer fragments differ from each other by one or more monomers of known mass, and the difference μ corresponds, with a chosen confidence level, to the known mass-to-charge ratio of one or more different monomers.

3.2 Document D6 constitutes the closest prior art. It discloses that the sequence of the bases in DNA is determined by measuring the molecular mass of DNA fragments in a mass spectrometer. It requires that four separate collections of DNA fragments are prepared with

the aid of four different base-specific reactions. The spectra of the collections are recorded under conditions under which no further fragmentation occurs, so that for positive ions the molecular weight is that of the fragment plus one proton, and for the negative ions that of the fragment minus a proton (column 2, lines 41 to 68). There is a passing mention that, as an alternative, sequence information could be obtained from a single mass spectrum, but this alternative is not discussed in any detail and is dismissed as giving inferior accuracy (column 3, lines 1 to 10). Differences in the mass-to-charge ratios between two fragments are referred to, but only in the context of detecting and correcting sequence errors (document D6 at col. 7, lines 25 to 42). There is no mention in document D6 of any statistical correlation between known monomers and those differences.

3.3 It follows that the invention as claimed in claim 1 is new with respect to the disclosure in document D6.

3.4 Claim 13

The method claimed in claim 1 is novel and relates to the technical problem of obtaining sequence information. Accordingly claim 13, which relates to a kit of parts that includes a computer program to enable a computer to obtain sequence information according to the method steps of claim 1, is also novel.

4. *Inventive Step*

4.1 Based on the differences between the claimed invention and the nearest prior art, the objective problem to be

solved by the invention is to find a method of obtaining sequence information which does not require the preparation and evaluation of different sets of polymer fragments.

4.2 The invention as claimed in claim 1 of the main request solves this problem by forming the polymer fragments with the aid of hydrolysis, obtaining a mass spectrum of the polymer fragments and statistically correlating - at a chosen confidence level - the measured differences in the mass-to-charge ratios of the fragments with asserted differences that correspond to monomers of known mass-to-charge ratios.

4.3 Refusing the application, the examining division considered the claimed invention to be obvious over a combination of the teaching of document D6 and the use of statistics as taught by any one of documents D1 to D3 and D7. The examining division relied in particular on the text in column 3, lines 1 to 10 of document D6 to show that it was known that repetition would improve accuracy, on the text in column 7, lines 9 to 21 to show that the use of a computer was known, and on the text in column 7, lines 21 to 45 to show that in document D6 the differences between adjacent peaks of the spectrum were measured.

4.4 However, as stated in paragraph 3.2 above, in document D6 the difference in the mass-to-charge ratio between two fragments is used to correct sequence errors, e.g., to interpolate an undetected small peak in the spectrum (column 7, lines 30 to 36). In contrast to the invention claimed in claim 1, the method of document D6 requires that four different, base-specific reactions

are performed on portions of the DNA molecules to be sequenced by techniques which are themselves known (D6, col. 2 lines 46 to 48) as standard techniques. "These standard procedures produce from each section of DNA to be sequenced **four separate collections of DNA fragments**, each set containing only one or two of the four bases." (D6, col. 2, lines 48 to 52, emphasis added by the board). There is also no mention in document D6 of the statistical correlation with known monomers claimed claim 1. The method claimed in claim 1 cannot therefore be derived in an obvious manner solely from what is taught in document D6.

4.5 Document D1 briefly mentions statistics in the context of interpreting with the aid of a statistical bond-breaking model the secondary ion spectra of alkali halides (page 278, left-hand column last two lines to right-hand column end of first paragraph).

4.6 Document D2 discusses the use of statistical methods to evaluate spectra for uranium isotopes 234 to 238. The statistical analysis disclosed there involves similar statistical tools in the form of the Student-t-test (page 66, lines 1 to 19 of the chapter headed "STATISTICAL EVALUATION"), but gives the skilled person no indication what correlations should be established for the purpose of obtaining sequence information from polymer fragments.

4.7 Document D3 refers to an improved accuracy because the molecular mass can be determined as the average of that determined for a number of peaks (page 416, left-hand column, lines 5 to 8). Document D3 therefore cannot be seen to disclose anything other than the broad

principle of applying statistics to the results of the mass spectrometry of proteins.

4.8 Document D7 discusses the velocity distributions of high mass polypeptide molecule ions in combination with matrix assisted laser desorption, and that the mass dependence of the ion translational energy distribution may have consequences for the design of time-of-flight mass spectrometers (page 481, last paragraph). There is neither a disclosure of any specific techniques for obtaining sequence information about a polymer, nor any mention of correlating mass-to-charge differences with monomers of known mass for the purpose of doing so.

4.9 The application is based on the appreciation that it is unnecessary to separate out different fragments because the application of statistical techniques, which are themselves known, permits obtaining of the sequence information. As claimed in claim 1 of the main request, a statistical correlation is established between the measured and asserted differences in mass-to-charge ratios, and hence between the measured differences and the mass-to-charge ratios of known monomers. This correlation which is obtained from an undifferentiated sample, that is, a sample containing all the polymer fragments obtained by hydrolysis, and is based on a confidence level to be chosen for it, cannot be derived from any of the documents D1 to 3, D6 or D7. Consequently, the skilled person would not arrive at the claimed method by merely combining the teaching of document D6 with the teaching in any one of documents D1 to D3 and D7.

4.10 The board also examined the three documents, C1, C2 and C3 and found that none of them suggested obtaining sequence information by the claimed method, whether read alone or together with any one of the other cited documents.

5. For the foregoing reasons the board concludes that neither the method claimed in claim 1 of the application in suit nor the kit of parts claimed in claim 13 for performing that method is obvious over the cited prior art.

6. In the judgement of the board the application according to the main request complies with the EPC.

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 on the basis of the main request submitted during the oral proceedings and comprising the following documents:

claims

1 to 13

description

pages 1 to 3, 5, 6, 8 to 11, 13, 16 to 22, 24, 26 to 33, 35 to 42, 44 to 51 as originally filed

pages 4,7,12,14,15,23,25,34,43 as filed during the oral proceedings

drawings

as originally filed.

Registrar Chair

S. Sánchez Chiquero R. G. O'Connell