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**Datasheet for the decision
of 4 September 2014**

Case Number: T 2225/10 - 3.3.08

Application Number: 04813618.8

Publication Number: 1692486

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Language of the proceedings: EN

Title of invention:
Biosensors for detecting macromolecules and other analytes

Applicant:
Saint Louis University

Headword:
Biosensors/SAINT LOUIS

Relevant legal provisions:
EPC Art. 56
EPC R. 139

Keyword:
Inventive step (yes)
correction of error (Figure 27) (no)
Correction of error (Page 42) (yes)

Decisions cited:

Catchword:



**Beschwerdekammern
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Case Number: T 2225/10 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 4 September 2014

Appellant: Saint Louis University
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Decision under appeal: **Decision of the Examining Division of the
European Patent Office posted on 11 June 2010
refusing European patent application No.
04813618.8 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman M. Wieser
Members: T. J. H. Mennessier
C. Heath

Summary of Facts and Submissions

- I. The applicant (appellant) lodged an appeal against the decision of the examining division dated 11 June 2010, whereby the European application number 04 813 618.8 was refused. The application, entitled "*Biosensors for detecting macromolecules and other analytes*", originated from the international application published as WO 05/59509 (hereinafter referred to as the application as filed).
- II. The decision was based on the main request and the first and second auxiliary requests, all filed under cover of the letter of 30 April 2010, as well as the third auxiliary request filed at the oral proceedings of 4 May 2010. All requests were refused for lack of inventive step (Article 56 EPC) in the light of the disclosure in document D2.
- III. Together with its statement setting out the grounds of appeal, the appellant filed submissions which were accompanied by a new main and a new first auxiliary request. Moreover, a correction under Rule 139 EPC of page 42 of the description (paragraphs [000131] and [000132]) and of Figure 27 was requested.
- IV. The board issued a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), which was sent together with the summons to oral proceedings.
- V. On 1 August 2014, in reply to the board's communication the appellant submitted additional comments and filed new auxiliary requests 2 to 5.

VI. Oral proceedings took place as scheduled on 4 September 2014. The appellant filed a new main request and withdrew all its previous claim requests.

VII. The main request consisted of 11 claims of which claims 1, 3 and 10 read:

"1. A method of detecting a polypeptide in a sample comprising the steps of

(a) contacting a sample with a first molecular-recognition construct, and a second molecular-recognition construct, and

(b) detecting an association of the first molecular-recognition construct, the second molecular-recognition construct, and the polypeptide by a detection method; wherein

(c) the first molecule-recognition construct is capable of binding to a first epitope of the polypeptide and the second molecular-recognition construct is capable of binding to a second epitope of the polypeptide,

(d) the first molecular-recognition construct comprises (i) a first epitope-binding agent that can bind to the first epitope, (ii) a first signaling oligo and (iii) a first label, **the first epitope-binding agent being covalently attached to the first signaling oligo via a first flexible linker**, and

(e) the second molecular-recognition construct comprises (iv) a second epitope binding agent that can bind to the second epitope, (v) a second signaling oligo, which is complementary to the first signaling oligo but which only forms a stable association with

the first signaling oligo when brought into close proximity thereto through binding to the polypeptide, and (vi) a second label, **the second epitope-binding agent being covalently attached to the second signaling oligo via a second flexible linker,**

wherein the first and second label produce a different signal when the first molecular-recognition construct, the second molecular recognition construct and the polypeptide associate, thereby bringing the first and second label into close proximity, than they do when the first molecular-recognition construct, the second molecular-recognition construct and the polypeptide are not associated, and further wherein

- i) **the first epitope-binding agent and the second epitope-binding agent are each an aptamer, or**
- ii) **the first epitope-binding agent is a double stranded polynucleotide and the second epitope-binding agent is an aptamer, or**
- iii) **the first epitope-binding agent is an aptamer and the second epitope-binding agent is an antibody.**

(emphasis added by the board to show the difference with claim 1 of auxiliary request 2 underlying the decision under appeal)

"3. The method of claim 2 which is used to detect an analyte in a sample, the method comprising as step (a) contacting a sample with a first aptamer construct, a second aptamer construct and a polypeptide, wherein **in the presence of the analyte,** the first aptamer construct is capable of binding to a first epitope of the polypeptide **and the second aptamer construct is**

capable of binding to a second epitope of the polypeptide, so that the said different signal is indicative of the presence of the analyte."

(emphasis added by the board to show the difference with claim 4 of auxiliary request 2 underlying the decision under appeal)

10. A molecular beacon comprising a first molecular-recognition construct and a second molecular recognition construct: wherein

(a) the first molecule-recognition construct is capable of binding to a first epitope of a polypeptide and the second molecular-recognition construct is capable of binding to a second epitope of the polypeptide,

(b) the first molecular-recognition construct comprises (i) a first epitope-binding agent that can bind to the first epitope, (ii) a first signaling oligo and (iii) a first label, **the first epitope-binding agent being covalently attached to the first signaling oligo via a first flexible linker**, and

(c) the second molecular-recognition construct comprises (iv) a second epitope-binding agent that can bind to the second epitope, (v) a second signaling oligo which is complementary to the first signaling oligo but which only forms a stable association with the first signaling oligo when brought into close proximity thereto through binding to the polypeptide, and (vi) a second label, **the second epitope-binding agent being covalently attached to the second signaling oligo via a second flexible linker**,

wherein the first and second label produce a different signal when the first molecular-recognition construct, the second molecular recognition construct and the polypeptide associate, thereby bringing the first and second label into close proximity, than they do when the first molecular-recognition construct, the second molecular-recognition construct and the polypeptide are not associated; wherein

- i) the first epitope-binding agent and the second epitope-binding agent are each an aptamer, or**
- ii) the first epitope-binding agent is a double stranded polynucleotide and the second epitope-binding agent is an aptamer, or**
- iii) the first epitope-binding agent is an aptamer and the second epitope-binding agent is an antibody."**

(emphasis added by the board to show the difference with claim 11 of auxiliary request 2 underlying the decision under appeal)

Claims 2 and 4 to 9 referred to preferred embodiments of the method of claim 1, claim 11 referred to a preferred embodiment of the molecular beacon of claim10.

VIII. The following document is referred to in this decision:

(D2) WO 00/70329 (published on 23 November 2000)

IX. The submissions made by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

Corrections requested under Rule 139 EPC

The constructs described in paragraphs [000129] and [000130] were for use in a sensor as shown in Figure 26A. The molecular beacon referred to in paragraph [000131] comprised two epitope-binding agents, one being a double stranded DNA and the other being an aptamer. The molecular beacon referred to in paragraph [000132] comprised two epitope-binding agents, one being an antibody and the other being an aptamer. It was obvious for a skilled reader that the reference in these paragraphs to Figures 17B and 17C was not correct. Firstly, because Figure 17B showed a sensor comprising only aptamers and, secondly, because Figure 17C did not exist in the application. Furthermore, it was clear to the skilled reader that nothing else but a reference to Figures 26B and 26C was intended instead, showing exactly the sensors described in paragraphs [000131] and [000132], respectively.

It was stated in paragraph [00043] that Figure 27 depicted the experimental demonstration of the sensor design shown in Figure 26F. However, Figure 27 of the application as filed clearly contained some elements which had been erroneously replicated. It would have been immediately evident to the skilled person that Figure 27 was erroneous and nothing else was intended instead amended Figure 27 attached to appellant's letter of 1 August 2014.

Inventive step

The problem to be solved in view of the disclosure in document D2 was the provision of a two-component molecular beacon for the detection of a polypeptide causing reduced background signals and, as a

consequence, allowing more accurate detection methods. This problem was solved by the provision of the method of claim 1 using the molecular beacon of claim 10. Said beacon contained two molecular recognition complexes, each comprising an epitope-binding agent (aptamer, double stranded polynucleotide, antibody) and a signaling oligo, wherein the signaling oligos were complementary to each other but associated stably only when the two molecular recognition complexes bound to the polypeptide to be detected. This was achieved by the inclusion of flexible linkers, covalently attaching each of the epitope-binding agents to a signaling oligo. This solution to the underlying technical problem could not be derived in an obvious way from the disclosure in document D2.

- X. The appellant requested that the decision under appeal be set aside and that a patent be granted based on claims 1 to 11 of the new main request filed at the oral proceedings. The appellant further requested a correction under Rule 139 EPC in paragraphs [000131] and [000132] on page 42 of the application and of Figure 27 of the application.

Reasons for the Decision

Admissibility of the main request

1. The main request is based on auxiliary request 4, filed with letter dated 1 August 2014, in reply to an objection under Article 123(2) EPC raised in the board's communication. The main request was filed in direct reply to a clarity objection against claim 2 of

previous auxiliary request 4 raised by the board during oral proceedings. The essential feature of claim 17 as filed (see WO 05/59509) that in the presence of the analyte also the second aptamer construct is capable of binding to a second epitope of the polypeptide has been introduced into claim 2. This amendment, which satisfies the requirements of Article 123(2) EPC, does not raise any complex technical or legal issues. Therefore, the board, exercising the discretion conferred to it by Article 13(1) RPBA, decides to admit the main request into the proceedings.

2. The decision under appeal is solely concerned with Article 56 EPC and did not raise any objections under Articles 123(2), 83 and 84 EPC against, *inter alia*, auxiliary request 2 then on file. However, as the main request contains several amendments (see Section VII, *supra*), the board has to assess whether it meets the requirements of these articles.

Article 123(2) EPC

3. The main request differs from auxiliary request 2 underlying the decision under appeal essentially in that - in addition to the amendment referred to at point 1 *supra* - (i) it has been specified in claims 1 and 10 that each of the first and second epitope-binding agent is **covalently** attached to its signaling oligo via a flexible linker, and (ii), in addition to the use of two aptamers, it contains the further options of (a) the first epitope-binding agent being a double stranded polynucleotide and the second epitope-binding agent being an aptamer, and (b) the first epitope-binding agent being an aptamer and the second epitope-binding agent being an antibody.

4. The covalent attachment is directly and unambiguously disclosed in paragraphs [00093] and [000112] in the application as filed (see WO 05/59509) while paragraphs [000131] and [000132] as filed provide clear support for the combined use, respectively, of an aptamer and a double stranded polynucleotide, and of an aptamer and an antibody.

5. Therefore, the main request meets the requirements of Article 123(2) EPC.

Article 83 EPC

6. The examples clearly outline several ways of providing a flexible linker, described as such on page 39, lines 13 to 14, which points out that the "*[e]xperiments with thrombin as a model protein presented here provided a proof-of-principle evidence for the feasibility of this design*". Furthermore, paragraph [000131] discloses how the skilled person may obtain a molecular recognition construct comprising a double stranded polynucleotide as an epitope-binding agent also comprising a flexible linker and paragraph [000132] discloses how he/she may obtain a molecular recognition construct comprising an antibody as an epitope-binding agent also comprising a flexible linker. The appellant, having demonstrated in the experimental part of the description the usefulness of the flexible linkers with aptamers used as epitope-binding agents, has provided the skilled person with sufficient information that the invention can be worked with molecular recognition constructs comprising an aptamer and either an antibody or a double stranded polynucleotide.

7. Therefore, the board is convinced that the main request meets the requirements of Article 83 EPC.

Article 84 EPC

8. Contrary to the examining division's opinion expressed in the decision under appeal (see page 4), in the board's view "flexible" as used in the context of the present invention is not to be regarded as a relative term and has to be given its routine meaning in the absence of any specific definition in the description, namely, non-rigid and capable of movement. The term is used in the context of the required outcome that, within the molecular recognition complex, the signaling oligo attached to the epitope-binding agent via the flexible linker must be able to stably associate with the complementary signaling molecule when the epitope-binding agent binds the polypeptide. The skilled reader would, therefore, clearly understand that a flexible linker is any linking molecule which provides sufficient relative movement between the parts of the molecular recognition complex so that the required function can be achieved.

9. The board is satisfied that the main request meets the requirements of Article 84 EPC.

Inventive step (Article 56 EPC)

10. Document D2 represents the closest state of the art for the assessment of inventive step. It discloses, *inter alia*, a two-component aptamer beacon system for detecting analytes including polypeptides (see page 25, lines 13 to 29 and Figure 9B). The system comprises two aptamers, i.e. oligonucleotides specifically binding to the polypeptide, each linked to a fluorescent molecule by oligonucleotide sequences which are complementary to one another. The complementary sequences are intended

- to bind to one another when the aptamers bind to the target analyte, bringing the fluorescent molecules together such that a change in fluorescence signal could be detected.
11. The skilled person would immediately have recognised that the system disclosed on page 25, paragraph 3 of document D2 would not work properly. Document D2 teaches that hybridisation could be achieved by using poly-A and poly-T tails which would, therefore, be complementary. They would bind strongly together as the binding of nucleotides A and T is of higher affinity than the binding of nucleotides G and C. Document D2 also teaches that alternatively "complicated complementary sequences" could be used to reduce the likelihood of non-specific binding, suggesting that the sequences should have a high degree of specificity and complementary and, again, therefore, very strong bonding. Thus document D2 teaches the skilled person that complementary sequences, which bind strongly together, are required. In consequence, the complementary sequences of document D2 would clearly bind to one another strongly, even in the absence of target analyte polypeptide, generating a strong background signal of false positives.
 12. The objective technical problem underlying the patent in suit in the light of the disclosure in document D2 is seen in the provision of means for the detection of a polypeptide producing reduced background signals.
 13. As a solution to this problem the patent application proposes the method of claim 1 which relies on the use of a molecular beacon comprising two molecular recognition constructs (see claim 10). Each of the constructs comprises an epitope-binding agent that can

bind to a given epitope of the polypeptide to be detected, a signaling oligo and a label. The epitope-binding agent is covalently attached to the signaling oligo via a flexible linker. In one of the constructs the epitope-binding agent is an aptamer while in the other one it is an aptamer, a double stranded polynucleotide or an antibody. The signaling oligos are complementary.

14. In view of the disclosure made in particular in Examples 2 and 4 which describe the preparation of appropriate aptamer molecular beacons and in Example 3 which describes their use for the detection of a polypeptide, the technical problem is considered to have been credibly solved.
15. It remains to be answered whether a skilled person, in the light of document D2 (the only document on which the examining division relied for its assessment of inventive step), would have arrived at the claimed solution in an obvious way.
16. The association of the complementary signaling oligos of the molecular recognition constructs is controlled such that the association is insufficiently stable to occur in the absence of binding of the molecular recognition construct to the target polypeptide. This outcome is achieved by specific design of the signaling oligos to provide the correct binding characteristics, together with the inclusion within each molecular recognition complex of a flexible linker joining the epitope binding agent to the signaling oligo. By providing a flexible linker in the construct the suitable signaling oligos are readily selected so that they only associate stably when the molecular recognition complexes are brought into proximity by

polypeptide binding, because the flexibility of the linker facilitates their interaction when they are brought into proximity by binding of the epitope-binding agents to the polypeptide. The presence of the flexible linker ensures that the overall design of the molecular recognition complexes enables the association of the signaling molecules when the complexes are bound to the polypeptide, which in turn reduces background signals and allows more accurate detection.

17. In document D2, there is not the slightest hint to such a method making use of a flexible linker. Therefore, a skilled person being aware of this prior art document, when facing the objective technical problem to be solved, would not have arrived at the solution provided in claim 1 in an obvious way.
18. The method of detecting a polypeptide according to claim 1 as well as the molecular beacon of claim 10 involve an inventive step. The same conclusion applies to the subject-matter of dependent claims 2 to 9 and 11.

Corrections requested under Rule 139 EPC

19. According to Rule 139 EPC, errors in documents filed with the EPO may be corrected on request. However, *"if the request for such correction concerns the description, claims or drawings, the correction must be obvious in the sense that it is immediately evident that nothing else would have been intended than what is offered as the correction"*.
20. The appellant requests that page 42 of the description in the published application be replaced by an amended page 42 filed with the letter of 1 August 2014. In

paragraph [000131] of the amended page the reference to Figure 17B has been replaced by a reference to Figure 26B and in paragraph [000132] the reference to Figure 17C has been replaced by a reference to Figure 26C.

21. Paragraph [000131] describes the preparation of a molecular beacon according to claim 10 wherein one of the two epitope-binding agents is a double stranded polynucleotide and the other is an aptamer, which beacon is said to function in sensors depicted in Figure 17B. It is obvious that this reference to Figure 17B is erroneous as it depicts molecular beacons based on two aptamers. It is clear to the board that nothing else has been intended than a reference to Figure 26B in which a beacon as described in paragraph [000131] is represented.
22. Paragraph [000132] describes the preparation of a molecular beacon according to claim 10 wherein one of the two epitope-binding agents is an antibody and the other is an aptamer, which beacon is said to function in sensors depicted in Figure 17C. However, said Figure does not exist. Therefore, it is obvious that this reference to Figure 17C is erroneous. It is clear to the board that nothing else has been intended than a reference to Figure 26C in which a beacon as described in paragraph [000132] is represented.
23. Replacement of drawing page 27/34 (Figure 27) by the amended drawing page 27/34 filed with the letter of 1 August 2014 is also requested by the appellant, which in its part A differed from Figure 27 as filed (see WO 05/59509).

24. According to paragraph [00044] of the description Figure 27-part A depicts the principle underlying the functioning of the sensor shown in Figure 26F.

25. The appellant argued that Figure 27-part A includes an erroneous repetition of some elements. It is obvious that the originally filed figure depicts more than the principle underlying the functioning of the sensor shown in Figure 26F and that the application failed to provide any explanation therefor. However, the board is not convinced that the figure actually contains an error that would justify its amendment in accordance with rule 139 EPC.

26. Therefore, whereas the correction of page 42 of the description is allowed, the correction of Figure 27 is refused.

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 claims 1 to 11 of the new main request filed at the oral proceedings, and a description to be adapted thereto.
3. The correction under Rule 139 EPC of page 42 of the description (paragraphs [000131] and [000132]) as filed with letter dated 1 August 2014 is allowed.

The Registrar:

The Chairman:



A. Wolinski

M. Wieser

Decision electronically authenticated