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# Datasheet for the decision of 25 August 2010

**Case Number:** T 2116/09 - 3.3.08

**Application Number:** 04798537.9

**Publication Number:** 1692297

**IPC:** C12Q 1/00

**Language of the proceedings:** EN

**Title of invention:**
Nitroreductase biosensors for detecting nitro-compounds

**Applicants:**
University of Wales, Bangor, et al

**Headword:**
Biosensors/WALES

**Relevant legal provisions:**
EPC Art. 83, 84, 111(1), 123(2)

**Relevant legal provisions (EPC 1973):**
-

**Keyword:**
"Main request: clarity (yes)"
"Sufficiency of disclosure (yes)"
"Added subject-matter (no)"
"Remittal (yes)"

**Decisions cited:**
G 0010/93

**Catchword:**
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

**Case Number:** T 2116/09 **-** 3.3.08

# D E C I S I O N
**of the Technical Board of Appeal 3.3.08**
**of 25 August 2010**

**Appellants**: University of Wales, Bangor
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**Representative**: Walsh, David Patrick
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**Decision under appeal:** **Decision of the Examining Division of the European Patent Office posted 30 April 2009 refusing European application No. 04798537.9 pursuant to Article 97(2) EPC.**

**Composition of the Board:**

**Chairman:** L. Galligani
**Members:** T. J. H. Mennessier
B. Günzel

## Summary of Facts and Submissions

I. The applicants (appellants) lodged an appeal against the decision of the examining division dated 30 April 2009, whereby the European patent application No. 04 798 537.9 was refused. The application, entitled "*Nitroreductase biosensors for detecting nitro-compounds*", was filed as the international patent application with publication number WO 05/56815.

II. Basis for the refusal was the main request and the first and second auxiliary requests as filed with the letter of 20 March 2009.

III. The main and the second auxiliary requests were refused for reasons of presence of added matter (Article 123(2) EPC) and insufficiency of disclosure (Article 83 EPC), and the first auxiliary request for reasons of insufficiency of disclosure (Article 83 EPC). In the decision under appeal, novelty (Article 54 EPC) and inventive step (Article 56 EPC) were not discussed.

Claim 1 of the **first auxiliary request** read as follows:

"1. A sensing device comprising an electrode comprising a noble metal layer, on which layer is located a nitroreductase enzyme, wherein the enzyme comprises a plurality of introduced cysteine residues."

IV. On 10 July 2009, the appellants filed a notice of appeal in which they requested that the decision under appeal be set aside and a patent be granted.

V. The statement setting out the grounds of appeal was filed on 9 September 2009. It was accompanied by a main request and a first auxiliary request. The main request corresponded exactly to the first auxiliary request refused by the examining division. The first auxiliary request differed from the second auxiliary request refused by the examining division in that a dependent claim had been added.

VI. The examining division did not rectify its decision and referred the appeal to the Board of Appeal (Article 109 EPC).

VII. On 31 May 2010, the board issued a summons to oral proceedings to which a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) was attached. In that communication, which provided an outline of the issues to be discussed at the upcoming oral proceedings, the opinion was expressed that the requirements of Article 83 EPC were met by the main request. In addition, the board, exercising its discretionary power (decision G 10/93 (OJ EPO, 1995, 172), raised objections under Articles 84 and 123(2) EPC against the main request. The appellants were further informed that, as the examining division did not take position in the decision under appeal on the issues of novelty and inventive step, the appellants could expect, that, if a request meeting the requirements of Articles 83, 84 and 123(2) EPC were filed, the board would remit the case to the examining division for further prosecution in order to give them the benefit of two levels of jurisdiction.

VIII. On 9 August 2010, in reply to the board's communication, the appellants filed two sets of claims as their new main and first auxiliary requests. They further maintained their request for oral proceedings in case the refusal of the application be confirmed.

IX. The main request, which had been derived from the claims of the first auxiliary request refused by the examining division, consisted of 24 claims.

Claim 1 read as follows:

"1. A sensing device comprising an electrode comprising a noble metal layer, on which layer is located a nitroreductase enzyme, wherein the enzyme comprises a plurality of introduced cysteine residues **at a location which does not substantially interfere with its activity**."

Claims 2 to 16 were dependent on claim 1 and directed to particular embodiments thereof.

Claim 17 was directed to a method of detecting nitro group containing compounds which comprised inter alia the step of providing a sensing device of one of claims 1 to 15 and a reference electrode.

Claims 18 to 19 were dependent on claim 17 and directed to particular embodiments thereof.

Claims 20 and 21 read as follows:

"20. A protein comprising a nitroreductase enzyme which has been modified to comprise a plurality of cysteine

residues **at a location which does not substantially interfere with its activity** incorporated into its structure, which cysteine residues are not present in the native enzyme."

"21. An isolated nucleic acid sequence comprising a nitroreductase gene modified by the addition of a plurality of codons for cysteine residues **incorporated in the nucleotide sequence encoding the nitroreductase in such a way that the cysteine residues do not substantially interfere with the activity of the enzyme**."

Claim 22 was dependent on claim 20 and directed to a particular embodiment thereof.

Claim 23 read as follows:

"23. A nucleic acid construct comprising:

(a) a promoter for the expression of a nitroreductase gene;
(b) **a nucleotide sequence of a nitroreductase gene having** a plurality of codons for cysteine residues **incorporated in the nucleotide sequence encoding the nitroreductase in such a way that the cysteine residues do not substantially interfere with the activity of the enzyme**."

Claim 24 was dependent on claim 23 and directed to a particular embodiment thereof.

(in claims 1, 20, 21 and 23 above the emphasis in bold type added by the board shows the amendments carried

out on the first auxiliary request refused by the examining division)

X. The following document is referred to in the present decision:

(D3) Database Geneseq [Online], 27 February 1996, "E. coli nitro reductase gene, retrieved from EBI accession No. GSN:AAQ92891, database accession No. AAQ92891

XI. The appellants' arguments, insofar as they are relevant for the decision, can be summarised as follows:

Main request

*Requirements of Article 123(2) EPC*

The main request of 9 September 2009 having been amended to take into account the preliminary opinion of the board in its communication of 31 May 2010 should now meet the requirements of Article 123(2) EPC.

*Requirements of Articles 83 and 84 EPC*

The claimed subject-matter was in relation to the surprising finding of an improvement in sensitivity obtained when a plurality of cysteine residues was used to link the enzyme to the noble metal layer. This improvement had been demonstrated in the examples. The cysteine residues did not affect the biochemical properties of the enzyme itself. It was clear from the specification that the cysteine residues should be located where they do not interfere with the enzyme

activity. However, this did not mean that the cysteine residues could not still bind the enzyme to the noble metal layer even if they did not interfere with the enzyme activity. Therefore, the binding of the enzyme to the noble layer had been amply demonstrated. Furthermore, it was clear that one of the cysteine residues would still be effective in acting as a linking agent so that the recitation of the optimal number of cysteine residues was not necessary as the appellants had demonstrated the efficacy of the introduced cysteine residues.

Furthermore, the skilled person could ascertain the structure of the nitroreductase enzyme which consisted of a pair of monomers providing together two N-terminal ends and two C-terminal ends. It was clear that the N-terminal ends were on the surface of the enzyme and each of them represented therefore a possible active site for affixing the enzyme to the metal surface. There were also other possible sites for attachment which could be identified without extensive trial and error.

Other nitroreductases had been cloned by the appellants to which it would be easy to apply the teachings of the invention.

XII. The appellants request that the decision under appeal be set aside and that the case be remitted to the examining division for consideration of novelty and inventive step on the basis of either the main request or the auxiliary request, both filed on 9 August 2010.

## Reasons for the Decision

Main request

1. The main request is an amended version of the first auxiliary request refused by the examining division. A prerequisite for it to be examined further as to the criteria of patentability is its compliance with the requirements of Articles 84 and 123(2) EPC.

*Requirements of Article 84 EPC*

2. The board is satisfied that the claims are clear and concise. It is noted in particular that the amendments they contain, which were made in response to the board's objection in its communication of 30 May 2010, make it clear where in the enzyme the plurality of cysteine residues is to be included, namely at a location that does not substantially interfere with its activity.

*Requirements of Article 123(2) EPC*

3. In its communication of 30 May 2010, the board, in the exercise of its discretionary power (decision G 10/93, *supra*), had raised objections under Article 123(2) EPC against the then pending main request, for the reason that the claims implied that the plurality of cysteine residues could be located anywhere in the nitroreductase. This was in contravention with the statement on page 7, lines 16 to 21, which is the only passage in the description (see the international application WO 05/56815) where a sensing device comprising a layer of nitroreductase enzyme is

generally defined, that said enzyme **includes a plurality of cysteine residues at a location which does not substantially interfere with its activity**.

4. The board notes that the present claims 1, 20, 21 and 23 contain amendments which overcome the objection (see Section IX *supra*). It is concluded that the main request complies with the requirements of Article 123(2) EPC.

*Requirements of Article 83 EPC*

5. In the decision under appeal, the examining division raised two lines of objections under Article 83 EPC. It was considered that the application did not teach how to obtain proteins/enzymes other than the two specific nitroreductase enzymes modified with the addition of six consecutive cysteine residues at the N-terminal end it described. The examining division further held that the application did not specify how many cysteine residues should be added, where they should be added in the enzyme and whether they should be consecutive or not.

6. The invention relies on the finding that the incorporation of cysteine residues in a nitroreductase enables its incorporation onto a noble metal electrode, which in turn allows the detection of picomolar concentrations of nitrocompounds (to be compared with the nanomolar concentrations of the prior art). The conjugation of the nitroreductase to the noble metal electrode via cysteine linkages should enable optimal orientation of the enzyme on the electrode, leading to

enhanced sensitivity (see from page 11, line 29 to page 12, line 12 of WO 05/56815).

7. The invention is exemplified with the nitroreductase encoded by the *nfnB* gene of Escherichia coli K12. The preparation of a plasmid containing the *nfnB* gene modified by addition of codons for a sequence of six contiguous cysteine residues within the N-terminal part of the protein, the expression of the modified enzyme, its purification, as well as the preparation and the testing, for the detection of 2,4-dinitroethylbenzene, of a biosensor utilising the same are described in detail in the experimental part of the description (see Examples 1 to 6 on pages 14 to 29).

8. The complete amino acid sequence of two modified nitroreductases according to the invention and the correspondent nucleic acid sequences are described in the listing sequence.

The amino acid sequence SEQ ID NO:4 encodes a modified nitroreductase with a sequence of six contiguous cysteine residues (at positions 35 to 40) located just before a sequence of 216 amino acid residues (at positions 41 to 256) which correspond to amino acid residues 2 to 217 of the nfnB nitroreductase of E. coli K12, said reductase being encoded by SEQ ID NO:1.

The amino acid sequence SEQ ID NO:6 encodes another modified nitroreductase with a sequence of six contiguous cysteine residues (at positions 35 to 40) located just before a sequence of 273 amino acid residues (at positions 41 to 313) which correspond to amino acid residues 2 to 274 of the pnrA nitroreductase

of Pseudomonas putida JLR11 (erroneously recorded in the sequence listing as the "prnB" [sic] nitroreductase of the same bacterial strain), said nitroreductase being encoded by SEQ ID NO:2.

9. As regards the plurality of cysteine residues, the skilled person would find in the description the important information that it should be located in the enzyme sequence in such a way that it does not substantially interfere with the activity of the enzyme (see page 7, lines 20 to 21), a preferred location being within the N-terminal part thereof (see page 7, lines 27 to 29 and page 8, lines 3 to 4). The skilled person would also derive from the exemplified sequences that under the expression "plurality of cysteine residues" it is preferably meant a sequence of six contiguous cysteine residues (see residues 35 to 40 in SEQ ID NO:4 and SEQ ID NO:6).

10. Nitroreductases were available to the skilled person at the filing date as evidenced *inter alia* by document D3 which contains a reference to a Japanese patent application published in 1995, i.e. prior to the relevant filing date of the application at issue, in which the nucleic acid sequence encoding the nfnB nitroreductase of Escherichia coli K12 is represented. It may be reasonably assumed that he/she, as an ordinary practitioner aware of what was common general knowledge in the art at the time of filing the application, and also having access to everything in the state of the art and having at his/her disposal the normal means and capacity for routine work and experimentation, would have been in a position to prepare without undue burden a modified enzyme

therefrom which incorporates a plurality of cysteine residues.

11. It can be concluded that at least one way is clearly indicated which states the features that are essential to carrying out the invention such that a skilled person can understand them and put them into practice without undue burden. Therefore, the requirements of Article 83 EPC are satisfied.

Remittal

12. As the main request has remedied the defects underlined in the decision under appeal and in the board's communication of 30 May 2010 and thereby meets the requirements of Articles 83, 84 and 123(2) EPC, in order to give the appellants the benefit of two levels of jurisdiction for the assessment of novelty and inventive step, with respect of which the examining division did not take position in the decision under appeal, the board, exercising the discretional power conferred to it by Article 111(1) EPC considers it as appropriate to remit the case to the examining division for further prosecution.

13. In such circumstances, there is no need for the board to examine the first auxiliary request. Furthermore, the conditional request for oral proceedings becomes obsolete.

## Order

**For these reasons it is decided that:**

1. The decision under appeal is set aside.

2. The case is remitted to the first instance for further prosecution on the basis of the main request as filed with the letter of 9 August 2010.

The Registrar | The Chairman

A. Wolinski | L. Galligani