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Datasheet for the decision of 27 January 2023

Case Number: T 0933/18 - 3.3.08

Application Number: 11156649.3

Publication Number: 2380980

IPC: C12N15/09, C12M1/00, C12N1/15, C12N1/19, C12N1/21, C12N5/10, C12N9/04, C12Q1/32

Language of the proceedings: EN

Title of invention:
Coenzyme-linked glucose dehydrogenase and polynucleotide encoding the same

Patent Proprietor:
Ikeda Food Research Co., Ltd.
PHC Corporation

Opponents:
Toyobo Co., Ltd.
Roche Diabetes Care GmbH

Headword:
Method for preparing a biosensor/IKEDA FOOD RESEARCH PHC CORPORATION

Relevant legal provisions:
EPC Art. 100(a), 100(b), 100(c)

Keyword:
Patent as granted - requirements of the EPC met - (yes)

Decisions cited:
G 0002/88, G 0001/92, G 0001/15, T 0243/89, T 0666/89, T 0601/92, T 1566/12, T 1742/12, T 0989/16

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 0933/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 27 January 2023

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted on 25 January 2018 rejecting the opposition filed against European patent No. 2380980 pursuant to Article 101(2) EPC**

Composition of the Board:

Chair T. Sommerfeld
Members: M. Montrone
D. Rogers

Summary of Facts and Submissions

I. The appeals are against the decision of an opposition division to reject the oppositions against European patent No. 2 380 980. This patent is based on European patent application No. 11 156 649.3 ("patent application") which is a divisional application of European patent application No. 06 730 146.5 ("parent application") that was filed as International patent application published as WO 2006/101239.

II. With their statement of grounds of appeal, opponent 01 ("appellant I") raised objections against the subject-matter of the claims as granted under added subject-matter, insufficiency of disclosure, lack of priority, lack of novelty, and lack of inventive step. In support of their case document D5b was submitted.

III. With their statement of grounds of appeal, opponent 02 ("appellant II") raised objections against the subject-matter of the claims as granted under added subject-matter, insufficiency of disclosure, and lack of inventive step.

IV. In their reply to the appeal, the patent proprietor ("respondent") submitted new documents D115, D116a, D116b, D117 to D119, D119a, D120, D120a, D120b and D120c.

V. In various further replies to each others' submissions, appellant I and the respondent submitted additional documents (appellant I: documents D121 to D125, D129 and D130; respondent: D36b, D126 to D128, D131 and D131a).

VI. In a communication in preparation of oral proceedings, the parties were informed of the board's provisional, non-binding opinion on the issues of the appeal.

VII. The respondent and appellant II replied to the communication of the board. Appellant II submitted a further document (D67a).

VIII. Oral proceedings before the board were held on 26 and 27 January 2023 in the presence of the parties. At the oral proceedings, appellant I submitted two questions of law to be referred to the Enlarged Board of Appeal.

IX. Claim 1 as granted (main request) reads:

"1. A method for preparing a biosensor for measuring glucose in a sample liquid comprising:

producing a flavin adenine dinucleotide (FAD)-linked glucose dehydrogenase (GLD) by a method comprising cultivating a transformed cell prepared using a recombinant vector carrying a polynucleotide encoding the GLD having an amino acid sequence with a homology of at least 60% to an amino acid sequence set forth in SEQ ID NO: 2 or an amino acid sequence set forth in amino acid 20 to amino acid 592 of SEQ ID NO: 2, and having an activity towards maltose of 5% or less with respect to an activity towards glucose, and a total content of galactose, glucose, mannose, and arabinose of the GLD is 10 µg or less per µg of protein; and

preparing the biosensor encompassing an enzyme reaction layer containing the FAD-linked glucose dehydrogenase (GLD)".

Dependent claims 2 to 8 are directed to specific embodiments of the method of claim 1.

X. The following documents are referred to in this decision:

D3: EP 1 862 543

D4: JP 2005 089884 (priority document of patent in suit and D3)

D4a: Translation of D4

D5a: EP 1 584 675

D8: Frederick, K.R. *et al.*, J. Biol. Chem, 1990, Vol. 265, pp.3793-3802

D11: Bak T.-G., Bioch. Biophys. Acta, 1967, Vol. 139, pp.277-293

D15a: EP 1 739 174

D23: Kojima S. *et al.*, Chem. Sensors, 2004, Vol. 20, Suppl. B, pp. 768-769

D25: Ferri, S. *et al.*, J. Diabet. Sci. Technol., 2011, Vol. 5, pp. 1068-1076

D28: Machida, M. *et al.*, Nature, 2005, Vol. 438, pp.1157-1161

D36: Hata, Y., J. Agricult. Chem., 2002, Vol. 76, pp.715-718

D36a: Translation of D36

D36b: Fig. 3 of D36

D37: Edge, A.S.B., Biochem. J., 2003, Vol. 376, pp.339-350

D43: Roche Catalogue, 2005, (extract), pp. 530-541

D56: Yang, Y., *et al.*, Enzyme Microbial Techn., 2015, Vol. 68, pp.43-49

D61: Edge, A.S.B. *et al.*, Anal. Biochem., 1981, Vol. 118, pp.131-137

D66: Declaration by Ms. Yada, dated 18 March 2016

D66b: List of homologous GLD sequences and sequence alignments

D67: EP 2 003 199

D67a: Sequence listing of D67

D69: Translation of opponent 01's submission to the JPO concerning JP2008-178380A (Japanese Patent application No. 200775019)

D77: WO 2015/060150

D86: Declaration by Mr. Kawai, dated 7 October 2016

D87: Declaration by Mr. Kishimoto, dated 8 October 2016

D88: Declaration by Prof. Nishiya, dated 6 October 2016

D112: US 9 663 811 B2

D115: Sequence Analysis in a Nutshell, Markel S. and Léon D. eds., 1st. Ed., 2003, pp. 158, 159

D116a: "way-back machine" screenshot of EMBL-EBI, March 17, 2005 (mainpage)

D116b: "way-back machine" screenshot of EMBL-EBI, March 17, 2005 (dropdown menu)

D117: Product Information Sumizyme PX

D118: Product Information Sumizyme ARS

D119: Declaration by Prof. Ohta, dated 12 October 2018

D119a: Translation of D119

D120: Declaration by Prof. Pasut, dated 16 October 2018

D120a: Methods of Biochemical Analysis, ed. Glick D., Vol. III, 1956, pp. 112-152

D120b: Instruction Manual GlycoLink™ Coupling Catalyst

D120c: Sigma product information for Sodium borohydride

D121: Definition of "encompass" by the Oxford English Dictionary

D122: Declaration by Dr Kinkeldey, dated 1 March 2019

D123: Declaration by Prof. Nishiya, dated

26 February 2019

D124: Definition of "eluate" by The Free Dictionary

D125: Merry, T. and Astrautsova, S., Methods on Molecular Biology, Capillary Electrophoresis of Carbohydrates, Chapter 2, 2003, Vol. 213, pp.27-40

D126: Definition of "encompass" by Merriam Webster's Dictionary

D127: Lottspeich, F. and Zorbas, H. eds., Bioanalytik, 1998, Chapter 9, pp.195-198

D128: Ausubel, F.M. *et al.* eds., Current Protocols in Molecular Biology, 2003, pp. 1.13.4-1.13.6

D129: Declaration by Mr Kawai, dated 28 February 2022

D130: Ellaiah, P. *et al.*, Process Biochemistry, 2002, Vol. 38, pp. 615-620

D131: NRBC search for "Aspergillus" and "soil"

D131a: NRBC database entries for hits 1-3 of D131

XI. The appellants' submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request (claims as granted)

Substantive procedural violation

The opposition division committed a procedural violation in the decision under appeal by not providing

a proper reasoning. Firstly, the reasoning on novelty that concerned the non-enabling disclosure of document D5a referred erroneously to inventive step. Secondly, the opposition division did not discuss other starting points for inventive step other than document D5a although alternative lines of arguments using documents D15a and D23 as closest prior art had never been withdrawn during the first instance proceedings. The failure to provide a reasoned decision on inventive step based on these two documents deprived appellant I of their right to appeal the decision on this ground.

Admission of documents D36b, D67a, D121, D122, D124 to D131, and D131a into the appeal proceedings

Document D67a disclosed document D67's sequence listing and merely completed document D67's disclosure. Document D67 was introduced by the respondent during the opposition proceedings and relied on in their reply to the statements of grounds of appeals. This document should thus be admitted into the proceedings.

Documents D124 and D125 were submitted in direct response to the respondent's reply to the statements of grounds of appeals. Document D124 addressed the respondent's interpretation of the term "*eluate*" in Example 2 of document D5a, which was discussed for the first time in the proceedings. Document D125 addressed the respondent's submissions on "*Sumizyme*" mentioned in Example 6 of the patent. Both documents were *prima facie* relevant and should therefore be admitted. Documents D129 and D130 were submitted to address a new argument submitted by the respondent. Document D129 disclosed experimental evidence which assessed the issue of whether different solid culturing conditions affected the molecular weight of GLD, and thus GLD's

glycosylation level. Document D130 disclosed a further example of a solid-cultured Aspergillus strain used for producing an enzyme.

Added subject-matter

The application as filed provided no direct and unambiguous basis for the combination of features in claim 1, namely the combination of the features "*having an amino acid sequence with a homology of at least 60% to an amino acid sequence set forth in SEQ ID NO: 2*" (hereinafter "identity" feature), "*having an activity towards maltose of 5% or less with respect to an activity towards glucose*" (hereinafter "activity" feature), and "*a total content of galactose, glucose, mannose, and arabinose of the GLD is 10 µg or less per µg of protein*" (hereinafter "amount" feature) in claim 1. These features were selected from different lists in the absence of appropriate pointers. Moreover, claim 1 combined the least preferred "identity" and "activity" features, with the second-least preferred "amount" feature, instead of selecting features that were constantly indicated as "*most preferred*". The skilled person thus had to ignore the explicit disclosure of the application as filed for selecting the features of claim 1. Already the selection of the specific embodiment in paragraph [0038] represented a choice from a first list, namely from among the embodiments listed in paragraphs [0036] to [0040]. In addition, limitations were missing in the claim, such as that the GLD did not utilize oxygen as an electron acceptor (as required in paragraph [0038] and in granted claim 8) and the reference to "*wild type GLD*" as regards the "amount" feature, contrary to the basis in paragraph [0026] of the application as filed. The omission of this reference in claim 1 added subject-matter too,

because this was a further requirement that was not necessarily fulfilled since there were wild type GLDs that had already a lower sugar content (document D87).

The following features in claim 1 also lacked a basis in the application as filed:

- "a *polynucleotide encoding the GLD*" required for the recombinant production of GLD,
- the omission of a process step collecting the GLD,
- the feature "*preparing the biosensor encompassing an enzyme reaction layer*" (emphasis added) and
- *"A method for preparing a biosensor*".

As regards the subject-matter of claims 2 to 5 and 7, appellant II stated as follows "*The same analysis applies for dependent claims 2-5 and 7 as pointed out in the decision at section 17.2 which is incorporated by references in order to avoid unnecessary repetitions*".

Sufficiency of disclosure

The method of claim 1 was insufficiently disclosed in the patent for several reasons:

Firstly, the patent referred repeatedly to GLD as a "*sugar-embedded-type enzyme*" (see e.g. paragraph [0011]), while evidence for the existence of this GLD form was not available. This cast serious doubts on whether or not the experiments disclosed in the patent were correct.

Secondly, obtaining substantially all GLD homologs having the functional requirements as defined in claim 1 amounted to undue burden, in particular the finding of GLD homologs characterised by a low maltose

activity. The patent disclosed one working example falling within claim 1 only, while claim 1 covered a plethora of sequence variants (homologs being at least 60% identical to SEQ ID NO: 2) which all had to fulfill the functional requirements claimed, i.e. a certain maximum maltose activity (5% or less) relative to the enzyme's glucose activity. GLD enzymes with these properties were not known from the prior art.
Document D56 disclosed the sequence of a GLD which was 98% identical to the amino acid sequence of SEQ ID NO: 2 in claim 1. Despite this high sequence identity, the GLD had a high maltose activity (see page 46, left column, last paragraph). The sole provision of GLD's sequence information in the patent was thus not sufficient to find other GLD variants with low maltose activity. Since the patent was silent on markers for low maltose activity, and provided no pointers as to where GLD variants with this property might be found, the skilled person using the information in the patent was unable to obtain GLD variants with the claimed properties.
In this situation, a straightforward remaining approach for the skilled person was a homology search in public sequence databases to find structural homologs of GLD. This search provided the GLD sequence of the *Aspergillus oryzae* (*A. oryzae*) strain RIB40 because the genome sequence of this strain was publicly available at the effective date of the patent (see document D28). The GLD of the RIB40 strain however was enzymatically inactive (see document D67, page 33, lines 44 and 45). Thus this approach failed. The repeated failure in obtaining GLD variants that fell within claim 1 demonstrated undue burden.

Documents D66b, D67, D77 and D112 provided likewise no evidence that GLD homologs with the desired properties

were obtainable at the effective date. These documents were all post-published relative to the patent, i.e. their sequence information was not available and would not have been found in a sequence search.
Irrespective of the publication date, document D112 disclosed GLD enzymes of several *A. oryzae* strains (see column 20, Table 1). However, document D112 solely disclosed that the GLD of strain NBRC 5375 had glucose activity combined with low maltose activity (see column 15, lines 36 to 38, column 21, Example 7), while GLDs from the other strains (see Table 1) were not tested for maltose activity. Document D112 thus provided evidence for only one GLD enzyme. Document D67 disclosed a single GLD enzyme that fell within claim 1 too. The sequence of this enzyme was isolated from the *A. oryzae* TI strain which was isolated from "*soils*", and hence found by chance only (see paragraph [0264]).

Thirdly, the skilled person faced undue burden in determining the percentage of homology defined in claim 1 ("identity" feature), because a particular program/ algorithm or parameter was not defined. Since different parameters and/or programs resulted in different sequence homologies, the skilled person did not know which one to use.

Novelty

The method of claim 1 lacked novelty over the parent application (D3). Claim 1 was not entitled to priority, because the feature "*total content of galactose, glucose, mannose and arabinose of the GLD is 10 µg or less per µg of protein*" (i.e. the "amount" feature) was not disclosed in the priority document (D4/D4a). Claim 1 belonged therefore to the so-called "AND" claim category as defined in decision G 1/15 published in OJ

2017, 82, Reasons 5.2.1 which were not entitled to partial priority.
Example 3 of the parent application (D3) was identical to Example 2 of the priority document (D4/D4a), and therefore entitled to priority. The GLD obtained from Example 3 of the parent application (D3) was produced in *E. coli* and lacked any glycosylation. Since Example 3 disclosed a product produced by a process, GLD's lacking glycosylation was not immediately apparent to the skilled person, contrary to what was required for an "*implicit*" feature. If such a feature was implicit, any amendment in a claim that concerned a structural feature of a product defined by a process necessarily had a basis in a patent application. This was not the case. GLD's lacking glycosylation was thus an "*intrinsic*" feature which fell within claim 1. The facts of the case were different from those described in G 1/15, and since intrinsic features were not assessed, G 1/15 did not apply to the present case.

Two questions of law should thus be referred to the Enlarged Board of Appeal for assessing the impact of intrinsic features on "poisonous priorities".

Question 1 under Article 88 EPC read as follows:

"*In order to determine whether a claim is entitled to a priority date, is it essential to determine whether the features of the claim are present in the priority document from which priority is claimed?*"

Question 2 under Article 88 EPC read as follows:

"Where a first priority document discloses a feature A and where there is a feature B which is intrinsic to the disclosure in the first priority document but which

is neither implicitly nor explicitly disclosed in the first priority document and where a second priority document discloses feature B to be used together with feature A then can a claim to A+B (i.e. an "AND" claim) enjoy a partial priority from the first priority date?"

Furthermore document D5a anticipated the method of claim 1.

The case law held that information as to the internal structure of a prior art product was made available to the public and became part of the state of the art if direct and unambiguous access to such information was possible by means of known analytical techniques. Neither the likelihood of analysing such a product nor the degree of burden (i.e. the amount of work and time involved in carrying out such an analysis) was relevant for determining what constituted the state of the art (see Case Law, I.C.3.2.4.d, and G 1/92). Relevant was solely that "*it was possible*" to obtain the structure.

Document D5a disclosed all the features of the method of claim 1, except for GLD's amino acid sequence. This sequence represented the internal structure of GLD, i.e. of a protein that was publicly available and analysable at the effective date of the patent. Moreover, document D86 disclosed that the skilled person obtained the amino acid sequence from liquid-cultured *A. terreus*. It was irrelevant that the purification protocols in documents D86 and D5a were different, since the key issue was whether the skilled person obtained GLD's amino acid sequence by means known at the effective date and commonly used for this purpose. This was confirmed by document D88.

Even if the skilled person failed in sequencing GLD obtained from liquid-cultured *A. terreus*, document D5a disclosed solid culturing as an alternative to liquid culturing (see paragraph [0038]). The patent reported in paragraph [0020] that GLD could be sequenced after shifting *A. terreus* from liquid to solid culturing. In using GLD from solid-cultured *A. terreus* as disclosed in document D5a, the skilled person necessarily obtained a sequence-able GLD.

The failure reported in document D67 to sequence GLD from a different Aspergillus strain was irrelevant for the present case since the notional skilled person of the case law was fictitious contrary to a real group of scientists. Document D67 was moreover post-published and lacked, for this reason, any probative value as expert opinion. Even if document D67 was considered, the document did not state that GLD's sequencing was impossible. Paragraph [0266] solely reported that the authors applied a different strategy to obtain the sequence.

Inventive step

Document D5a represented the closest prior art. The method of claim 1 lacked an inventive step either on the teaching of document D5a alone or combined with document D36/D36a.

The method of claim 1 differed from that of document D5a solely in the provision of GLD's amino acid sequence.
There was no evidence on file that all GLD variants falling within claim 1 had an improved reactivity and dried easier on the biosensor surface compared to the GLD of document D5a. Moreover, the improved reactivity

of GLD reported in the patent was achieved under specific reaction conditions that were missing from claim 1. Thus no beneficial effects could be ascribed to all GLDs falling within claim 1.
The technical problem to be solved was thus either the provision of a sequence-able form of GLD, or the provision of an arbitrary method for preparing a biosensor.
Since the method of claim 1 comprised an arbitrary selection of non-functional biosensors, and serious doubts existed about the biosensor's functionality, the claimed method lacked an inventive step already for these reasons.
Furthermore, the provision of GLD's sequence as specified in claim 1 was not inventive. The skilled person following the teaching of document D5a would have necessarily obtained GLD's amino acid sequence from solid-cultured *A. terreus*, as shown in the patent. Solid culturing was explicitly mentioned in document D5a and therefore served as a valid alternative starting point. Moreover, since the availability of sufficient protein amounts for sequencing was an issue, the use of solid culturing of Aspergillus was obvious for the skilled person because this provided large protein amounts (see document D36a, page 3, first paragraph).
Even if GLD from liquid-cultured *A. terreus* was used for sequencing, the deglycosylation of a protein followed by sequencing was routine and known from numerous studies (see document D37, abstract and page 347, right column, last sentence). The deglycosylation problems of GLD reported in the patent (see Example 6) were artificial and caused by unreliable enzymes, wrong conditions, or uncommon chemical means (see e.g. document D88, page 7, items 2.2 and 2.3), instead of using well-established methods, such as TFMS (see

document D61, page 339, left column, Introduction). Evidence was available too that the skilled person by applying routine deglycosylation methods obtained a sequence-able GLD (see document D86, points 1.1.3 and 1.2, and document D43, page 1).

XII. The respondent's submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request (claims as granted)

Substantive procedural violation

Appellant I's right to be heard was not violated with regard to documents D15a and D23. Although a line of argument under lack of inventive step based on document D15a was raised in appellant I's notice of opposition, this line was not maintained during the written phase of the proceedings or at the oral proceedings before the opposition division. No arguments based on document D23 had been raised at all. The opposition division had no obligation to provide an additional reasoning under inventive step based on these two documents (see e.g. T 1742/12). In appeal, appellant I never substantiated their case on inventive step based on documents D15a and/or D23 but focused exclusively on document D5a as starting point.

Admission of documents D36b, D67a, D121, D122, D124 to D131, and D131a into the appeal proceedings

Document D36b disclosed an enlarged figure of document D36 to increase the figure's legibility.

Document D67a was late filed and no reasons were advanced why this document could not have been filed

earlier, let alone exceptional circumstances justified by cogent reasons. The document should thus not be admitted.

Document D121 should be admitted into the proceedings only if document D126 was admitted as well.

Document D122 was submitted in support of an allegation that the decision under appeal was not properly reasoned and that the claimed method lacked an inventive step. This submission did not respond to a new point but supplemented arguments already advanced in appellant I's statement of grounds of appeal. Reasons justifying the document's late submission were not provided. Document D122 should not be admitted.

Document D124 was submitted in support of a new line of argument that the gradient elution step of document D5a did not differ from the protocol disclosed in document D86. This reverted the appellant's position because before the protocol differences were uncontested. Document D124 should thus not be admitted.

As regards document D125, there were already many documents on file that concerned the deglycosylation of proteins. Document D125 did not add anything to these documents and should therefore not be admitted.

Document D129 concerned new experimental evidence regarding the impact of solid culturing on GLD's molecular weight, i.e. its glycosylation level. The issue of whether solid culturing affected glycosylation was already discussed at first instance. The data were late and should not be admitted. The same applied to document D130.

Added subject-matter

The method of claim 1 was primarily based on paragraph [0049] combined with paragraphs [0043] and [0038] of the application as filed (patent application). Paragraph [0049] disclosed both sequence variants of claim 1, including the "homology" feature, and referred to functional equivalents of GLD mentioned previously which included paragraph [0038]. Paragraph [0049] also mentioned a "*gene recombinant method*" which necessarily implied a polynucleotide encoding *inter alia* the "homology" feature. Paragraph [0038] further disclosed literally the "activity" feature of claim 1. Paragraph [0038] disclosed four functional properties. The FAD used as coenzyme in GLD necessarily excluded the use of oxygen (see application as filed, paragraph [0003], and document D25). Although document D25 was post-published, the document was a review article that summarised the skilled person's knowledge at the filing date. If FAD dehydogenases were found in the future that used oxygen contrary to known enzymes, then the definition of the enzyme would have to be amended too. Thus the omission of feature "*2)*" in claim 1, although cited in paragraph [0038], did not add any subject-matter because a redundant feature was removed. Paragraph [0038] further disclosed "*80 µg*" as upper limit of the "amount" feature instead of "*10 µg*" (see claim 1). This amendment limited the claimed method, had a basis in paragraph [0025] of the application as filed, and was the sole claimed feature selected from a list. Claim 1 did therefore not single out any new subject-matter. Furthermore, the omission of "*wild-type GLD*" from paragraph [0025] in claim 1 in conjunction with the "amount" feature did not add any subject-matter either. The sole wild type GLD disclosed in the application as filed was that of document D5a (cited as

"*Patent Document 1*", paragraph [0011]), i.e. the sequence of SEQ ID NO: 2. An upper limit of "*10 µg*" in claim 1 was however necessarily lower than that of this GLD isolated from liquid-cultured *A. terreus.* Any reference to "*wild-type GLD*" could thus be omitted from claim 1. Whether document D87 disclosed wild type GLDs with lower sugar or not was irrelevant; it was clear from the patent (paragraph [0011]), that the reference wild type GLD had large amounts of sugar.
The term "*encompassing*" of claim 1 was equivalent to the term "*contains*" in paragraph [0090] of the application as filed. Although both terms might have a different meaning too (documents D121 and D126), the context of the terms was held decisive. In the context of claim 1 both terms had the same meaning.

Sufficiency of disclosure

The method of claim 1 did not refer to a "*sugar-embedded-type enzyme*". The objection raised under insufficiency was thus irrelevant for the claimed method.

The patent provided the skilled person with sufficient guidance to find further GLD homologs with the properties defined in claim 1. Several other strains were indicated in the patent as sources for finding GLD homologs (see paragraphs [0012], [0028] and [0060]). Furthermore, the patent taught that from strains having GLD activity, nucleic acids encoding GLD's gene could be cloned (see paragraph [0034]). Example 11 disclosed degenerated primers to amplify and clone GLD encoding genes. Based on this teaching in the patent, the skilled person had to screen in a first step all available *Aspergillus* strains and select those strains with the desired enzymatic activities. In a second

step, the GLD encoding genes were cloned. This approach was routine for the skilled person and led to success (see e.g. document D112, column 14, Example 1, column 20, Table 1, and column 21, Example 7). The unavailability of many genomic sequences from *Aspergillus* or other fungi at the effective date was irrelevant, since by applying a classical screening/ cloning approach using the sequence information provided in the patent, this sequence data was not needed. The post-published documents D66b, D67, D77 and D112 provided evidence that many GLD homologs falling within claim 1 existed. Contrary to the appellants' submissions the finding of these homologs did not depend on chance, but merely required routine experimentation. Nor was a marker for low maltose activity required. It was sufficient to screen for Aspergillus strains having GLD enzymes with low maltose activity. The patent taught the skilled person how to determine these enzyme activities (see pages 22 and 23, paragraphs [0141], [0148], and [0149]). Lastly, the existence of non-functional GLD homologs (document D67, see paragraph [0271]), or of GLD homologs with high maltose activity (see document D56) was irrelevant too, since the skilled person applying a screening/cloning approach did not select these strains.

As regards sequencing, the patent provided a definition of "*homology*" as "*identity to the full-length*" sequence, and mentioned standard programs for determining sequence homology, e.g. BLAST and FASTA to be run by default parameters (see paragraph [0045]). It was not contested that these programs and default parameters belonged to the skilled person's common general knowledge.

Novelty

The method of claim 1 was novel over the disclosure of document D3 (parent application) and document D5a. The appellants' submissions on "intrinsic" features vs "implicit" features as regards their entitlement to priority were late and should not be admitted. Accordingly, the questions of law submitted by appellant I should not be admitted either.

As regards document D3, decision G 1/15 referred in the catchword to the concept of implicit disclosure being entitled to partial priority. Example 2 of the priority document was identical to Examples 3 of the parent application (D3) and the patent application. These working examples disclosed the recombinant production of GLD in *E. coli*. It was immediately apparent to the skilled person that the GLD produced in *E. coli* lacked any glycosylation, since bacteria did not have enzymes that glycosylate proteins. This was common general knowledge. It was established case law that a method of making a product (here GLD) provided the product as such. All structural features of this product were implicit (see T 666/89, Reasons 6). Since glycosylation was a structural feature of GLD, the lack of GLD's glycosylation was an implicit feature of this enzyme. Consequently, decision G 1/15 applied to the present case. Contrary thereto, "*intrinsic/inherent*" features were non-structural and concerned an activity of a product which revealed itself only by interaction with outside conditions, for example, the pharmaceutical effects of a product.

A GLD lacking any glycosylation was an embodiment of claim 1 which was implicitly disclosed in Examples 2 and 3 of the priority and parent/patent application, respectively. Since this embodiment belonged to the

"OR" claim category as defined in G 1/15, it was entitled to partial priority.

The method of claim 1 was also novel over the disclosure of document D5a, because this document did not disclose any sequences of GLD. Although nucleic acid and amino acid sequences of GLD were mentioned in document D5a, this disclosure was not enabling as evidenced by documents D66, D67 and D69. Documents D86 and D88 did not provide evidence to the contrary since the purification protocol used differed significantly from the protocol disclosed in document D5a. Moreover, the sequencing of GLD in document D86 required an additional enzymatic step (deglycosylation) which was absent from document D5a. The "was it possible" test for novelty as advanced by the appellants was wrong. G 1/92 held that the disclosure of a prior art document had to enable the skilled person to obtain a product without undue burden by taking common general knowledge into account (see Reasons 1.4). This excluded as a matter of principle that it sufficed to analyse the product by any means as long as they were known and used in the field. In the present case undue burden was required to obtain the sequence information of the GLD disclosed in document D5a.

Inventive step

Document D5a represented the closest prior art. The skilled person trying to obtain GLD's sequence would follow the purification protocol of document D5a. The established facts (documents D67 and D69) provided evidence that the skilled person failed to obtain the sequence of GLD. What would the skilled person have done next with a reasonable expectation of success? The use of an additional SDS gel-based purification step

was based on hindsight because document D5a was silent on this step. Even if a SDS gel was run prior to the sequencing of GLD, sequencing failed (see document D69 page 10, third and fourth paragraph).
Even if the skilled person by running a SDS gel had recognised that GLD was a glycoprotein, this by itself did not foreshadow a sequencing problem. Numerous examples were known where glycoproteins were directly sequenced without any problems (see e.g. document D8, page 3797, right column, second paragraph).
Furthermore, indications were lacking from any of the documents on file that GLD's glycosylation was most likely responsible for the failure. This assertion was based on hindsight again. As regards sequencing problems of glycosylated proteins, document D37 mentioned solely a problem that affected internal protein sequencing. This did not affect N-terminal protein sequencing which was preferred for cloning purposes because it provided a sequence that was complementary to one end of the gene.
The patent disclosed that the sequencing of GLD failed because the protein was extremely strong glycosylated. All approaches trying to deglycosylate GLD failed (see patent, Example 6). Moreover, evidence was lacking from the file that any GLD purified according to Example 2 of document D5a could be deglycosylated. The alleged evidence shown in document D86 was irrelevant because it depended on a purification protocol for GLD that differed substantially from document D5a. The use of this different protocol enriched a GLD fraction with a lower glycosylation level that could be deglycosylated. Document D5a contained no pointers that by using a different purification protocol a sequence-able GLD could be obtained. Nor did document D5a indicate that by using the protocol of Example 2 the skilled person obtained a low yield of GLD. On the contrary, document

D5a disclosed that sufficient amounts of GLD were purified to characterise the enzyme. Any argument concerning low GLD yield was based on hindsight. Even if the skilled person would have considered the use of solid culturing of *A. terreus* because this was mentioned in document D5a, there were no indications on file that this provided higher yields of GLD (see document D36a, Figure 3). The teaching of document D36a rather disencouraged the skilled person to use solid culturing because the purification of enzymes was stated to be "*very difficult*" (see page 2, second paragraph). Since impure proteins provided several unrelated protein sequences, the skilled person would have avoided solid culturing. Ignoring nevertheless this statement in document D36a required hindsight knowledge of the patent. Irrespective of this, document D5a did not disclose the specific solid culturing conditions of the patent but generic ones only. Thus there was no evidence on file that GLD obtained from any solid-cultured *A. terreus* was sequence-able, irrespective of the culture conditions used. The appellants' assertions in this respect were speculative only.

Remittal to the opposition division

There was no reason apparent why the case should be remitted to the first instance.

XIII. Appellants I and II requested that the decision under appeal be set aside and the patent be revoked. Appellant I further requested that the appeal fee be refunded in view of their right to be heard having been violated by the opposition division, and that the case be remitted to the opposition division. Furthermore, appellant I requested that the following new documents

be admitted: D5b (filed with the grounds of appeal), D121 to D125 (filed with letter of 5 March 2019), and D129 and D130 (filed with letter of 28 February 2022). Appellant I further requested the submission of questions to the Enlarged Board of Appeal. Appellant II further requested that document D67a (filed with letter of 18 January 2023) be admitted.

XIV. The respondent requested that the appeals be dismissed (main request), or alternatively that the patent be maintained on the basis of any of the auxiliary requests that had been filed during the first instance proceedings, i.e. auxiliary requests 1 to 4 submitted with the letter dated 21 March 2016, and auxiliary requests 5 to 9 submitted with the letter dated 29 September 2017. The respondent further requested that documents D67a, D121 to D125 and D129 and D130 and any new arguments based thereon not be admitted. Furthermore, the respondent requested that certain arguments of appellant II as regards insufficiency of disclosure not be admitted into the proceedings. The respondent requested that the following new documents be admitted: D115, D116a, D116b, D117 to D119, D119a, D120, D120a, D120b and D120c (filed with the reply to the grounds of appeal), D36b and D126 to D128 (filed with letter of 6 December 2019), and D131 and D131a (filed with letter of 14 June 2022). The respondent requested that appellant I's request for refund of the appeal fee in view of an alleged substantial procedural violation be refused.

Reasons for the Decision

Substantive procedural violation: requests for remittal of the case to the opposition division and for refund of the appeal fee

1. Appellant I advanced two lines of arguments as regards a violation of right to be heard. Firstly, the decision under appeal was not properly reasoned because the opposition division provided their reasoning under novelty regarding the non-enabling disclosure of document D5a by reference to inventive step; and secondly, no reasoning under inventive step was provided based on documents D15a and D23 as alternative closest prior art. Both failures deprived appellant I of the opportunity to give reasoned arguments on these issues.

2. As regards the first objection, the decision under appeal states in the respective passage on page 11, second paragraph as follows: "*For the sake of completeness it is added that even if in general such a disclosure of a vector was novelty destroying, in the specific case of D5a this statement can not destroy novelty, since D5a is not an enabling disclosure. As explained in detail below under inventive step, the provision of the nucleotide sequence cannot be considered as a routine procedure but was dependent on trial and error and on inventive skills*" (emphasis added).

3. While this passage represents an unfortunate choice of formulation, it does not affect the matter under dispute in substance. The passage contains the very essence of why the opposition division considered that document D5a provided no enabling disclosure, while the

detailed reasons were given under inventive step. A procedural violation would have occurred if appellant I was not heard on this issue or if no reasons for the decision had been given at all. This, however, is not the case.

4. With regard to the second objection, it is established practice under the EPC to use the problem-solution approach to examine inventive step; according to this approach, the most promising document to arrive at the claimed invention, i.e. the closest prior art, should be selected in a first step. While document D15a was advanced by appellant I in their notice of opposition as an alternative starting point under inventive step (see page 38, point 4 to page 40, point 4.2), this line of argument was never referred back to, let alone further substantiated or defended in reply to the preliminary opinion of the opposition division annexed to the summons which stated that "*it is noted that all parties seem to agree that document D5a may be considered as closest prior art document*" (see page 9, point 12.4, emphasis added).

4.1 The minutes of the oral proceedings report under inventive step on page 2, in point 5 that "*After O1's request for a short break to deliberate on the strategy, OD announced a break from 15:10 to 15:20*". After resuming the oral proceedings the minutes disclose that "*all parties agreed on D5a as closest prior art document*" (see page 2, point 5, emphasis added). Thus appellant I had been given the opportunity to consider their case and decided to continue with document D5a as the closest prior art. This course of events does not imply that appellant I maintained their line of arguments under inventive step based on

document D15a. Nor are any other indications derivable from the documents on file in this respect.

4.2 As regards D23 as alternative closest prior art, appellant I never advanced any line of argument under inventive step based on document D23 during the first instance proceedings. Nor are indications derivable from the evidence on file that appellant II maintained their alternative line of argument starting from document D23. The same applies for the appeal proceedings where none of the appellants used any document other than D5a to substantiate their case on lack of inventive step.

4.3 The case law has established that if a piece of prior art can be identified as the closest prior art or the most promising springboard and it can be shown that, starting from this prior art, the claimed invention is non-obvious, then the invention must be even less obvious starting from any other prior art. In these circumstances a detailed inventive step assessment starting from the other prior art document(s) can be dispensed with (see e.g. T 1742/12, Reasons 6.3).

4.4 As set out above, all parties agreed on document D5a as the closest prior art. There was thus no need for the opposition division to assess inventive step starting from any other prior art document. Therefore, appellant I's second objection is not convincing either.

5. Since the board is not convinced that a substantial procedural violation occurred in the present case, appellant I's requests for refund of the appeal fee and remittal of the case to the opposition division are rejected.

Admission of documents D36b, D67a, D121, D122, D124 to D131, and D131a into the appeal proceedings

6. Documents D36b and D126 have been submitted by the respondent in reply to appellant I' reply, while documents D121, D122, D124 and D125 were submitted by appellant I in response to the respondent's reply to the statements of grounds of appeals. Since the statements of grounds of appeal were filed before the date of entry into force of the RPBA 2020, the transitional provisions set out in Article 25(2) RPBA 2020 apply. Hence the discretion of the board in admitting these documents has to be exercised in accordance with Article 13(1) RPBA 2020.

6.1 Document D36b concerns solely a blow-up figure of a figure of document D36 which improves legibility. This document was thus admitted.

6.2 Document D121 concerns an excerpt from the Oxford English Dictionary for the term "encompass". Since this document reflects the skilled person common understanding of the term "encompass", document D121 was admitted.

6.3 Document D122 concerns an opinion of a former member of the Boards of Appeal in support of arguments under enablement and inventive step. Reasons are not apparent why document D122 was not submitted with the statement of grounds of appeal but at a later stage of the proceedings. Nor have any such reasons been provided by appellant I. Document D122 was thus not admitted.

6.4 Appellant I argued that documents D124 and D125 were submitted in direct response to the respondent's reply to the statements of grounds of appeals which discussed

for the first time the meaning of "*eluate*" in Example 2 of document D5a and the functioning of Sumizyme disclosed in Example 6 of the patent.

6.5 The board considers that the admission of documents D124 and D125 adds further complexity to the case since the disclosure of these documents and arguments based thereon concern matter that has not been assessed in the first instance proceedings. Moreover, both documents do not resolve any of the issues that are at stake under inventive step. Documents D124 and D125 were thus not admitted.

6.6 Moreover, since documents D124 and D125 were not admitted, there was no need to admit documents D127 and D128 into the proceedings either, because both documents were submitted by the respondent in reply to documents D124 and D125.

6.7 Document D126 concerns a Merriam Webster Dictionary excerpt for the term "encompass". Since this document reflects the skilled person common understanding of the term "encompass", document D126 was admitted.

7. Documents D129 and D130 and arguments based thereon have been filed by appellant I in a further letter in reply to the respondent's submissions. In reply thereto, the respondent submitted documents D131 and D131a. Their consideration/admission is also at the discretion of the board pursuant to Article 13(1) RPBA 2020.

7.1 Document D129 concerns new experimental evidence which assessed the issue of whether different solid culturing conditions affect the molecular weight of GLD, and hence, its glycosylation level. Document D130 discloses

a further example of a solid-cultured Aspergillus strain for the production of an enzyme. Appellant I argued that these documents addressed a new argument raised by the respondent. However, solid culturing of Aspergillus has been discussed already during the first instance proceedings (see e.g. Appellant II's notice of opposition, page 10, point 3.2.2.(a), or at the oral proceedings, see decision under appeal, point 20.3.1). Consequently, the submission of documents D129 and D130 at this late stage of the proceedings did not address a new line of argument of the respondent. Document D129 and D130 were therefore not admitted.

7.2 Moreover, since documents D129 and D130 were not admitted, there is no need to admit documents D131 and D131a into the proceedings as well, because these documents were submitted by the respondent in reply to documents D129 and D130.

8. Document 67a was submitted by appellant II with the letter dated 18 January 2023, i.e. after notification of the summons to oral proceedings and after the board had issued the communication under Article 15(1) RPBA 2020. Thus, the submission of this document constitutes an amendment to the party’s appeal case within the meaning of Article 13(2) RPBA 2020.

8.1 In support of admittance, appellant II argued that document D67a completed the disclosure of document D67 by providing the document's sequence listing. Furthermore, document D67 had been submitted by the respondent itself in reply to the notices of oppositions and was relied upon by the respondent in reply to the statements of grounds of appeals.

8.2 Exceptional circumstances justifying the late filing of document D67a into the proceedings were not advanced by appellant II. It is evident that this document could have been filed during the first instance proceedings. The board exercised thus its discretion under Article 13(2) RPBA 2020 and did not admit document D67a.

Main request (claims as granted)

Claim construction - claim 1

9. The method of claim 1 is directed to the preparation of a biosensor for measuring glucose in a liquid, which comprises at least the following process steps:
(i) the recombinant production of a FAD-linked glucose dehydrogenase (GLD) by "*cultivating a transformed cell*", and
(ii) preparing a biosensor that encompasses an enzyme reaction layer containing said GLD.

9.1 Claim 1 indicates two GLD alternatives to be used on the biosensor which are structurally defined by the amino acid sequences:
(iii) having a "*homology of at least 60% to an amino acid sequence set forth in SEQ ID NO: 2*", or
(iv) from "*amino acid 20 to amino acid 592 of SEQ ID NO:2*";
moreover, the GLD should have
(v) a maximum total content of galactose, glucose, mannose and arabinose of 10 µg per µg of protein ("*10 µg or less per µg of protein*"). Since the term "*total content* of *galactose, glucose, mannose and arabinose*" in claim 1 is not further defined, the skilled person would give this feature its ordinary meaning, i.e. any amount of these sugars falling within the claimed range wherein 10 µg/µg protein defines the upper limit and 0

µg/µg protein (i.e. no such sugars) the lower limit of the range.

9.2 The two GLD alternatives are further functionally defined by
(vi) a maximum activity for maltose ("*5% or less*") relative to glucose.

Added subject-matter

10. In the following all references to the application as filed refer to the published patent application (EP 2 380 980 A1).

11. The appellants submitted several lines of arguments under added subject-matter.

12. The appellants disputed in a first line of arguments that the application as filed provided a basis for the combination of the features "*having an amino acid sequence with a homology of at least 60% to an amino acid sequence set forth in SEQ ID NO: 2*" ("identity" feature), "*having an activity towards maltose of 5% or less with respect to an activity towards glucose*" ("activity" feature), and "*a total content of galactose, glucose, mannose, and arabinose of the GLD is 10 µg or less per µg of protein*" ("amount" feature) cited in claim 1.

These features were selected from different lists in the absence of pointers or by taking the second-least preferred feature. Thus, the combination of the "identity", "activity" and "amount" features in claim 1 was not directly and unambiguously derivable from the application as filed.

12.1 The board does not agree. All three disputed features are literally disclosed in the application as filed:
- "identity" feature, see paragraph [0043], lines 44 and 45, and paragraph [0049], lines 17 to 20, in conjunction with, for example, paragraph [0019], lines 35 and 36;
- "activity" feature, see paragraph [0024], lines 9 and 10;
- "amount" feature, see paragraph [0025], lines 16 and 17.

12.2 The "identity" feature concerns only feature (iii) cited in claim 1, (see claim construction above), i.e. one of the two cited GLD sequence alternatives.

12.3 As regards the combination of these three features, the question is whether or not the skilled person can derive this combination directly and unambiguously, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the application as filed (see Case Law of the Boards of Appeal 10th ed., 2022 ("Case Law"), II.E.1.3.1).

12.4 Since there are no specific pointers to this feature combination under dispute in the application as filed, it is relevant to assess whether this combination pertains to separate embodiments or a single embodiment of the application as filed. It is established case law that an application is not a mere reservoir from which features of separate embodiments can be artificially combined to create a particular, new embodiment (see Case Law, II.E.1.6.1a)).

12.5 As set out above only one of the two GLD enzyme alternatives in claim 1 is structurally and

functionally defined by the feature combination under dispute.

12.6 Paragraph [0049] of the application as filed reads as follows: "*Also, the present invention provides a GLD containing an amino acid sequence set forth in amino acid 20 to amino acid 592 of SEQ ID NO. 2 or an amino acid sequence with a homology of at least 60% to the amino acid sequence, having a function equivalent to that of the above-mentioned GLD, and being produced by a peptide synthesis method or a gene recombinant method*" (emphasis added).

12.7 Although "*SEQ ID NO. 2*" is missing as reference sequence for the amino acid sequence that is 60% homologous in paragraph [0049] above (i.e. the identity feature under dispute), it is evident that nothing else than SEQ ID NO. 2 is meant here as amino acid sequence. SEQ ID NO. 2 is the sole amino acid sequence with GLD activity disclosed in the application as filed. Moreover, the preceding paragraphs [0043] and [0045] disclose the same wording, except that SEQ ID NO. 2 is present.

12.8 Furthermore paragraph [0049] discloses that the two GLD alternatives mentioned in claim 1 are produced by "*a gene recombinant method*", which necessarily implies that polynucleotide sequences encoding the amino acid sequence variants of SEQ ID NO: 2 are cloned into a vector and expressed in a transformed cell.

12.9 Paragraph [0038] discloses a GLD of the invention with a defined set of functional properties, including the "activity" feature referred to in claim 1. The functional properties of the GLD mentioned here differ from the feature combination under dispute in the

disclosure of (1) "*80 µg*" as the upper limit of the "amount" feature instead of "*10 µg*", and (2) a GLD that is "*not utilizing oxygen as an electron acceptor*", i.e. an additional property. This property, however, is an implicit property of all GLD enzymes and can thus be omitted from claim 1 without adding subject-matter (see application as filed, paragraph [0003], line 18, and document D25, page 1069, left column, first full paragraph).

12.9.1 Appellant I argued that document D25 was not prior art - but post-published, and that it was not excluded that GLD enzymes in the future might be able to utilise oxygen. The board construes appellant I's submission that while future GLD enzymes might be able to use oxygen, it is not contested that the present GLD enzymes are unable to do so. Irrespective thereof, the term glucose "*dehydrogenase*" in paragraph [0003] of the application as filed renders it unambiguous that this enzyme cannot utilise oxygen, in contrast to a likewise mentioned "*glucose oxidase*". A further argument that granted claim 8, by including this feature, rendered clear that the GLD of claim 1 did not necessarily have this limitation is not convincing either. The board follows the respondent's argument that, in view of the patent's teaching and of common general knowledge (e.g. document D25) claim 8 is in fact redundant. Appellant I's arguments are thus not convincing.

12.9.2 Appellant I further submitted that the choice of paragraph [0038] already represented a selection from a first list of five GLD alternatives disclosed in paragraphs [0036] to [0040] of the application as filed. The board does not agree. Each of the five GLD alternatives of paragraphs [0036] to [0040] represents an individualised and singled out embodiment of the

application as filed. This is not changed by the separate mentioning of GLD's structural information in paragraph [0049], since the application as filed discloses solely a single GLD that is structurally characterised by the nucleic acid and amino acid sequences encoded by SEQ ID NOs: 1 and 2. In light of the application as filed as a whole, the skilled person derives directly and unambiguously that this structural information of GLD refers to each of the five singled out embodiments disclosed in paragraphs [0036] to [0040]. The combination of paragraphs [0038] and [0049] does therefore not represent a selection from a first list of embodiments.

12.10 Consequently the combined disclosure of paragraphs [0038] and [0049] differ from the feature combination under dispute solely in the mentioning of "*80 µg or less*" instead of "*10 µg or less*", i.e. the upper limit of the "amount" feature. As mentioned above, paragraph [0026] of the application as filed discloses a list of total sugar contents of GLD, of which "*10 µg or less*" is indicated as "*preferably*".

12.10.1 Appellant I further argued that paragraph [0026] did not refer to an amino acid sequence but to "*the GLD encoded by the GLD polynucleotide*" and therefore could not provide a basis for the "amount" feature in the context of granted claim 1. This is not convincing. The expression "*GLD encoded*" in this statement can be interpreted only in that it refers to the protein and, hence, to the respective amino acid sequence of SEQ ID NO: 2 as the sole GLD protein sequence disclosed in the application as filed.

12.10.2 Appellant II submitted that paragraph [0026] of the application as filed did not solely disclose that the

total content of the four sugars cited in claim 1 must not exceed "*10 µg or less*", i.e. an absolute value, but further required that the total content of these sugars was "*different from that of a wild type GLD*", i.e. a relative property. Since the 60% homology of the "identity" feature of claim 1 was not limited to a wild type GLD, GLDs might fall within claim 1 that were not different from wild type. Wild type GLDs with sugar contents that were lower than 10 µg/µg protein were known from other Aspergillus strains (see document D87). The board does not agree. As set out above, the application as filed discloses as sole wild type GLD the one purified from *Aspergillus terreus* (*A. terreus*), i.e. the specific GLD characterised by amino acid sequence SEQ ID NO. 2. The skilled person would thus derive from the application as filed as a whole that the difference in sugar content must be relative to this GLD. It is uncontested that the GLD of *A. terreus* is characterised by a very high sugar content, and that the upper limit of "*10 µg or less*" as referred to in claim 1 is by far lower. Thus all GLDs falling within claim 1 necessarily have a lower (and hence different) sugar content compared to the GLD encoded by SEQ ID NO: 2 and obtained from *A. terreus*.

12.11 In light of these considerations, the board agrees with the opposition division that solely a selection of "*10 µg or less*" from a single list is required for selecting the disputed feature combination. The disputed feature combination is therefore directly and unambiguously disclosed in the application as filed.

13. The appellants raised further objections under added subject-matter against the following features in claim 1:

- "a *polynucleotide encoding the GLD*" required for the recombinant production of GLD,
- the omission of a process step collecting the GLD,
- the feature "*preparing the biosensor encompassing an enzyme reaction layer*" (emphasis added) and
- *"[a] method for preparing a biosensor*".

13.1 The feature "a *polynucleotide encoding the GLD*" has a basis in paragraph [0049] (see point 9.8 above). Furthermore paragraph [0047] of the application as filed states: "*Moreover, the present invention provides a recombinant vector carrying any one of the above-mentioned polynucleotides according to the present invention, a transformed cell prepared using the recombinant vector, a method for producing the GLD characterized in that the transformed cell is cultivated followed by collecting the GLD having a glucose dehydration activity from the cultivated product, and the GLD produced by the method*" (emphasis added).

13.2 Furthermore, paragraph [0047] mentions the step of "*collecting the GLD having a glucose dehydration activity from the cultivated product*". The appellants submitted that the omission of this step in claim 1 added subject-matter. The board does not agree. As set out above under claim construction, claim 1 comprises at least two process steps, i.e. the recombinant production of GLD (feature (i)), and the preparation of the biosensor that encompasses a reaction layer that contains GLD (feature (ii)). It is necessary for preparing the biosensor that the recombinantly produced GLD has been collected from the cell culture. The omission of an explicit collection step in claim 1 does therefore not add any subject-matter.

13.3 The appellants further submitted that the exchange of the term "*contains*" (see application as filed, page 13, line 8 of paragraph [0090]) by "*encompassing*" in claim 1 added subject-matter because both terms had a different meaning, i.e. "*have or hold within*" compared to "*surround and have or hold within*", respectively (see also dictionary definitions of D121 and D126).

13.3.1 The board does not agree. The skilled person usually interprets the terms "*encompassing*", "*containing*", or "*comprising*" in patent claims as having the same meaning. This is also in line with the ordinary construction of claim 1. That document D121 discloses that "*encompass*" means "*surround and have or hold within*" and "*include comprehensively*", and document D126 discloses that this term means "INCLUDE", "COMPREHEND", "ENVELOP", "ENCLOSE", (or, in a different context which is not the one of the claim, "BRING ABOUT" or "ACCOMPLISH"), does not change the board's view on the interpretation of these terms.

13.3.2 The respective sentence in paragraph [0090] of the application as filed states as follows: "*The biosensor according to the present invention contains the GLD according to the present invention as an enzyme in a reaction layer, and is a glucose sensor for measuring glucose concentration in sample liquids*".

13.3.3 If, as submitted by appellant I the term "encompass" means predominantly "*surround and have or hold within*" the question is whether or not this meaning adds any new information that cannot be derived from paragraph [0090] of the application as filed. It is relevant that neither the first sentence of paragraph [0090] nor claim 1 define the form or design of the biosensor, as long as it contains/encompasses GLD in a reaction

layer. Thus, and irrespective of the use of "encompassing" or "contains", the biosensor can have any form/design relative to the reaction layer. Also for this reason the term "*encompassing*" in claim 1 does not add any subject-matter.

13.4 As regards the "*method for preparing a biosensor*" according to claim 1, the appellants submitted that the application as filed did not disclose such a method, but the biosensor only. Moreover, if a method for preparing the biosensor was disclosed in the application as filed (see paragraph [0090]), then the biosensor was characterised by further features that were, however, lacking from claim 1.

13.4.1 The board is not convinced by this argument. It is established case law that the change of a product claim to a method claim is normally allowable under Article 123(2) EPC, even if the method itself is not explicitly disclosed in a patent application (see e.g. T 243/89, Reasons 3, and T 601/92, Reasons 6.1.1).

13.4.2 Moreover, paragraph [0090] of the application as filed (see above) discloses the purpose of the biosensor ("*for measuring glucose concentration in sample liquids*") and that the biosensor "*contains the GLD according to the present invention as an enzyme in a reaction layer*". This implies the production of GLD (as sole enzyme disclosed in the application as filed for achieving this purpose) and the preparation of the biosensor. That paragraph [0090] discloses in the second sentence a specific biosensor defined by further technical features is irrelevant for assessing added subject-matter, since this disclosure starts with "*For example*".

13.5 In summary, the method of claim 1 has a direct and unambiguous disclosure in the application as filed.

14. Appellant II submitted a sweeping reference to the opposition case as regards further added subject-matter issues against claims 2 to 5 and 7 (see statement of grounds of appeal, page 7, point 2.5.3). Such a reference is insufficient for establishing why the contested decision should be overturned on this point. Under Article 108 EPC, Rule 99(2) EPC, and Article 12(3) RPBA 2020, appellant II has to present a complete case in their statement of grounds of appeal so as to allow the board and the other party to understand why the contested decision should be overturned without having to make any further investigations of their own (see, *inter alia*, T 1566/12, Reasons 20, and T 989/16, Reasons 1). Appellant II's submission against claims 2 to 5 and 7 is thus disregarded.

15. No separate/independent objections under added subject-matter based on the parent application (WO 2006/101239 / EP1862543 A1) were raised by the appellants.

16. In light of the considerations above, the board concludes that Article 100(c) EPC does not prejudice the maintenance of the patent as granted.

Sufficiency of disclosure

17. It is established case law under sufficiency of disclosure that the claimed invention must be sufficiently disclosed at the effective date of the patent, based on the patent as a whole in consideration of the common general knowledge of the skilled person over substantially the whole breadth of the claim

without undue burden (see Case Law, II.C.1. and II.C. 5.4).

18. As set out above under claim construction, claim 1 comprises *inter alia* as embodiment a method of preparing a biosensor for measuring glucose, comprising a step of producing functional GLDs defined by a "*homology of at least 60% to an amino acid sequence as set forth in SEQ ID NO: 2*", and a step of preparing the biosensor. This embodiment will be considered in the following only since the other embodiment claimed, i.e. the preparation of a biosensor comprising a step of producing "*amino acid 20 to amino acid 592 of SEQ ID NO:2*" is uncontested under sufficiency of disclosure.

19. The putting into practise of the embodiment under consideration of claim 1 requires that the skilled person obtains sequence homologs of GLD across substantially the whole breadth of the claim without undue burden.

20. It is uncontested that the patent discloses experimental data demonstrating that the biosensor obtained by the method of claim 1 is suitable for determining glucose in liquid samples (see page 24, Example 10). The patent also discloses methods for recombinantly producing GLD, including GLD's nucleic acid and amino acid sequences (see SEQ ID NOs: 1 and 2, Examples 2 to 5 on pages 16 to 18, and Example 11 on page 24). The GLD obtained from different production hosts contains a total sugar content that falls within the range cited in claim 1 (see page 21, Table 1). Examples 7 and 8, respectively disclose methods for determining the sugar content of GLD, and the activity of GLD towards glucose and maltose (see paragraphs [0137] to [0140], [0142], [0148] and [0149]).

21. The appellants advanced in essence three lines of arguments under insufficiency of disclosure.

22. In the first line of argument, the appellants argued that the lack of evidence in the art for a "*sugar-embedded-type enzyme*" (see patent, paragraph [0011]) casts serious doubts on the correctness of the experimental data disclosed in the patent. The board does not agree. Claim 1 does not refer to a GLD that is a sugar-embedded-type enzyme. Accordingly, the question of whether or not such an enzyme exists in the art is of no relevance for assessing sufficiency of disclosure.

22.1 As regards the second line of argument, the appellants contested that the patent provided sufficient information for the skilled person to obtain substantially all GLD homologs falling within the scope of claim 1.

22.2 The patent discloses that nucleic acid sequences encoding GLDs with the desired properties can be isolated "*from a filamentous fungi or a basidiomycete, such as, for example, a microorganism belonging to the genus Aspergillus Penicillium, or the genus Ganoderma, and is particularly a polynucleotide isolated from Aspergillus terreus (A. terreus)*" (see paragraph [0028]). Other Aspergillus strains serving as potential GLD source are mentioned, such as *"Aspergillus japonicus (A. japonicus), and Aspergillus oryzae (A. oryzae)*" (see paragraph [0060]). Moreover, the patent mentions that GLD enzymes with low maltose activity were already described in the prior art before the effective date (see paragraph [0012]; the "*Non-patent Document 1*" mentioned here is document D11 in these

proceedings). Furthermore, the patent teaches that nucleic acids encoding GLD homologs with the desired properties can be cloned from *"a microorganism having GLD productivity*" which preferably belongs to the genus of Aspergillus and has been cultivated on solid medium (see paragraph [0034]).

22.3 In other words, the patent teaches the skilled person in these paragraphs to cultivate Aspergillus strains, screen them for desired enzyme activities, and after identifying respective strains to clone GLD(s) gene(s). For cloning, primers can be used, such as the oligonucleotides set forth in SEQ ID NOs: 13 and 14 (see Example 11, paragraph [0158]). Alternatively, GLD can be purified from solid-cultured strains and a partial amino acid sequence from "*the N-terminal or internal sequence of the GLD*" can be determined. After back translating the sequence into the corresponding nucleic acid sequence, the gene can be cloned (see paragraphs [0060] to [0064]).

22.4 The screening and cloning procedures outlined above are routine for the skilled person using the nucleic acid sequence disclosed in the patent. Moreover, a screening of strains which express a GLD with desired enzyme properties neither requires the availability of sequence information, nor a marker for low maltose activity. Accordingly, the appellants' objections that no Aspergillus genome sequences were available at the effective date, except for strain RIB40 (see document D28), and that the patent disclosed no marker for low maltose activity is of no relevance for assessing sufficiency of disclosure in the present case.

22.5 Likewise the existence of GLD enzymes fulfilling the structural requirements of claim 1, but having either

no GLD activity (see document D67, page 33, lines 44 and 45), or a too high maltose activity (see document D56, page 46, left column, last paragraph) is of no relevance for sufficiency of disclosure since the screening process outlined above does not select strains with these enzyme activities.

22.6 It is uncontested that the post-published documents D66b, D67, D77 and D112 disclose that further GLD enzymes falling within claim 1 exist in various Aspergillus strains. Document D77 is available in the Japanese language only. The probative value of this document is therefore limited. The non-availability of the sequence information encoding the GLDs disclosed in these documents - as argued by the appellants - is of no relevance for sufficiency of disclosure. This is so because the availability of the Aspergillus strains disclosed in these documents is uncontested. The skilled person applying thus the screening/cloning approach outlined above would have obtained GLD sequences from these strains even if their sequence information was not available at the effective date of the patent. Document D112 discloses that at least one Aspergillus strain (NBRC 5375) expresses a GLD with the required functional properties (see Examples 1 and 7 in columns 14 and 21). The appellants argued that the GLD enzymes of the other Aspergillus strains disclosed in Table 1 (see column 20) were not tested for their maltose activity, and that document D112 thus provided no evidence that further GLD enzymes with the required properties existed. The board does not agree. The absence of a test in document D112 is not an evidence for the non-existence of further GLDs with the required properties. The board is thus convinced that the skilled person following the teaching in the patent

would have obtained further GLD homologs falling within the scope of claim 1 without undue burden.

23. As regards the third line of argument, the appellants argued that the skilled person was faced with undue burden in determining the percentage of homology referred to in claim 1 absent any specification of a particular program/algorithm for determining homology.

23.1 Claim 1 indicates that a recombinant vector is used that carries a "*polynucleotide encoding the GLD having an amino acid sequence with a homology of at least 60% to an amino acid sequence set forth in SEQ ID NO: 2*". In the absence of further indications in claim 1, the skilled person would give this feature its ordinary meaning.

23.2 The skilled person would thus reasonably interpret the minimum homology of 60% in claim 1 as the result of a sequence comparison based on the use of a program/algorithm commonly used for this purpose, provided it is performed under standard parameter conditions. Knowing, moreover, the strengths and weaknesses of such programs/algorithms, the skilled person would not use a program/algorithm that leads to nonsensical results.

23.3 The patent mentions on page 7, lines 10 to 12 that the sequence length has to be considered in determining the percent homology of the sequence claimed. This passage states that the *"nucleotide sequence with a homology of at least 60% to a polynucleotide composed of the nucleotide sequence set forth in SEQ ID NO.1" refers to a nucleotide sequence of which the identity to the full-length nucleotide sequence set forth in SEQ ID NO. 1 is at least 60%, ...*" (emphasis added). A similar disclosure is found on page 8, lines 32 to 34.

23.4 Furthermore, the patent discloses standard exemplary programs ("*BLAST, FASTA, or GENETYX*" which "*may be run with default parameters*") for determining a sequence homology (see paragraphs [0031] and [0045]).

24. In light of the considerations above, the board is convinced that the embodiment under consideration could be put into practice by the skilled person at the effective date across substantially the whole breadth claimed without undue burden.

25. Article 100(b) EPC does not therefore prejudice the maintenance of the patent as granted.

Novelty

26. The appellants argued that the method of claim 1 lacked novelty over the disclosure of the parent application (document D3) and document D5a.

27. The board does not agree with the appellants for the following reasons.

28. As regards the parent application (D3), appellant I submitted that because the method of claim 1 of the patent was not entitled to priority contrary to the disclosure of Example 3 of the parent application, the latter anticipated the claimed method due to a "poisonous priority".

29. It is uncontested that the disclosure of Example 2 of the priority document (D4/D4a) is identical to Examples 3 of the parent application and the patent application.

30. It is however contested, whether or not decision G 1/15, published in OJ 2017, 82 applies to the present case at all, and if G 1/15 applies, whether claim 1 as granted belongs to the so called "AND" or "OR" claim category (see G 1/15, Reasons 5.2.1).

31. Appellant I contested that G 1/15 applied to the present case because the glycosylation level of the GLD enzyme disclosed in Example 2 of the priority document and in Examples 3 of the parent application and the patent application was an "*intrinsic*" feature of GLD and not an "*implicit*" one. Since intrinsic features were not assessed in decision G 1/15, let alone their impact on the concept of a "poisonous priority", G 1/15 was irrelevant for the present case.

31.1 The board does not agree for the following reasons.

31.2 The case law has established that an intrinsic/inherent feature of a product normally relates to a technical effect caused by an interaction with specifically selected outside conditions, i.e. a certain use of the product (see decision G 2/88, published in OJ 1990, 93, Reasons 10.2), while structural features of a product are normally implicit to that product (see opinion G 1/92, published in OJ 1993, 277, Reasons 3).

31.3 Example 2 of the priority document discloses the transformation of an *E. coli* strain with a recombinant vector encoding a GLD gene for the production of an active GLD enzyme (see document D4a, page 42, lines 6 to 21). It is uncontested that proteins recombinantly produced in *E. coli* are not glycosylated ("sugar-free", i.e. lack any galactose, glucose, mannose and arabinose residues as referred to in claim 1), because *E. coli* does not contain the enzymes required for

glycosylation, i.e. for adding sugar residues to a protein. This belongs to the common general knowledge of the skilled person. Furthermore, the absence or presence of sugar residues on a protein are a structural feature of this protein.

31.4 A skilled person reading Example 2 of the priority application (and Examples 3 in the parent application and the patent application) therefore immediately understands that the GLD recombinantly produced in *E. coli* is sugar-free (i.e. not glycosylated) although this is not explicitly mentioned. The production of sugar-free GLD in *E. coli* is thus the clear and unambiguous consequence of the explicit disclosure of this working example in view of *E. coli*'s generally known inability to produce glycosylated proteins. It is established case law that such a feature is implicit (see Case Law, I.C.4.3, T 666/89, Reasons 6). Appellant I's arguments are therefore not convincing.

31.5 This means that G 1/15 applies to the present case. For this reason the second question of law submitted by appellant I during the oral proceedings (see item XI, page 12 above) which refers to an intrinsic feature is of no relevance for deciding the "poisonous priority" issue of the present case and is therefore rejected.

32. As regards the "AND" or "OR" claim category as defined in G 1/15 (Reasons 5.2.1), claim 1 as granted relates to a method for preparing a biosensor. This method comprises as an embodiment the use of GLD or variants thereof that lack any galactose, glucose, mannose and arabinose since the content of these sugars is defined as "*10 µg or less per µg of protein*", which includes 0 µg/µg GLD, i.e. a "sugar-free" GLD.

32.1 As set out above, it is uncontested that Example 2 of the priority document is identical with Examples 3 of the patent application and the parent application.

32.2 If therefore as asserted by appellant I, the disclosure of a sugar-free GLD in Example 3 of the parent application (D3) falls necessarily within the subject-matter of claim 1, then this applies likewise to the sugar-free GLD of Example 3 of the patent application too. Moreover, since both Examples 3 are identical to Example 2 of the priority document (D4/D4a), claim 1's embodiment of a sugar-free GLD is present in the priority document too.

32.3 This finding answers the first question of law submitted by appellant I (see item XI, page 12 above), and moreover corresponds to the practise under Article 88 EPC established by the case law. The request for a referral of this first question of law to the Enlarged Board of Appeal is therefore rejected too.

32.4 In light of these considerations, the embodiment of claim 1 using a sugar-free GLD for the preparation of a biosensor must be regarded as an "OR" claim as defined in G 1/15 (Reasons 5.2.1), since sugar-free GLD is an implicitly disclosed feature in Examples 2 and 3 of the priority document and the patent application, respectively. Consequently, this embodiment of claim 1 is entitled to partial priority (see decision G 1/15, Reasons 6.4). Therefore, the parent application (D3) cannot anticipate the claimed method.

33. As regards document D5a, it is uncontested by the parties that this document discloses a GLD enzyme that is obtained from the same deposited *A. terreus* strain mentioned in the patent. Furthermore, document D5a

discloses likewise a method for producing a biosensor that uses this GLD enzyme. It is likewise uncontested that this GLD of document D5a is glycosylated to an extent that exceeds the maximum content of sugars defined in claim 1.

34. Appellant I submitted that since document D5a mentioned the recombinant expression of GLD by a vector in *E. coli* (see paragraph [0039]), the nucleic acid sequence encoding GLD was implicitly disclosed therein. Moreover, the expression of this protein in *E. coli* led to the provision of "sugar-free" GLD (see above). Although document D5a did not disclose a specific nucleic acid and amino acid sequence, this information was obtainable by the skilled person as a matter of routine. Experimental evidence thereof was submitted (see documents D86 and D88) which demonstrated that "it was possible" for the skilled person to obtain this sequence information, thus rendering the enzyme characterised by this amino acid sequence state of the art (see G 1/92, OJ 1993, 277).

35. The board shares the opposition division's finding that document D5a provides a non-enabling disclosure for a recombinantly produced GLD.

36. It is established case law that the subject-matter described in a document (here the expression of the GLD enzyme of document D5a in *E. coli* which requires the availability of the undisclosed nucleic acid sequence of GLD) can only be regarded as having been made available to the public, and therefore as comprised in the state of the art pursuant to Article 54(1) EPC, if the information given in that document is sufficient to enable the skilled person at the effective date to reduce the subject of the document into practise

without undue burden taking common general knowledge into account (see Case Law, I.C.4.11, and G 1/92, headnote and Reason 1.4).

37. The issue to be assessed is thus whether the skilled person is able to obtain the nucleic acid sequence of GLD mentioned in document D5a without undue burden.

37.1 In reading document D5a, the skilled person might *prima facie* assume that GLD's nucleic acid sequence is disclosed in an enabling manner. This document indicates (1) the source of GLD (*A. terreus* deposited under the accession number FERM BP-08578), (2) a protocol for purifying GLD from this source (Example 2), and (3) biochemical properties of this enzyme (Example 3). The provision of this information normally suffices to obtain a partial amino acid sequence of the protein by routine methods, such as N-terminal sequencing or internal sequencing, which after back translation into a corresponding nucleic acid sequence is used to clone the encoding gene by routine means. Document D5a does also not foreshadow any potential problems as regards the sequencing of GLD, albeit that this document is silent on any GLD sequencing attempts.

37.2 However, a purified protein is not necessarily always sequence-able by routine methods.

37.3 In the present case it is relevant that another group of scientists encountered independently from the inventors of the patent problems in sequencing GLD obtained from liquid-cultured *A. terreus* and *A. oryzae* when applying routine sequencing methods (see document D67, paragraphs [0154], [0265] and [0266]). Although document D67 is post-published relative to the patent (the filing date of document D67 is 12 month later than

the filing date of the patent), the similarity of the problems encountered and the closeness of the filing dates of both documents are indicative of the circumstances the fictitious skilled person would have encountered in trying to sequence the GLD of document D5a. Appellant I's argument that problems encountered by real persons cannot be exemplary for the fictitious skilled person defined in the case law is not convincing.

37.4 Document D67 discloses that since the problems of sequencing *inter alia A. terreus* (i.e. the same species mentioned in document D5a) GLD could not be overcome, sequencing attempts were abandoned and a different and independent route to obtain GLD's nucleic acid sequence was chosen (see paragraph [0155]). This is confirmed by document D69 (see page 7, last paragraph to page 9, last paragraph).

37.5 The sequencing problems described in documents D67 and D69 are consistent with those encountered by the inventors of the patent using the GLD protein of document D5a (see document D66, points 8 to 14).

37.6 Thus, based on the evidence on file, the board finds that the skilled person using the GLD of document D5a and applying routine sequencing methods would have failed to obtain a partial amino acid sequence of GLD. The board agrees with the opposition division that the reason for this failure is not self-evident (see decision under appeal, point 20.4.1, page 17, penultimate paragraph to page 18, first paragraph). The skilled person faced a situation where several equally likely causes might be responsible for the failure, including for example, insufficient GLD purity, low

amount, N-terminal blockage, mixed-up amino acid sequence information, or protein glycosylation.

37.7 Since many reasons might be responsible for the failure, and document D5a is silent on sequencing, document D5a provides no guidance to the skilled person on how to make an educated guess on the most likely reason for failure. The skilled person has thus to find out by trial and error the underlying cause, without knowing whether or not he/she would succeed. Although a reasonable amount of trial and error experimentation is acceptable to obtain GLD's sequence without undue burden, this presupposes that sufficient information is available that leads the skilled person directly towards success through the evaluation of initial failures. In the absence of such guidance, document D5a lacks an enabling disclosure for the amino acid and nucleic acid sequence of GLD.

38. Appellant I argued that according to G 1/92 any evidence that demonstrated that GLD's sequence was obtainable by methods generally known in the art and used in the field (as provided in documents D86 and D88) supported the argument that document D5a anticipated the claimed method.

38.1 The board does not agree. The criterion to be applied under enablement is not the use of every means as long as it is generally known and applied in the field. The criterion to be applied is the availability of sufficient information in document D5a, together with common general knowledge, which leads directly towards success through the evaluation of initial failures. In the absence of guidance (because many equal reasons for failure exist, see above) that allows the skilled person at least an educated guess about the most

promising way to success, the skilled person is left with trial and error to convert failure into success. This requires a research project which amounts to undue burden. For this reason, appellant I's arguments must fail.

38.2 Document D86 discloses a purification protocol for GLD that is different from that described in Example 2 of document D5a, as confirmed by document D88 (see Table under point 1.1. on pages 2 and 3). Document D86 does not explain why the protocol of document D5a has been changed. Nor are indications derivable from document D5a that the protocol of Example 2 should be changed to that of document D86 to obtain a sequence-able GLD. On the contrary, document D5a discloses that active GLD is obtained in sufficient quantities (see Examples 3 and 4). Solely the purified GLD of document D86 is sequence-able after applying a further deglycosylation step (see pages 3 and 4, points 1.1.3, 1.2 and 2), while based on the facts on file, the GLD of document D5a is not sequence-able (see point 37.6 above), nor can it be deglycosylated (see patent, Example 6). In the absence of any indication that the purification protocol of document D5a is responsible for the failure, let alone any pointer to the specific conditions used in document D86, the skilled person has to try out a myriad of potential alternations which might or not lead to success.

39. Lastly appellant I argued that in case of failure the skilled person would have changed the liquid culture conditions disclosed in Example 1 of document D5a to overcome the sequencing problem, and would have used instead solid-culturing since this was the sole other culture form disclosed in document D5a (see paragraph [0038]).

40. The board does not agree. Firstly, indications are missing from document D5a that the culture conditions are responsible for the observed failure. Secondly, the solid culture conditions reported in paragraph [0038] of document D5a are very generic, while specific conditions for solid culturing are not disclosed. Thirdly, evidence is lacking from the file that any GLD obtained from a solid-cultured *A. terreus* is sequence-able irrespective of the condition used. In other words, evidence is missing that a sequence-able GLD is the necessary consequence of growing *A. terreus* on solid-culture in general. A mere reference to a general statement in the patent (see paragraph [0020]) is thus of no help. Nor is it of help that the patent discloses specific solid culture conditions (see paragraphs [0060], [0061] and Example 1) to obtain a sequence-able GLD, since document D5a is silent on these conditions. Thus a sequence-able GLD is no implicit disclosure of document D5a even if the GLD is obtained from solid-grown *A. terreus* as disclosed in paragraph [0038].

41. Consequently, the method of claim 1 is novel over the disclosure of document D5a.

42. Article 100(a) EPC in combination with Article 54 EPC does not therefore prejudice the maintenance of the patent as granted.

Inventive step

Closest prior art and technical problem to be solved

43. It is uncontested that document D5a represents the closest prior art.

44. The difference between the method of claim 1 and the method of document D5a is the use of a recombinantly produced GLD characterised by the lower total sugar content as defined in claim 1 compared to the GLD obtained from an *A. terreus* culture for the preparation of the biosensor.

45. The appellants argued that the claimed method and the method of document D5a differed in the provision of the nucleic acid/amino acid sequence of GLD. The technical problem to be solved was thus the provision of a sequence-able form of GLD. Furthermore, they submitted there was no evidence that all GLD variants falling within claim 1 resulted in the production of a sensor that benefited from a facilitated drying and improved reactivity. Even if the patent disclosed such an improvement then this was due to specific reaction conditions not specified in claim 1.

46. The board does not agree. The difference as set out above resides in the use of a GLD for preparing a biosensor which is defined by the presence of a maximum amount of certain sugar residues. This amount is several fold lower than that of GLD obtained from liquid-cultured *A. terreus* (see patent, page 21, Table 1). The patent discloses that the lower total sugar content *inter alia* facilitates the drying of GLD on the biosensor (see paragraphs [0011], [0015]). Sugar is a hygroscopic molecule. Therefore it is plausible that enzymes with an absolute lower sugar content dry easier on a surface compared to an enzyme with a higher sugar content. This effect is achieved across the whole breadth of the claim, since drying depends on the enzyme's total sugar amount which is the same for all GLD variants compared to the GLD of document D5a.

47. In light of the considerations above, the technical problem to be solved by the claimed method is the provision of an improved method for preparing a biosensor.

48. For the reasons set out above the board is satisfied that the method of claim 1 plausibly solves this problem.

Obviousness

49. It remains to be assessed whether or not the skilled person, starting from the method for preparing a glucose biosensor of document D5a and facing the problem defined above, would have arrived at the method of claim 1 in an obvious manner.

50. The case law has held that a course of action can be considered obvious within the meaning of Article 56 EPC if the skilled person would have carried it out in expectation of some improvement or advantage. This implies the ability of the skilled person to predict rationally, on the basis of the knowledge existing before a research project is started, the successful conclusion of this project within acceptable time limits. In other words a reasonable expectation of success follows from the scientific appraisal of available facts (see Case Law, I.D.7.1).

51. The appellants argued that the claimed method lacked an inventive step either in light of the teaching of document D5a alone or when combined with document D36a.

52. The board agrees with the appellants that the skilled person had a motivation to obtain GLD's sequence in view of document D5a because such a sequence is not

provided, while the sequence's use in transforming *E. coli* is explicitly suggested (see paragraph [0039]).

53. The question that arises is thus whether the skilled person would have a reasonable expectation of success when embarking on this task.

54. As set out above under novelty, the skilled person based on the facts on file would have failed to obtain the sequence of GLD if he/she followed the teaching of document D5a using routine methods.

55. The board also disagrees with the appellants' arguments that, in case of failure, it would have been obvious for the skilled person to change the purification protocol of Example 2 in document D5a, and by applying standard protein purification methods, the skilled person would have arrived at a sequence-able GLD as shown in document D86. As set out above under novelty, document D5a provides no pointers for the skilled person that the purification protocol of Example 2 might be responsible for the sequencing failure. On the contrary, document D5a discloses that the protocol of Example 2 provides enzymatically active GLD in sufficient amounts to prepare a biosensor (see Examples 3 to 6). Nor does document D5a provide pointers for using the amended protocol of document D86 for solving the problem.

56. In a further line of argument, the appellants submitted that in case of failure, the skilled person would have considered that GLD's glycosylation was the prime cause. That GLD was a glycoprotein was evident from the use of SDS gels which were run routinely during a protein purification to check the protein's purity. Moreover, numerous prior art studies disclosed that a

direct sequencing of glycosylated proteins was difficult (see document D37, page 341, right column, second paragraph, page 347, right column, last sentence). Glycosylated proteins had thus to be deglycosylated first by standard means (see document 43) before they could be sequenced.

56.1 The board does not agree. As a matter of routine the skilled person applying the protocol of Example 2 of document D5a might have run a SDS gel to check the purity of GLD before the sequencing, even if such a step is not mentioned in this document. By doing so, the skilled person would have recognised that GLD is a glycosylated protein because this creates a smear on SDS gels, while non-glycosylated proteins form sharp bands (see e.g. document D8, page 3798, Figure 4, lanes 3 and 4, and lanes 9 to 12 respectively). However, the mere use of SDS gels as a further purification step in Example 2 of document D5a does not result in a sequence-able GLD, as demonstrated by document D69 (see page 6, first and third paragraphs).

56.2 Does the finding that GLD is a glycoprotein suggest to the skilled person that glycosylation is most probably responsible for the sequencing failure? The skilled person knows that sequencing of glycosylated proteins "*can be difficult*" and that the "*Sequencing of proteins after chemical deglycosylation has been performed in numerous studies*" (see e.g. document D37, page 341, right column, first full paragraph, page 347, right column, last sentence). This disclosure implies that sequencing of glycoproteins might be difficult, but is possible. The skilled person knows likewise that glycosylated proteins can be directly sequenced by routine means (N-terminal sequencing and internal sequencing) without prior deglycosylation (see e.g.

document D8, page 3797, right column, second paragraph). In light of these teachings, the board concludes that the skilled person had no reason to assume that glycosylation represents the prime cause for GLD's sequencing failure.

56.3 Even assuming that the skilled person might have tried to deglycosylate GLD and then to sequence it, the following is relevant. The patent discloses that the skilled person would have failed in deglycosylating GLD (see Example 6). The appellants submitted that the deglycosylation approaches in the patent were designed to fail, while document D86 disclosed that GLD could be deglycosylated. Reference in this respect was made to documents D43, D61 and D88.

56.4 The board is not convinced by these arguments. Example 6 of the patent provides credible evidence of failure, while the appellants have not provided any evidence that the GLD obtained from the purification protocol of Example 2 of document D5a using the methods disclosed in documents D43, D61 and D88 can be deglycosylated at all. Reference to other methods (for example TFMS in document D61), or uncommon means (see document D88) remain mere speculative assertions in the absence of evidence that GLD purified according to the protocol of document D5a can be deglycosylated. It is established case law that each of the parties to the proceedings bears the burden of proof for the facts it alleges (see Case Law, I.C.3.5.1). The appellants referred as experimental evidence solely to document D86. However, as set out above under novelty, document D86 uses a substantially different purification protocol, and solely this GLD protein can be deglycosylated. Since, however, it cannot be excluded that the GLD purified according to document D86 differs significantly from

the GLD of document D5a, document D86 lacks probative value.

56.5 Therefore, as held by the opposition division (see decision under appeal, page 18, first paragraph), the skilled person in this situation knows, as already set out above under novelty, that the sequencing problems of GLD might be caused by several equally likely reasons. Document D5a is however silent on any pointers for the skilled person on which route he/she should embark to solve the sequencing problem with a reasonable expectation of success.

57. In a further line of argument, the appellants submitted that the skilled person in case of failure was aware that more protein was required for sequencing. The skilled person would have then switched to solid culturing of *A. terreus* which produced higher protein amounts (see document D36a). Moreover, GLD obtained from solid-cultured *A. terreus* was readily sequence-able as shown in the patent, i.e. sequencing problems did not even exist, and the skilled person would have automatically arrived at the method claimed. Alternatively, the skilled person would have started right-away with solid culturing to obtain GLD's sequence, since solid culturing was mentioned in document D5a (paragraph [0038]).

57.1 The board does not agree. Firstly, as set out in point 55 above, there are no indications derivable from document D5a that the GLD obtained from liquid-cultured Aspergillus is available in small amounts only. Nor does the sequencing of a protein normally requires industrial amounts of a protein as referred to in document D36a.

57.2 Secondly, there are no indications derivable from any of the documents on file that the yield of GLD obtained from solid-grown Aspergillus is higher compared to a liquid-grown Aspergillus. Nor is evidence thereof available. The appellants referred to document D36a. However, document D36a does not disclose that any enzyme, let alone GLD obtained from solid-grown Aspergillus is produced in higher amounts compared to liquid culturing. Page 3 of document D36a discloses that "*certain types of proteins, such as (...)enzymes for foods and digesting agents*" are produced by solid-state culturing, and that based on experience, "*enzymes effective for brewing processes are produced in large amounts*". Page 4, line 1 mentions "*glucoamylase*" and "*tyrosinase*" as specifically expressed proteins in solid culturing. GLD belongs to none of these enzyme classes. Based on these facts it is not convincing that the skilled person had any reasonable expectations that GLD would be produced in high quantities by a solid-cultured Aspergillus strain compared to liquid culturing.

57.3 Thirdly, document D36a discloses that "*in solid-state culture, separating a desired product, such as an enzyme, from cells or medium is difficult, and obtaining a high-purity product is very difficult*" (see page 2, second paragraph, emphasis added). Since for sequencing the use of pure proteins is of utmost importance to avoid wrong or confusing results, the skilled person faced with sequencing problems would rather avoid using GLD produced by a solid-grown Aspergillus.

57.4 Thus, although growing of Aspergillus on solid culture is mentioned in document D5a, the skilled person would not follow this route to overcome GLD's sequencing

problems. Let alone that there are indications implying that the skilled person would have obtained a sequenceable GLD at all from a solid-cultured Aspergillus. The specific conditions of solid culturing in the patent (paragraphs [0060], [0061] and Example 1) are not disclosed in document D5a, nor are indications available that the conditions disclosed in the patent can be generalised to any solid culturing.

58. In light of the considerations above, the board considers that the skilled person would not have arrived at the method of claim 1 in an obvious manner. Article 100(a) EPC in combination with Article 56 EPC does not therefore prejudice the maintenance of the patent as granted (main request).

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chair:

L. Malécot-Grob T. Sommerfeld

Decision electronically authenticated