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Datasheet for the decision of 27 July 2022

Case Number: T 1973/18 - 3.3.08

Application Number: 11741181.9

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Language of the proceedings: EN

Title of invention:
MODIFIED PEPTIDE DISPLAY

Patent Proprietor:
Miti Biosystems GmbH

Opponent:
LanthioPep B.V

Headword:
Modified peptide display/MITI BIOSYSTEMS

Relevant legal provisions:
EPC Art. 56
RPBA 2020 Art. 12(2)

Keyword:
"Main Request - requirements of the EPC met (yes)"

Decisions cited:

Catchword:

This datasheet is not part of the Decision
It can be changed at any time and without notice

Beschwerdekammern

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Case Number: T 1973/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 27 July 2022

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted on 25 May 2018 revoking European patent No. 2603586 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman B. Stolz
Members: D. Pilat
D. Rogers

Summary of Facts and Submissions

I. European patent No. 2 603 586 is based on European patent application No. 11 741 181.9, (published as WO 2012/019928 on the 16 February 2012). The patent, entitled "MODIFIED PEPTIDE DISPLAY", was opposed on the grounds of Article 100(a) in conjunction with Articles 54 and 56 EPC, and of Articles 100(b) and (c) EPC. An opposition division considered that the main request did not fulfil the requirements of Article 56 EPC, while the auxiliary request was not admitted because it prima facie failed to overcome the objection under Article 56 EPC. The patent was revoked.

II. The patentee (appellant) lodged an appeal against the decision of the opposition division. With its statement of grounds of appeal, appellant submitted a main request and a first to third auxiliary request. The main request is identical to the main request in opposition proceedings.

III. The respondent (Opponent) replied to appellant's statement of grounds of appeal.

IV. As a subsidiary measure, oral proceedings were requested by the parties.

V. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA 2020, the parties were informed of the board's provisional, non-binding opinion, especially on issues concerning Article 56 EPC.

VI. The respondent informed the board with its letter dated 5 July 2022 that it would not attend the scheduled oral proceedings.

VII. Oral proceedings before the board were held on 27 July 2022, in the absence of the respondent.

VIII. Claim 1 according to the main request reads as follows:

"1. Replicable genetic package displaying a peptide having at least one intramolecular cyclic bond between two atoms of amino acid side chains, wherein said cyclic bond is between C-N, C-0, C-S, N-N, N-0, N-S, 0-0, 0-S, wherein the replicable genetic package is not a bacterium."

Dependent claims 2 to 7 define particular embodiments of the replicable genetic package of claim 1.

Independent claim 8 relates to a method of preparing a replicable genetic package according to any of claims 1 to 7, and claims 9 to 11 to specific embodiments thereof. Claim 12 relates to a method of producing a library of replicable genetic packages, wherein the replicable genetic package is according to any of claims 1 to 7 and claim 13 relates to a library of replicable genetic packages obtainable by a method according to claim 12.

IX. The following documents are cited in this decision:

D14 PhD Thesis "Microbial production of thioether -stabilized peptides" Anneke Kuipers, University of Groningen, 10 September 2010.

X. The **appellant's** submissions, insofar as relevant to the present decision, may be summarized as follows:

Main request
Article 56 EPC

Document D14 described the production of (poly)cyclic polypeptides and suggested their presentation with phage display (see page 99, lines 8 to 25). It used gram-positive bacterial cells for the production and secretion of thioether-stabilized peptides, e.g. nisin, using the cellular nisin regulatory system and NisT for cellular transport. It investigated other bacterial secretion systems, like the Sec transport pathway for optimized secretion. It showed that dehydration of the peptides and cyclisation was not inhibited by N-terminal fusion of a foreign signal peptide to the nisin leader sequence and that N-terminal extensions to the leader sequence could be a promising tool.

The difference between claim 1 and document D14 was that the replicable genetic package displays (poly)cyclic peptides comprising at least one intramolecular cyclic bond between two heteroatoms of amino acid side chains, wherein said cyclic bond is between C-N, C-O, C-S, N-N, N-O, N-S, 0-0, and O-S, wherein the replicable genetic package is not a bacterium.

The technical problem was to establish the conditions under which a replicable genetic display package displaying a peptide with (poly)cyclic structures was enabled.

The solution was the non-bacterial replicable genetic display package displaying cyclic peptides of claim 1.

Document D14 described three options for generating (poly)cyclic peptides comprising at least one intramolecular cyclic bond between two heteroatoms of amino acid side chains. A first option consisting of a method of production of (poly)cyclic peptides using only an *in vivo* step; a second option consisting of a method using only an *in vitro* step and a third option consisting of a method using both an *in vivo* and an *in vitro* step.

Although it was not disputed that the proper formation of the (poly)cyclic polypeptides, the correct formation, folding, and/or secretion of the fusion proteins for display and the correct assembly and secretion of the replicable genetic display packages might be complex to achieve, there was no clear evidence why the skilled person had to select the second or third option specified above, without hindsight knowledge of the present invention, in the light of the technical disclosure provided in document D14.

The lantibiotics lacticin 481 and the two peptide lantibiotic haloduracin were both modified by incubation of the precursor peptide with the LanM enzymes *in vitro*. In addition, the dehydrated precursor of nisin was successfully cyclized by incubation with NisC *in vitro* (see page 16, second half of the last paragraph). There was however no indication in document D14 whether the precursor peptide fused to a pIII displayed on a phage would be processed in the same way by the LanM enzymes *in vitro*.

Document D14 disclosed the use of *Lactococcus lactis* (Gram positive bacteria) for producing thioether

stabilized peptides, whilst Gram-negative bacteria were commonly used for the production of peptides on the surface of filamentous phages in phage display, e.g. *E. coli*.

Given the differences between Gram-positive and Gram-negative bacteria, it could not be assumed that the *in vivo* enzymatic poly(cyclic) processing of the peptide in *Lactococcus lactis* would necessarily be the same as the processing of the peptide in E. coli. The different protein folding machinery, different membrane translocation systems, different types of cell membranes and different redox potentials found intracellularly and/or in the periplasm of Gram-positive and Gram-negative bacteria prevented the conclusion that the processed fusion protein displaying the peptide would be obtained regardless of the host cell used.

The *in vivo* dehydratase activity in document D14 was only measured in *Lactococcus lactis* strains. The post-translational enzymatic dehydration of precursor peptide via a dehydratase was only reported for prenisin or peptides having a similar length but not for more complex proteins, such as peptide fused pIII proteins. Moreover, it could not be excluded that an intermolecular rather than intramolecular reaction was preferred in the more oxidizing periplasm than in the cytoplasm. It was therefore impossible either to determine or to infer that the protein displaying the peptide would not also be dehydrated, cyclized and covalently conjugated in an undesired manner preventing thereby the proper production of phage display particles.

The skilled person would not have arrived at the solution claimed with a reasonable expectation of success by simply following the teaching of the prior art. Although the experimental results obtained in the prior art were useful for predicting future applications and for formulating hypotheses for developing new technologies, their implementation could not be regarded as obvious, just because they were mentioned as being an interesting area to explore.

Document D14 reported that in the Sec mediated transport of post-translationally dehydrated peptides in *Lactoccocus lactis*, the transport of the fully modified prenisin via the Sec pathway was impaired. On the other hand, the translocation of peptides with a single lanthionine via the Sec pathway might still be possible, as the results obtained with the therapeutic peptides could not establish if ring formation occurred intra- or extracellulary (see document D14, page 75 and paragraph bridging pages 75 and 76).

Document D14 demonstrated that an N-terminal fusion to the nisin leader of a signal peptide up to 44 amino acids long did not prevent dehydration by NisB and cyclization by NisC. This feature had a high potential. The N-terminal extensions to the leader peptide could therefore be a promising tool in the development of combining the thioether modification technology with phage display. With these united techniques huge libraries of thioether-constrained peptides could be made and screened for dedicated purposes (see page 99, first full paragraph). However, there were still many obstacles to overcome to realise the described theoretical project and arrive at the claimed solution.

XI. The **respondent's** submissions, insofar as relevant to the present decision, may be summarized as follows:

Main request
Article 56 EPC

Document D14 represented the closest prior art.

It disclosed how to generate lanthipeptides comprising a cyclic thioether bond (C-S) and that specific enzymes, such as NisB (dehydratase) and NisC (cyclase) or bifunctional enzymes, LtnM1 or LtnM2, were capable of modifying natural target peptides (e.g. Nisin, LtnA1 or LtnA2 respectively) as well as artificial nonlantibiotic peptides (e.g. angiotensin).
The *in vitro* activity of NisC (cyclization) was shown to be independent of the NisB and NisT enzymes of the multimeric lanthionine synthase complex NisBTC required for thioether bridge formation.
The Sec signal sequence at the N-terminal end of the peptide to be modified (e.g. thioether-bridged Azurin fragment) did not interfere with the post-translational modification and allowed its translocation via the Sec-pathway (see page 75, 2nd paragraph).
The transportation of unmodified and modified peptides (dehydrated and thioether-bridged) to which different signal sequences were added occurred via natural transportation routes or via the bacterial Sec pathway (see Chapters 2 to 7). The *in vivo* cyclization of an Azurin fragment and the *in vitro* cyclization of *the* precursor peptide of lacticin 481 and haloduracin with the LanM enzymes or of the dehydrated precursor of nisin incubated with NisC were successful (see page 16, last paragraph; page 22, 2nd paragraph, last sentence; page 59, 6th paragraph, line 5).

> "The use of the Sec-system provided a greater versatility to produce peptides with dehydroresidues followed by *in vitro* cyclization of the dehydroresidues to cysteines, either chemically or enzymatically. Taken together these findings underline the large potential to apply lantibiotic enzymes for the biotechnological production of modified peptides." (see, page 76, lines 1-5).

Even though lacticin 3147 enzymes LtnT and LtnM2 were used *in vivo* for the introduction of thioether bridges into non-natural peptide substrates, this system was more complex to examine than the NisBTC capable of modifying small thioether-bridged peptides and the more complicated substrate with the sequence ITPGCKATVECKITGPCKATVECK. The secretion of the studied peptides appeared to be the bottleneck. The *in vitro* introduction of thioether bridges in the peptides by lantibiotic enzymes could avoid this drawback (see page 97, 1st paragraph, lines 3 to 5).

The reconstitution of lacticin M *in vitro* for the synthesis of lacticin 481 was described. The enzyme LctM and the substrate LctA were both produced in *E. coli*. When the His-tagged substrate peptide LctA and the modifying enzyme LctM were incubated *in vitro* in the presence of adenosine triphosphate (ATP) and Mg^{2+}, dehydration and cyclization of the substrate occurred effectively. Though LctM does not display an evident ATP-binding domain, ATP was necessary for functionality of LctM. Also *in vitro* reconstitution of the two-component haloduracin has been successful. After incubation of the prepeptides HalAl and HalA2 with the modifying enzymes HalMl and HalM2, respectively, a bioactive haloduracin was formed. Concerning the modifying enzymes NisB and NisC, only NisC had

successfully been reconstituted *in vitro*. Incubation of dehydrated prenisin with NisC *in vitro*, resulted in bioactive nisin after removal of the nisin leader (see page 97, first full paragraph). With the powerful *in vitro* thioether modification system, the substrate specificity and mechanistic aspects of the LctM 481 enzyme were further explored (see page 97, second full paragraph, first sentence).

The Sec pathway enabling translocation and secretion of cyclic peptides was key for any kind of display, such as linear or cyclic constrained structure. The N-terminal peptide extension added to the peptide allowed the Sec-mediated translocation (see document D14).
It was also a basic requirement for phage display. Phages having the peptide of interest fused to the phage coat protein were assembled and generated in *E. coli* and the secretion of phage coat proteins e.g. pIII was mediated via the Sec pathway. The phage display vector usually had an N-terminal extension with a Sec signal sequence to direct the N-terminally displayed peptide fused to the pIII protein through the Sec pathway.
Since the Sec-mediated translocation of a peptide with an added N-terminal extension, even of thioether-bridged Azurin, was possible, the use of a phage display of peptides of interest displaying cyclic peptides was enabled.

Last but not least document D14 explicitly suggested the use of phage display technology (see page 99, second paragraph). It provided a literal pointer to the phage display for peptide presentation, as the N-terminal Sec signal did not negatively affect cyclization.

The skilled person using the *in vivo* or *in vitro* approach described in document D14 would therefore have arrived at a replicable package displaying a thioether-bridged peptide according to claim 1 with a reasonable expectation of success.

The difference between the subject matter of claim 1 and document D14 could be seen in that the latter did "not" sufficiently disclose the display and presentation of a cyclic peptide on the surface of a non-bacterial replicable genetic package.

In view of this difference, the objective technical problem was regarded as the provision of a non-bacterial replicable genetic package displaying a cyclic peptide.

The solution to this problem as defined in claim 1 was obvious to the skilled person.

The skilled person would have applied, based on his common general knowledge represented by D1 or D17, the teaching of document D14 to the phage display system by incubating *in vitro* the phage display with an enzyme.

Article 83 EPC

The claimed subject matter was not disclosed in a manner sufficiently clear and complete to be carried out by a person skilled in the art. It contravened Article 83 EPC. The arguments made in opponent's submissions during the opposition proceedings were maintained.

XII. The appellant (patentee) requested that the decision under appeal be set aside and the patent be maintained

on the basis of the main request or alternatively on the basis of any of the first to third auxiliary requests submitted with its statement of grounds of appeal.

XIII. The respondent (Opponent) requested that the appeal be dismissed and that auxiliary requests 1 to 3 not be admitted into the proceedings.

Reasons for the Decision

Article 113(1) EPC

1. In its communication to the parties in preparation for the oral proceedings, the board expressed a provisionally negative view on inventive step of the main request.

2. By its decision not to attend the oral proceedings and not to file substantive arguments in reply to the issues raised in the board's communication, the respondent has chosen not to make use of the opportunity to comment on the board's provisional opinion, either in writing or at the oral proceedings. According to Article 15(3) RPBA 2020, the board is not obliged to delay any step in the proceedings, including its decision, by reason only of the absence at the oral proceedings of any party duly summoned who may then be treated as relying on its written case.

   This decision is based on the same grounds, lines of argument and evidence on which the provisional opinion of the board was based.

Article 56 EPC

3. It was common ground between the parties that document D14 represents the closest prior art for the subject-matter of claim 1.

3.1 The decision under appeal stressed that document D14 related to the cyclization of peptides, namely thioether bridged peptides, to the transport of said modified peptides via the sec pathway and the possibility of an *in vitro* cyclization, which is carried out outside the cell (see page 75, last sentence to page 76 and page 97, first paragraph). The use of a phage display system combining the thioether modifications of the peptides with a subsequent screening step was also suggested (see page 99, second paragraph). All the technical features of the claimed subject-matter were provided in document D14 but the replicable genetic package displaying a peptide combining all the technical features of claim 1 was not exemplified.

3.1.1 The technical problem underlying the subject-matter of claim 1 was to provide a phage capable of displaying thioether modified peptides by putting into practice the teaching of document D14.

3.1.2 The skilled person had all the necessary technical knowledge required to produce such a phage display.

3.2 The board considers that document D14 describes in detail, how to express and generate lantibiotic and nonlantibiotic peptides containing an intracellular thioether bond (C-S). The system for the biosynthesis of lantibiotics is flexible. The enzymes are capable of modifying and producing peptides that are entirely different in size and sequence from their native substrates (see page 16, last paragraph and page 17,

first paragraph). Although new lantibiotics with increased bioactivity can be lethal to the producer cell, this problem was overcome by using a production system free of NisP, which cleaves the leader peptide. The use of an *in vitro* modification system was another solution. The lantibiotics lacticin 481 and the two peptide lantibiotic haloduracin were both modified by incubation of the precursor peptide with the LanM enzymes *in vitro*. In addition, the dehydrated precursor of nisin was successfully cyclized by incubation with NisC *in vitro*.

3.2.1 Chapter 2 of document D14 shows that NisT, the transporter of the lantibiotic nisin, was capable of transporting fully modified, dehydrated and unmodified prenisin and fusions of the leader peptide with nonlantibiotic peptides (see Table 2).
Chapter 3 shows that NisB, which is the dehydratase of Nisin, is capable of modifying peptides which are not naturally-occurring nisin, but artificial non-lantibiotic peptides (see Table 2).
Chapter 4 shows that enzymes capable of modifying Lacticin 3147 in nature (the LtnTM2 part of the lacticin 3147 synthetase) were also capable of modifying other peptides, such as angiotensin.
Chapters 5 and 6 show that the Sec pathway is an alternative secretion route for post-translationally modified peptides, e.g. dehydrated peptides and even a thioether bridged peptide fragment of azurin.
The insertion of an N-terminal extension including a Sec signal does not interfere with the post-translational modification. The NisC required for thioether bridge formation and the cyclization works *in vitro* and independently of the enzymatic machinery NisB and NisT (see page 75, 2nd paragraph). The Sec system having a broad substrate range can be used for the

export of peptides with dehydrated amino acids, although completely modified prenisin with its multiple thioether rings appears not to be tolerated. The use of the Sec-system provides a greater versatility to produce peptides with dehydroresidues followed by *in vitro* cyclization of the dehydroresidues to cysteines, either chemically or enzymatically (see pages 75 to 76, bridging paragraph).
When the His-tagged substrate peptide LctA and the modifying enzyme LctM are incubated *in vitro* in the presence of adenosine triphosphate (ATP) and Mg^{2+}, dehydration and cyclization of the substrate occurs effectively. Incubation of dehydrated prenisin with NisC *in vitro*, results in bioactive nisin after removal of the nisin leader (see page 97, 1st paragraph).
The N-terminal fusion to the nisin leader of a signal peptide up to 44 amino acids long did not prevent dehydration by NisB and cyclization by NisC. The N-terminal extensions to the leader peptide can be a promising tool in the development of combining the thioether modification technology with phage display. With these united techniques huge libraries of thioether-constrained peptides can be made and screened for dedicated purposes (see page 99, first paragraph).

3.3 The difference between claim 1 and the teaching of document D14 consists of a replicable genetic package displaying the cyclic peptide which is not a bacterium.

3.4 The objective problem to be solved starting from document D14 may be regarded as the provision of a non-bacterial replicable genetic package displaying cyclic peptides.

3.5 The board has no doubt that this problem is solved by applying the method described in example 1.

3.6 The solution to the problem is the replicable genetic package of claim 1.

3.7 Although document D14 suggests the use of alternative display systems such as phage display, the crucial question for the board is whether a skilled person would and not only could, given the technical problem described above, consider alternative display systems including phage display to arrive at the replicable genetic display packages defined in claim 1.

3.8 The respondent contended that document D14 explicitly suggested the use of phage display technology. It provided a literal pointer to phage display for peptide presentation by showing that an N-terminal Sec signal peptide did not negatively affect cyclization (see page 99, second paragraph). The Sec-system was a basic requirement for phage display. The phages were generated in *E. coli* and were constituted of peptide fused to phage coat protein whose secretion was commonly mediated via the Sec pathway. The phage display vector used for expressing the phage's recombinant fusion protein pIII typically comprised an N-terminal extension with a Sec signal sequence to direct its translocation through the Sec pathway. Since the translocation of the N-terminal Sec-signal peptide extended non-lantibiotic peptides did not interfere with the activity of post-translation modification enzymes and was used in phage display systems, the Sec translocation system to direct the phage's pIII protein together with the N-terminally displayed peptide in phage display was enabled.

Obviousness

3.9 Although it is not disputed that the proper formation of the (poly)cyclic polypeptides, the correct formation, folding, and/or secretion of the fusion proteins for display and the correct assembly and secretion of the replicable genetic display packages may be complex to achieve, it needs to be assessed whether this would still be the case in the light of the technical disclosure provided in document D14.

3.10 The board agrees with the appellant that document D14 identifies three options for generating (poly)cyclic peptides comprising at least one intramolecular cyclic bond between two heteroatoms of amino acid side chains. A first option consisting of a method of production of (poly)cyclic peptides using only an *in vivo* step; a second option consisting of a method using only an *in vitro* step and a third option consisting of a method using both an *in vivo* and an *in vitro* step.

First option: a method of production of (poly)cyclic peptides using only an in vivo step.

3.11 Document D14 discloses the *in vivo* post-translational modification of Lacticin 3147 and of other unrelated peptides such as angiotensin variants (1-7) (see document D14 chapter 4). A thioether-bridged Azurin is translocated via the Sec pathway in *L. lactis*.

3.12 Although document D14 mentioned that the use of a method for generating (poly)cyclic peptides comprising at least one intramolecular cyclic bond between two heteroatoms of amino acid side chains comprising an *in vivo* step in *Lactococcus lactis* is possible, the Sec pathway could only successfully transport linear dehydrated peptides and an azurin peptide fragment with a small methyllanthionine. The translocation of an *in*

vivo produced prenisin with its multiple thioether rings appeared not to be tolerated. It was estimated that the size of the Sec Y pore in *L. lactis* is too small for translocation of prenisin (see document D14, paragraph bridging pages 75 and 76; page 99, first full paragraph).

3.12.1 Even if document D14 reports that in *E. coli* the translocon Sec YEG could transport the polypeptide proOmpA with disulfides or labeled with a bulky fluorescent probe, it is also explicitly stated that the improved transport of bulky peptides via the Sec pathway in *L. lactis* will possibly be achieved by producing Sec Y mutants in the future. Thus, the Sec Y protein in *L. lactis* must be first adequately mutated to overcome the size limitation currently imposed on the translocated protein.

3.12.2 The board considers that the skilled person, in view of the content of document D14, faced with the technical problem identified above, would have refrained from using a method for generating (poly)cyclic peptides comprising only an *in vivo* step for producing a larger and bulkier thioether-bridged fusion protein displayed on a replicable genetic package.

Second option: a method using only an in vitro step.

3.13 According to the respondent, the skilled person, faced with the technical problem identified above, was motivated to use a method for generating (poly)cyclic peptides comprising at least one intramolecular cyclic bond between two heteroatoms of amino acid side chains comprising only an *in vitro step*.

3.13.1 Indeed, the *in vitro* introduction of thioether bridges in peptides with lantibiotic enzymes could avoid processing/translocation difficulties.

3.13.2 The respondent highlighted that "The lantibiotics lacticin 481 and the two peptide lantibiotic haloduracin were both modified successfully by incubation of the precursor peptide with the LanM enzymes *in vitro* (121, 226). In addition, the dehydrated precursor of nisin was successfully cyclized by incubation with NisC *in vitro* (106). Overall, the biosynthetic system used for the biosynthesis of lantibiotics seems to have a remarkable flexibility." (see document D14, page 16, last paragraph). Concerning the modifying enzymes NisB and NisC, only NisC had successfully been reconstituted *in vitro*. The enzymatic machinery required for thioether bridge formation and the cyclization via NisC was independent and capable of acting *in vitro* (see page 16, last paragraph; page 22, second paragraph, last line; page 59, last paragraph; page 75, second paragraph; page 97, line 3 to end of first full paragraph).

3.14 The board observes that the thioether bridged peptides disclosed in document D14 were all produced in *L. lactis* cells and transported and secreted via the *L. lactis* bacterial export machinery. Although it mentions that the *in vitro* introduction of thioether bridges in peptides with lantibiotic enzymes could avoid processing/translocation difficulties, it neither discloses nor suggests that a peptide fused to the protein III displayed on a phage particle expressed in *E. coli* could be modified the same way by LanM enzymes *in vitro* with a reasonable expectation of success.

3.15 Even if the post-translationally modified peptides can be transported via an endogenous Sec pathway in *L. lactis*, the transportation by the Sec system requires the presence of a N-terminal Sec signal peptide, which, when fused to the N-terminal end of the nisin leader, does neither prevent the dehydration by NisB nor the cyclization by the NisC of the peptide. As stated on page 99, lines 8 to 10, of document D14, "This feature has a high potential. For instance, N-terminal extensions to the leader peptide can be a promising tool in the development of combining the thioether modification technology with phage display."

3.15.1 The board considers the non-destructive effect of the N-terminal extensions, i.e. Sec signal peptide located upstream of the nisin leader, on the subsequent post-translational modification of the peptide to be the promising tool in the development of the combination of the thioether modification technology with the phage display. Albeit a promising one, this fact remains only a tool for use in the attempt to combine the thioether modification technology with the phage display technology.

3.15.2 From the disclosure of document D14 it emerges that the proper formation of the (poly)cyclic (poly)peptides, the correct formation, folding, and/or secretion of both short lantiopeptides and unrelated peptides is difficult to achieve in *L. lactis*. Hence, it can only be assumed that the above post-translational processes must prove all the more difficult for a much longer thioether modified fusion protein when expressed in a different type of bacterial cell.

Another and further layer of difficulty lies in the proper incorporation and assembly of the secreted

thioether modified fusion protein into the replicable genetic display packages.

3.15.3 The single sentence identified above referring to phage display is therefore far from providing the skilled person with a reasonable expectation of success in obtaining a recombinant phage capable of presenting on its surface a thioether modified peptide.

Third option: a method of production of (poly)cyclic peptides using both an in vivo and in vitro step.

3.16 The Sec-mediated transport of post-translationally dehydrated peptides in *L. lactis* such as prenisin was disclosed and could be exploited for the secretion of dehydrated variants of therapeutic peptides. The use of the Sec-system provided a greater versatility to produce peptides with dehydroresidues followed by *in vitro* cyclization of the dehydroresidues to cysteines, either chemically or enzymatically (see chapter 5 and paragraph bridging pages 75 and 76).

3.17 The board finds that document D14 discloses only *Lactococcus lactis* (Gram positive bacteria) for producing thioether stabilized peptides, whilst Gram-negative bacteria are used in phage display, e.g. *E. coli*. The phages having the peptide of interest fused to the phage coat protein are commonly assembled and generated in *E. coli*.

3.17.1 Given the differences between Gram-positive and Gram-negative bacteria, the board cannot assume that the *in vivo* enzymatic poly(cyclic) processing of the peptide in *Lactococcus lactis* is necessarily the same as the processing of the peptide in *E. coli*. The different protein folding machinery, different membrane

translocation systems -even if the Sec-translocation system is homologous-, different types of cell membranes and different redox potentials occurring intracellularly and/or in the periplasm of Gram-positive and Gram-negative bacteria do not allow the conclusion that the processed fusion protein displaying the peptide will first be obtained, and secondly, be present as a poly(cyclic) peptide in the host cell used.

3.17.2 The use of the same or homologous Sec translocation pathway system for (poly)peptides having different lengths and/or different three dimensional structures - covalently constrained and/or relaxed structures- across different cell membrane(s) does not allow to conclude that these peptides or proteins are actually and effectively transported, let alone to a similar degree.

3.17.3 The board observes that in document D14, post-translational enzymatic dehydration of precursor peptide via a dehydratase is only reported for prenisin or peptides having a similar length in *Lactococcus lactis* strains not in *E. coli,* and not for more complex proteins, such as peptides fused to pIII proteins.

3.17.4 Since the bacterial cell type to be used for the phage display is not the one used in document D14, the skilled person cannot determine and infer whether the protein displaying the peptide will not also be dehydrated, cyclized and covalently conjugated in an undesired manner, thereby preventing the proper production of phage display particles. Moreover, the skilled person cannot exclude that some undesired intermolecular rather than intramolecular reaction will preferably take place in the more oxidizing periplasm,

compared to the cytoplasm, before the fusion protein displaying the peptide reaches the extracellular space.

Thus, even if it was obvious for the skilled person to try to implement the proposed theoretical hypotheses, this does not necessarily mean that there was a "reasonable expectation of success" in arriving at the solution. A reasonable expectation of success should not to be confused with the understandable "hope to succeed".

3.18 The board considers that starting with document D14, the skilled person faced with the technical problem of providing means and methods for the selection and identification of (poly)cyclic polypeptides, despite being explicitly taught that an N-terminal fusion to the leader peptide can be a promising tool in the development of combining the thioether modification technology with phage display, must nevertheless be able to rationally predict, on the basis of the knowledge existing before the research project is started, the successful conclusion of said project within acceptable time limits.

3.18.1 The greater the differences between the assumptions made, and the experiments actually performed, in the prior art, the more difficult it is for the skilled person to make reasonable predictions about the outcome of each individual experimental step in a long series of experimental steps that need to be performed to achieve the desired end result. Without any guidance given in document D14, the skilled person appreciates that the achievement of the theoretical project (solution), proposed in document D14, depends not only on the success of the individual experimental steps performed, but also on their sequence and on his or her

own ability to take the correct decisions for each and every difficulty/choice encountered.

3.19 In the board's view, given the numerous potential technical problems highlighted above, the skilled person would have had no reasonable expectation of success in arriving at the replicable genetic packages defined in claim 1 by simply altering and combining the post translational modification system with the display system.

The above conclusion applies mutatis mutandis to the method of preparing a replicable genetic package according to claim 8, to the method of producing a library of replicable genetic packages according to claim 12 and to the library of of replicable genetic packages according to claim 13 obtainable by a method of claim 12.

3.20 The main request therefore fulfils the requirements of Article 56 EPC.

Article 12(2) RPBA 2007

4. Concerning its objections under Article 83 EPC, the opponent merely stated: "We maintain our opinion that the subject matter of the Contested Patent is not disclosed in a manner sufficiently clear and complete to be carried out by a person skilled in the art and thus contravenes Article 83 EPC. We therefore refer to our submissions made during the opposition proceedings and maintain our arguments." (see page 8 last paragraph of the statement of grounds of appeal).

4.1 According to Article 12(2) RPBA 2007, the statement of grounds of appeal shall contain the party's complete

case. This requirement is not fulfilled by a mere passing reference to facts and evidence put forward in opposition proceedings. It is not for the board to identify issues which arose in opposition proceedings and may (or may not) still be a matter of dispute in appeal proceedings, but for the appellant to put forward in the statement of grounds of appeal its line(s) of argument and each of the facts and evidence on which it relies.

4.2 Since the objection under Article 83 EPC was not substantiated in the appeal proceedings, neither in the statement of grounds of appeal nor at a later stage, and the appeal procedure is a judicial procedure that is less investigative than the opposition procedure (G 7/91 published OJ 1993, 356, G 8/91 published OJ 1993, 346), the unsubstantiated objection of the respondent needs no further consideration.

Order

For these reasons it is decided that:

5. The decision under appeal is set aside.

6. The case is remitted to the opposition division with the order to maintain the patent with the following claims and a description to be adapted:

Claim(s):
No. 1 to 13 of the main request filed under cover of a letter dated 3 October 2018.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated