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Datasheet for the decision of 3 April 2017

Case Number: T 0727/10 - 3.3.02

Application Number: 03744485.8

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Title of invention:
HYBRID AND SINGLE CHAIN MEGANUCLEASES AND USE THEREOF

Patent Proprietor:
CELLECTIS

Headword:
Meganucleases/CELLECTIS

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G 0009/92, T 0629/90, T 0789/89

Catchword:

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Beschwerdekammern
Boards of Appeal
Chambres de recours

European Patent Office
D-80298 MUNICH
GERMANY
Tel. +49 (0) 89 2399-0
Fax +49 (0) 89 2399-4465

Case Number: T 0727/10 - 3.3.02

D E C I S I O N
of Technical Board of Appeal 3.3.02
of 3 April 2017

Appellant: (Patent Proprietor)	CELLECTIS 102, route de Noisy 93230 Romainville (FR)
Representative:	Espinasse, Sylvain Cellectis 8, rue de la Croix Jarry 75013 Paris (FR)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 8 February 2010 concerning maintenance of the European Patent No. 1485475 in amended form.**

Composition of the Board:

Chairman	A. Lindner
Members:	T. Sommerfeld
	L. Bühler

Summary of Facts and Submissions

I. European patent No. 1485475, based on European patent application No. 03744485.8, which was filed as an international patent application published as WO 03/078619, was granted with 36 claims.

II. An opposition was filed against the granted patent by Precision BioSciences, Inc. (opponent) requesting revocation of the patent in its entirety on the grounds of lack of inventive step (Articles 56 EPC and 100(a) EPC), insufficiency of disclosure (Article 100(b) EPC) and added subject-matter (Article 100(c) EPC).

III. By an interlocutory decision announced at oral proceedings, the opposition division decided to maintain the patent in amended form on the basis of the third auxiliary request filed during the oral proceedings (Articles 101(3)(a) and 106(2) EPC).

The opposition division considered that the main request (claims as granted) did not comply with Article 123(2) EPC, that the first auxiliary request complied with Article 83 EPC but not with Article 56 EPC, and that the second auxiliary request contravened both Articles 123(2) and 84 EPC.

IV. Both the patent proprietor and the opponent lodged an appeal against that decision.

With the statement of grounds of appeal, the patent proprietor requested that the patent be maintained according to the main request or, alternatively, according to one of auxiliary requests 1 to 7, all

filed with the grounds of appeal. A new document, D51, was submitted.

With the statement of grounds of appeal, the opponent requested that the decision be set aside and the patent revoked in its entirety.

V. Both parties filed a reply to each other's grounds of appeal. With its response, the patent proprietor filed a new auxiliary request 7, the previous auxiliary request 7 becoming auxiliary request 8.

VI. With its letter dated 10 January 2017, the opponent withdrew both the opposition and the appeal.

VII. Oral proceedings before the board took place on 3 April 2017. During the oral proceedings, the appellant submitted new auxiliary requests 6, 7 and 8, and renumbered the previous auxiliary requests 6 and 7 as auxiliary requests 9 and 10. At the end of the oral proceedings, the chairman announced the board's decision.

The **main request** corresponds to the claims as granted, except that granted claim 32 has been deleted. Independent claims 1 and 22 of this request read as follows:

"1. A hybrid meganuclease comprising a first domain and a second domain in the orientation N-terminal toward C-terminal, said first and second domains being derived from two different initial dodecapeptide meganucleases, each domain being a polypeptide fragment comprising or consisting of a dodecapeptide motif and a DNA binding moiety, and wherein said hybrid meganuclease is capable of causing DNA cleavage."

"22. A single-chain meganuclease comprising a first and a second domain in the orientation N-terminal toward C-terminal, wherein said first and second domains are derived from the same mono-dodecapeptide meganuclease, each domain being a polypeptide fragment comprising or consisting of a dodecapeptide motif and a DNA binding moiety, and wherein said single-chain meganuclease is capable of causing DNA cleavage."

A claim identical to claim 22 of the main request is present also in **auxiliary request 1** (as claim 22) and in **auxiliary request 2** (as claim 10).

Claim 10 of **auxiliary request 3** differs from claim 22 of the main request by the following amendments (deletions struck through and insertions underlined):

"~~22~~10. A single-chain meganuclease comprising ~~a first and a second domain in the orientation N-terminal toward C-terminal, wherein said first and second domains are derived from the same mono-dodecapeptide meganuclease, each domain being a polypeptide fragment comprising or consisting of a dodecapeptide motif and a DNA binding moiety,~~ two sub-units from the same mono-dodecapeptide meganuclease, said sub-units being bound by a convenient linker and wherein said single-chain meganuclease is capable of causing DNA cleavage."

Claim 10 of **auxiliary request 4** differs from claim 22 of the main request by the following amendments:

"~~22~~10. A single-chain meganuclease comprising ~~a first and a second domain in the orientation N-terminal toward C-terminal, wherein said first and second domains are derived from the same mono-dodecapeptide~~

~~meganuclease, each domain being a polypeptide fragment comprising or consisting of a dodecapeptide motif and a DNA binding moiety,~~ two monomers of a mono-dodecapeptide meganuclease, said monomers being modified such as to introduce a covalent link between the monomers and wherein said single-chain meganuclease is capable of causing DNA cleavage."

Claim 10 of **auxiliary request 5** differs from claim 10 of auxiliary request 4 by the insertion of the feature "... said covalent link being introduced by creating a peptide bond between the two monomers ...".

Claim 22 of the main request has been amended by insertion of the features of dependent claim 23 in **auxiliary request 6**, while it has been deleted in **auxiliary requests 7 and 8**. Claim 1 of all these requests was identical to claim 1 of the main request.

In **auxiliary request 9**, claim 1 differs from claim 1 of the main request in that the hybrid meganuclease is to be selected from the group consisting of:

"- a hybrid meganuclease wherein said first domain is derived from the N-terminal domain of a first di-dodecapeptide meganuclease and said second domain is derived from the N-terminal domain of a second di-dodecapeptide meganuclease;
- a hybrid meganuclease wherein said first domain is derived from the N-terminal domain of a first di-dodecapeptide meganuclease and said second domain is derived from the C-terminal domain of a second di-dodecapeptide meganuclease; and
- a hybrid meganuclease wherein said first domain is derived from the N-terminal domain of a

di-dodecapeptide meganuclease and said second domain is derived from a mono-dodecapeptide meganuclease, wherein said first and second domains are bound by a convenient linker."

In **auxiliary request 10**, claim 1 differs from claim 1 of the main request as follows:

"1. A hybrid meganuclease comprising a first domain and a second domain in the orientation N-terminal toward C-terminal, said first domain is derived from the N-terminal domain of a di-dodecapeptide meganuclease and said second domain is derived from a mono-dodecapeptide meganuclease, said first and second domains being bound by a convenient linker, each domain being a polypeptide fragment comprising or consisting of a dodecapeptide motif and a DNA binding moiety, and wherein said hybrid meganuclease is capable of causing DNA cleavage."

VIII. The documents cited during the proceedings before the opposition division and the board of appeal include the following:

D5 Chevalier et al., Nat.Struct.Biol.2001,8,312-316
D8 Silva et al., J.Mol.Biol.,1999,286,1123-1136
D31 Epinat et al., Nucleic Acids Research,2003,31, 2952-2962
D32 Chevalier and Stoddard, Nucleic Acids Research, 2001,29,3757-3774
D51 Liang et al., PNAS USA,1993,90,7010-7014

IX. The appellant's submissions, in so far as relevant to the present decision, may be summarised as follows:

Regarding claim 22 of the main request, the problem to be solved was to expand the biodiversity of homodimeric

meganucleases, and the solution involved, as a first step, the creation of single-chain meganucleases (e.g. I-CreI), which had the advantages listed in the patent at page 21, lines 38 to 41. Starting from D5, which described the structure of I-CreI, the technical problem was to obtain meganucleases which were easier to manipulate. Once the single-chain molecules were available, one could introduce mutations, swap domains, etc.; this was also included in the claim, due to the term "derived". D5 in fact taught away from creating single-chain molecules because it made clear that I-CreI's structure was very complex, rendering it unpredictable how changes in the structure could affect or impair the activity; e.g. page 314, right column, first paragraph. The skilled person would not consider that the homodimeric meganucleases could be made to work like monomeric meganucleases since these two classes were very different. The statement on the last page of D51 was general knowledge but valid only for "a significant portion of proteins", and it was not clear for what kind of proteins: certainly not for meganucleases, which were not mentioned at all in D51. As regarded the corresponding claim in the auxiliary requests, a covalent linker (claim 10 of auxiliary requests 3 to 5) was commonly used when producing fusion proteins; in auxiliary request 5, the type of linkers used were specified.

In relation to claim 1 of auxiliary request 6, D32 did not provide any incentive to combine domains from two different dodecapeptides. In fact, D32 taught away from it by pointing out that the LAGLIDADG family was phylogenetically diverse (page 3759, bottom of left column; Figure 2 on page 3760, making apparent that the structure was very different from other endonucleases). There was a low sequence identity between different

meganucleases (page 3764, left column, penultimate paragraph), with a high divergence in both identity and position of active site residues (page 3767, left column, penultimate paragraph) and mutation of even only one residue might abolish activity (page 3767, right column, last paragraph). Too many possible architectures meant that any changes were likely to reduce or inhibit cleavage reactions. D32 did not suggest rearranging and combining domains but rather introducing random mutations into the domains and generating combinatorial libraries (page 3770, right column, penultimate paragraph). In fact, D32 did not disclose any means to screen or select new meganucleases with new specificities. In relation to the statement in D31, first paragraph of the Discussion (page 2958), meganucleases were very particular and, in the absence of a screening method, the suggestion to swap domains was not possible: the methods to swap protein domains were known but the global strategic idea of the patent, involving the creation of new meganucleases to use as starting tools, was not. As regarded claim 1 of auxiliary requests 9 and 10, specific selections were made to overcome the objection that the problem had not been solved; moreover, said selections were not suggested at all in D32.

X. The arguments of the former opponent, submitted in writing during appeal and in so far as relevant for the present decision, may be summarised as follows:

D32 was considered the closest prior art. It disclosed the structural similarities of various LAGLIDADG or dodecapeptide meganucleases, including the αββαββα fold shared by mono-dodecapeptide meganucleases such as I-CreI and di-dodecapeptide meganucleases such as I-DmoI (page 3759, right column, third paragraph). D32

further noted that even the di-dodecapeptide meganuclease monomers had a "pseudo-dimeric structure" like the mono-dodecapeptide dimers (page 3764, left column, third paragraph). It also stated that the endonucleases of the LAGLIDADG family should be used in engineering enzymes with novel DNA-binding properties (page 3770, right column, third paragraph, last two sentences). Starting from D32, there were two alternative technical problems to be solved: (a) the provision of new hybrid meganucleases with altered cleavage specificity derived from combinations of the various mono- or di-dodecapeptide (LAGLIDADG) meganucleases and (b) the provision of new single-chain meganucleases derived from the various mono-dodecapeptide meganucleases. D8 provided detailed information regarding the structural similarities of the I-DmoI and I-CreI meganucleases, including figure 6 showing the superposition of their "ribbon" structures. Based on this teaching, in combination with D32, it was obvious to produce either (a) a hybrid meganuclease derived from one domain of I-DmoI and one I-CreI monomer, or (b) a single-chain I-CreI meganuclease derived from the monomers of I-CreI and the interdomain "linker" of I-DmoI, simply by swapping or combining the domains at or near the positions that were superposed in Figure 6(a) of D8. D5 too disclosed the crystal structure of I-CreI bound to DNA and discussed the likely catalytic site structure and mechanism of action, and the structural and catalytic similarities amongst the LAGLIDADG meganucleases.

XI. The appellant (patent proprietor) requested that the decision under appeal be set aside and that the patent be maintained according to the main request filed with the statement of grounds of appeal, or, alternatively, according to one of the following requests:

- auxiliary requests 1 to 5, all filed with the statement of grounds of appeal, or
- auxiliary requests 6 to 8, filed during the oral proceedings of 3 April 2017, or
- auxiliary request 9 filed as auxiliary request 6 with the statement of grounds of appeal, or
- auxiliary request 10, filed as auxiliary request 7 with letter dated 28 December 2010.

Reasons for the Decision

1. The appeal is admissible.

2. Withdrawal of the appeal and of the opposition by the opponent

2.1 During appeal, the sole opponent withdrew both its opposition and its appeal. Hence the patent proprietor became the sole appellant and sole remaining party to the proceedings.

2.1.1 According to decision G 9/92 of the Enlarged Board of Appeal (OJ 1994, 875), if the patent proprietor is the sole appellant against an interlocutory decision maintaining a patent in amended form, the maintenance of the patent as amended in accordance with the interlocutory decision may not be challenged at appeal proceedings (prohibition of *reformatio in peius*).

2.1.2 As regards the withdrawal of the opposition by the opponent who is not the sole appellant, this does not affect the appeal proceedings, in so far as it is the principal task of the boards of appeal to review the decision under appeal on the basis of the appellant's requests. However, the withdrawal of an opposition by

the respondent means that the respondent ceases to be party to the appeal proceedings in respect of the substantive issues (T 789/89, OJ 1994, 482). The board may nevertheless take into account the submissions and evidence filed by the former opponent before the opposition was withdrawn (T 629/90, OJ 1992, 654).

3. Inventive step

3.1 The present patent is related to meganucleases, i.e. endonucleases which have recognition sequences, usually asymmetric, that span 12 to 40 base pairs (bp) of DNA in contrast to "classical" restriction enzymes which recognise much shorter stretches of DNA, in the 3-8 bp range, mostly with dyad symmetry. Due to the size of their recognition site, meganucleases are rare-cutting endonucleases, i.e. they have a very low recognition and cleavage frequency even in large genomes. Meganucleases fall into four distinct families on the basis of well conserved amino acid motifs. The largest of these families is the dodecapeptide family, also called LAGLIDADG, its members being defined by having one or two copies of the conserved dodecapeptide LAGLIDADG motif. Meganucleases with one dodecapeptide (D; mono-dodecapeptide) act as homodimers, while the vast majority have two dodecapeptides (DD; di-dodecapeptide) and act as monomers.

The fact that meganucleases are rare-cutting endonucleases makes them particularly interesting for use in molecular biology and genetic engineering methods (paragraphs [0002] to [0006] of the patent). The aim of the patent is thus to provide "new rare-cutting endonucleases with new sequence specificity for the recognition and cleavage" (paragraph [0009]).

3.2 Main request

3.2.1 Claim 22 of the main request is directed to a "single-chain meganuclease" which comprises two domains from the same mono-dodecapeptide meganuclease, each domain being a polypeptide fragment comprising or consisting of a dodecapeptide motif and a DNA binding moiety. According to the patent (paragraph [0129]), single-chain meganucleases have advantages over the corresponding homodimers in that they are easier to manipulate, are thermodynamically favoured, e.g. for the recognition of the target sequence, and allow the oligodimerisation to be controlled. As acknowledged by the appellant, the claimed single-chain meganucleases do not necessarily recognise new substrates in relation to the corresponding homodimers.

3.2.2 Document D5, which discusses the structure of I-CreI as one exemplary dimeric (i.e. mono-dodecapeptide) homing endonuclease (another designation for meganuclease), is the closest prior art. The difference to the claimed subject-matter is that D5 does not disclose a single-chain meganuclease as claimed, and the technical problem can hence be formulated as the provision of a meganuclease with improved properties such as those disclosed in the patent and listed above. Although the patent does not provide any comparative data allowing to conclude that the single-chain meganuclease as claimed does in fact present the alleged advantages over the naturally occurring homodimer, the board considers that such advantages are plausible, based on common general knowledge. The board is thus satisfied that the technical problem is solved by the claimed subject-matter.

3.2.3 The board however considers that said solution is not inventive. As mentioned above, it is common general knowledge (confirmed e.g. in D51, page 7014, first paragraph, lines 5 to 17) that single-chain molecules comprising the two subunits of a dimer are easier to manipulate and may have thermodynamical advantages over the corresponding dimers. Hence the skilled person, motivated to provide such advantageous meganucleases, would routinely consider engineering a molecule by fusion of both subunits, possibly linking them by a convenient linker: note that although the claim refers to domains of each subunit, it includes also, due to the open language "comprising", the whole subunit. To create such single-chain proteins would be a matter of routine experimentation. Moreover, in view of the fact that the vast majority of meganucleases are monomeric mononucleases, i.e. they have both LAGLIDADG motifs and both DNA-binding domains in one molecule, the skilled person would have no reason to doubt that such a single-chain configuration would be functional.

3.2.4 The appellant argued that D5 actually taught away from creating such a single-chain molecule, since it made clear that the structure was very complex. The board however notes that there is nothing in D5 suggesting that I-Cre's structure is more complex than the structures of other enzymes. While it might be difficult to predict which individual residues are responsible for binding or activity, D5 notes that the topologies of these enzymes (i.e. of the structurally solved LAGLIDADG meganucleases, including monomers and homodimers) are quite similar (page 313, left column, first paragraph) and makes clear where the relevant domains are and how they interact with each other. Again, in view of the fact that the claim also

encompasses fusion of the whole subunits, exact knowledge of the crucial domains and residues would not even be required.

3.2.5 The appellant further argued that, although single-chain proteins (fusion proteins) of some oligomeric proteins were available, the technique was not necessarily applicable to all oligomeric proteins, as was apparent from D51, page 7014, first paragraph. In view of the complex structure of the meganucleases, the skilled person would fear that any structural change could impair enzymatic activity. The board however notes that the above-mentioned passage of D51 in fact states that "for a significant portion of oligomeric proteins, it should be possible to connect the termini of different subunits by using a short loop without seriously affecting their enzymatic or binding functions" and that this "has been carried out for several proteins (29-33)", resulting in fusion proteins which "exhibited similar or enhanced enzymatic activities compared to the respective WT [wild-type] proteins" and which were stable (D51, page 7014, left column, lines 5 to 13). The concluding sentence of the quoted paragraph then reads: "These results, along with those presented here, indicate that the applicability of improving the stability of oligomeric proteins by subunit fusion to reduce unfolding entropy may be quite general". Hence, the skilled person would have no reason to doubt that the same strategy could be applied to dimeric meganucleases which, as mentioned above, are not necessarily more complex in structure than other proteins.

3.2.6 The main request is thus not allowable for lack of compliance with Article 56 EPC.

3.3 Auxiliary requests 1 and 2

3.3.1 Claim 22 of auxiliary request 1 and claim 10 of auxiliary request 2 are identical to claim 1 of the main request. Hence, for the same reasons as discussed above for the main request, these requests are also not allowable for lack of compliance with Article 56 EPC.

3.4 Auxiliary requests 3 to 5

3.4.1 Claim 10 of these requests is based on claim 22 of the main request, further specifying that the single-chain meganuclease comprises two subunits (auxiliary request 3) or two monomers (auxiliary requests 4 and 5) of a mono-dodecapeptide meganuclease, which are bound by a covalent linker; the covalent linker is further defined in auxiliary request 5 as "being introduced by creating a peptide bond between the two monomers".

3.4.2 The same arguments as discussed above for claim 22 of the main request also apply to claim 10 of these requests, since none of these amendments contributes to inventive step. In fact, the inventive-step discussion concerning claim 22 of the main request was already based on an embodiment wherein the two subunits (= monomers) were fused together, possibly by means of a covalent linker. The use of a covalent linker introduced by creating a peptide bond is, as acknowledged by the appellant, routine when producing fusion proteins.

3.4.3 Auxiliary requests 3, 4 and 5 are thus also not allowable for lack of inventive step (Article 56 EPC).

3.5 Auxiliary request 6

3.5.1 Claim 1 of this request is identical to claim 1 of the main request. It is essentially directed to functional (i.e. catalytically active) hybrid meganucleases comprising two domains derived from different initial dodecapeptide meganucleases, each domain comprising a dodecapeptide motif and a DNA-binding motif (for the exact claim wording, see section VII).

3.5.2 Document D32 is a review article about homing endonucleases (i.e. meganucleases). It specifically reviews the "attempts to engineer them to bind novel DNA substrates" (abstract, last sentence) and "the potential for engineering homing endonucleases with novel specificity" (page 3757, right column, lines 26 and 27), its last section being devoted to this subject ("Engineering endonucleases: Generation of novel enzymes with high specificity", starting at page 3770, bottom of left column). Hence it can be considered that D32 is directed to the same purpose as the patent (see section 3.1). D32 is thus the closest prior art for claim 1.

3.5.3 The difference compared with the claimed subject-matter is that a hybrid meganuclease as claimed, i.e. a hybrid meganuclease comprising two domains in the orientation N-terminal toward C-terminal, the domains being derived from two different initial dodecapeptide meganucleases and each being a polypeptide fragment comprising a dodecapeptide motif and a DNA-binding moiety, is not disclosed in D32. Instead, reference is made to other available strategies, e.g. the engineering of other endonucleases such as EcoRV or the joining of non-specific endonuclease domains, such as the cleavage

domain of FokI, to DNA-binding domains via a flexible linker (page 3770, right column, paragraphs 2 and 4). The patent does not disclose any advantages over the engineered endonucleases of the prior art for the hybrid meganucleases with the features as claimed. The technical problem can thus be formulated as the provision of further endonucleases, and the board is satisfied, based on the teachings of the patent, that the problem is solved by the claimed solution.

3.5.4 It hence has to be examined whether the skilled person would arrive at the claimed solution in an obvious manner.

3.5.5 As mentioned above, document D32 repeatedly refers to the possibility of engineering homing endonucleases to create novel specificities, and specifically teaches that the large LAGLIDADG family "should offer a strong foundation for engineering novel DNA-binding proteins" (page 3770, right column, lines 47 to 52). Moreover, D32 discusses the available information in the prior art concerning the structure and the cleavage mechanism of this class of enzymes. In particular, D32 refers to the available structural models of four such "widely divergent" enzymes as revealing the functional significance of the LAGLIDADG motif, the nature of the DNA-binding interface, the location of the two active sites, and details of the catalytic mechanism (page 3759, right column, second paragraph). D32 concludes from this structural data that "Despite limited sequence homology outside the LAGLIDADG motif(s), they all [the four enzymes] share a core topology that places the residues involved in DNA-binding and catalysis within the same domain" (page 3764, left column, lines 35 to 38). Also, when discussing in detail the mechanism of DNA cleavage by

the LAGLIDADG endonucleases, D32 states that "Many lines of evidence suggest this mechanism is conserved across the entire LAGLIDADG family" (page 3767, right column, lines 22 to 23), and supports this statement by listing a number of residues which are of importance for activity. D32 further identifies a "significant characteristic of this β-sheet DNA-binding motif of the homing endonucleases" as being "the close proximity within the primary sequence of many amino acids responsible for specificity of DNA recognition", which "presents the opportunity of replacing one or two stretches of DNA within the ORF with randomized sequences" (page 3770, right column, lines 40 to 45).

3.5.6 Prompted by D32 to use the LAGLIDADG family as source for new - recombinant - endonucleases, the skilled person would then turn to the available information in the prior art (including D32) concerning the structure and the cleavage mechanism of this class of enzymes. With the knowledge that this family of enzymes requires the presence of two LAGLIDADG motifs and two DNA-binding sites, each of the two binding sites being in fact a half-site (i.e. each recognising half of the target sequence) and that the two half-target sequences are not palindromic, the skilled person would expect to be able to create meganucleases with new binding specificities just by swapping the DNA-binding domains of different meganucleases. In fact, protein domain swapping strategies used to manipulate protein activities (including enzymatic activities) were common general knowledge, as acknowledged by the inventors in D31, the contemporary publication of the invention, (first paragraph of the "Discussion" section: page 2958, bottom of right column). At least when using as starting molecules those meganucleases whose three-dimensional structures had already been solved, the

skilled person would be in a position to identify the domains to be swapped without interfering in the structural stability. This was in fact the strategy used in the patent, which started by superimposing known structures: such superimposition of structures had already been performed for the structures of the homodimer I-CreI and the monomers I-DmoI and PI-SceI, as shown in Figure 6 of D8 and as discussed in D32 (page 3764, left column, third paragraph; reference 46 in D32 is document D8). Swapping the domains would then be a matter of using routine molecular biology (DNA recombination) methods.

3.5.7 Hence the board comes to the conclusion that the subject-matter of claim 1 lacks inventive step.

3.5.8 The appellant mainly argued that the skilled person would not necessarily consider using swapping strategies in the context of meganucleases because, as their structure was very complex, he would expect that even small modifications would render the enzyme inactive. Moreover, D32 suggested other methods to increase diversity of endonucleases and taught that the results obtained were limited and not in line with predictions (page 3767, right column, last paragraph).

3.5.9 The board considers that there is no evidence on file that the meganuclease structure is more complex than the structure of other enzymes which also have recognition sites and catalytic domains. Once such domains are identified, the skilled person would know that they can be exchanged among related proteins in an attempt to change their functional properties. It is true that D32 suggested other methods, namely random mutation of the DNA-binding site: such a method however is also not excluded from the claimed subject-matter,

which includes domains "derived" from the known domains. In fact, such a method is likely to produce more diversity, since it is not restricted to re-combining known DNA-binding half-sites. D32 nevertheless also teaches that it is possible to replace stretches of DNA (page 3770, right column, line 44), which points in the direction of using domain-swapping. The skilled person would furthermore recognise that the risk of losing enzymatic activity would be greater when using random mutation than when using domain swapping.

3.5.10 A further argument of the appellant was that, before the invention, there were no screening methods allowing to identify the new meganucleases. Since the skilled person could not identify the new specificities he would not be in a position to provide such new meganucleases. The board acknowledges that this could be a problem when using the method of random mutation suggested by D32, where it would be necessary to identify target sequences which might be completely new. However, for those embodiments, also falling within the claim, where no mutation has taken place, i.e. wherein two known domains of two known meganucleases are put together in one hybrid molecule, the resulting meganuclease is expected to recognise a DNA target sequence consisting of the two known half-target sequences of the original meganucleases: this could easily be tested with known screening methods, making use of artificially constructed DNA target sequences.

3.5.11 Auxiliary request 6 is thus not allowable for lack of compliance with Article 56 EPC.

3.6 Auxiliary requests 7 and 8

3.6.1 Claim 1 of auxiliary requests 7 and 8 is identical to claim 1 of the main request. Hence these requests also lack inventive step (Article 56 EPC).

3.7 Auxiliary requests 9 and 10

3.7.1 Claim 1 of auxiliary request 9 differs from claim 1 of auxiliary requests 7 and 8 essentially in that the hybrid meganucleases are to be selected from hybrid meganucleases comprising a first domain derived from the N-terminal domain of a first di-dodecapeptide meganuclease and a second domain derived from the N-terminal domain of a second di-dodecapeptide meganuclease, or comprising a first domain derived from the N-terminal domain of a first di-dodecapeptide meganuclease and a second domain derived from the C-terminal domain of a second di-dodecapeptide meganuclease, or comprising a first domain derived from the N-terminal domain of a di-dodecapeptide meganuclease and a second domain derived from a mono-dodecapeptide meganuclease. Claim 1 of auxiliary request 10 is essentially directed to the latter alternative.

3.7.2 The board comes to the conclusion that the same arguments as for claim 1 of auxiliary request 6 also apply to these amended claims. The restriction to these more specific combinations of domains does not contribute to inventive step because they are merely equally suitable alternative solutions based on the generally known concept of domain swapping. Also the combination of domains from a di-dodecapeptide enzyme and a mono-dodecapeptide enzyme is considered

straightforward in view of the known structural and functional characteristics of both groups of enzymes, as discussed in documents D32 and D5.

3.7.3 Auxiliary requests 9 and 10 are hence also not allowable for lack of compliance with Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

N. Maslin A. Lindner

Decision electronically authenticated