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Datasheet for the decision of 6 November 2017

Case Number: T 0540/12 - 3.3.04

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Title of invention:
Sequentially arranged streptavidin-binding modules as affinity tags

Applicant:
IBA GmbH

Headword:
Streptavidin-binding modules/IBA

Relevant legal provisions:
EPC Art. 56

Keyword:
"Main request - requirements of the EPC fulfilled (yes)"

Decisions cited:

Catchword:

-

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Case Number: T 0540/12 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 6 November 2017

Appellant: (Applicant)	IBA GmbH Rudolf-Wissell-Strasse 28 37079 Göttingen (DE)
Representative:	Schiweck, Weinzierl & Koch Patentanwälte Partnerschaft mbB Landsberger Straße 98 80339 München (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 28 September 2011 refusing European patent application No. 01274019.7 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairwoman	G. Alt
Members:	B. Claes
	L. Bühler

Summary of Facts and Submissions

I. The appeal lies from the decision of the examining division to refuse European patent application No. 01 274 019.7. The application was published as international application No. WO02/077018 with the title "*Sequentially arranged streptavidin-binding modules as affinity tags*".

II. The following documents are cited in this decision:

D4: US 6,103,493

D7: Schmidt *et al.* (1996), J. Mol. Biol., Vol. 255, Pages 753-766.

D8: Skerra & Schmidt (1999), Biomolecular Engineering, Vol. 16, pages 79 to 86.

D20: Experimental data filed as Annex A to the letter dated 3 June 2011.

D21: Experimental data filed as Annex B to the letter dated 27 June 2011.

D26: Experimental data filed with the letter dated 6 October 2017

III. The examining division held that claim 1 of the request under consideration (filed on 3 June 2011) did not meet the requirements of Article 123(2) EPC and lacked clarity (Article 84 EPC). It further held that the subject-matter of claim 1 did not involve an inventive step (Article 56 EPC). In addition, in an *obiter dictum* the examining division held that the subject-matter of

claim 7, which was dependent on claim 1, lacked an inventive step.

IV. With the statement of grounds of appeal the applicant (hereinafter "appellant") submitted a new main request and an auxiliary request (each comprising 23 claims) and arguments to the effect that claim 1 of those requests complied with the requirements of Articles 84 and 123(2) EPC and that its subject-matter involved an inventive step (Article 56 EPC). The appellant filed two further documents.

V. The appellant was summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the board expressed its preliminary view in relation to clarity, added subject-matter and inventive step.

VI. With a letter dated 6 October 2017, in response to the board's communication, the appellant submitted a new main request and eight auxiliary requests, further experimental evidence (document D26) and arguments in relation to clarity, added subject-matter and inventive step.

VII. The appellant was heard by the board during oral proceedings, at the end of which the appellant maintained a sole (main) request comprising 23 claims. Independent claims 1, 8, 10, 11, 13, and 19 to 21 read:

"1. Isolated linear streptavidin-binding affinity peptide having a total length of up to 56 amino acids, wherein the peptide comprises the sequential arrangement of two different or identical streptavidin-binding or/and streptavidin mutein-binding modules, wherein the streptavidin mutein has the sequence

Ile-Gly-Ala-Arg or Val-Thr-Ala-Arg at amino acid positions 44 to 47 of the amino acid sequence of wild-type streptavidin, wherein the distance between the two modules is at least 0 and not greater than 12 amino acids, wherein one binding module has 3 to 15 amino acids and comprises at least the sequence -His-Pro-Baa where Baa is glutamine, asparagine or methionine, and wherein the other binding module includes at least the sequence -Oaa-Xaa-His-Pro-Gln-Phe-Yaa-Zaa- where Oaa is Trp, Lys or Arg, Xaa is any amino acid and where either Yaa and Zaa are both Gly or Yaa is Glu and Zaa is Lys or Arg.

8. Fusion protein comprising a peptide according to any of claims 1 to 7 linked to a protein.

10. Expression vector comprising a nucleic acid sequence which codes for a peptide according to any of claims 1 to 7 and a restriction cleavage site which adjoins said nucleic acid sequence in 5' or/and 3' direction and which allows the introduction of another nucleic acid sequence coding for a protein or a protein part to be expressed.

11. Method for preparing a recombinant fusion protein, wherein a nucleic acid sequence which codes for a fusion protein according to either of claims 8 and 9 is introduced into a suitable host cell or into a cell lysate or into a cell extract.

13. Method for detecting or/and obtaining the fusion protein according to claim 8 or 9 in or from a sample, which comprises contacting the sample with a conjugate of streptavidin or a streptavidin mutein and a label or/and with a conjugate of streptavidin or a streptavidin mutein and a supporting material.

19. Nucleic acid coding for a peptide according to any of claim 1 to 7 or a fusion protein according to claim 8 or claim 9.

20. Use of streptavidin or/and a streptavidin mutein as receptor for binding a peptide according to any of claims 1 to 7 or a fusion protein according to claims 8 or 9.

21. Method for detection of a binding event between a protein and an analyte which is capable of binding to the protein by use of a biosensor, wherein streptavidin or a streptavidin mutein is immobilized on a surface of the biosensor, comprising the steps of

(a) contacting a first sample containing a protein which is linked to a peptide of any of claims 1 to 7 with the biosensor, thereby allowing the formation of a complex between said protein and streptavidin or a streptavidin mutein via the peptide of any of claims 1 to 7,

(b) contacting a second sample which can contain an analyte which is capable of binding to said protein with the biosensor, thereby allowing the formation of a complex between said protein and the analyte,

(c) detecting the binding of the analyte to the protein by use of a signal caused by the formation of the complex between said protein and the analyte."

Claims 2 to 7 were dependent on claim 1, claim 9 was dependent on claim 8, claims 14 to 18 were dependent on claim 13 and claims 22 and 23 were dependent on claim 21.

At the end of the oral proceedings the chairwoman announced the board's decision.

VIII. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the claims of the main request dated 6 November 2017.

Reasons for the Decision

1. The appeal is admissible.

Main request - claim 1

Article 123(2) EPC - added subject-matter

2. The board is satisfied that claim 1 of the main request finds a basis in the application as filed, in particular in claims 1, 7, 8 and 11 as filed and in the passages on page 8, lines 19 to 22, page 12, lines 32 to 35, page 14, lines 17 and 18, and page 16, lines 1 to 17.

3. The requirements of Article 123(2) EPC are accordingly complied with.

Article 84 EPC - clarity

4. The amendments to claim 1 have remedied the lack of clarity objected to in the decision under appeal, in that the claim now defines the streptavidin mutein by its particular sequence at position 44 to 47 of the amino acid sequence of wild-type streptavidin.

5. The board has no further objections in respect of clarity and is therefore satisfied that claim 1 meets the requirements of Article 84 EPC.

Article 56 EPC - inventive step

6. Claim 1 is for a streptavidin-binding affinity peptide (tag) of a maximum length of 56 amino acids, comprising two sequentially arranged streptavidin- or streptavidin mutein-binding modules, separated by 0 to 12 amino acids. The binding modules comprised in the claimed peptide are as such based on the so-called S*trep*-tag and S*trep*-tag II module sequences known in the prior art (see e.g. documents D7 and D8).

Closest prior art

7. In order to assess whether or not a claimed invention meets the requirements of Article 56 EPC, the boards of appeal apply the "problem and solution" approach, which requires as a first step the identification of the closest prior art. In accordance with the established case law of the boards of appeal, the closest prior art is a teaching in a document conceived for the same purpose or aiming at the same objective as the claimed invention (Case Law of the Boards of Appeal of the European Patent Office, 8th edition 2016, I.D.3.1).

8. The objective of the invention is stated in the application to be "*... to develop short peptide sequences which can be linked to a recombinant protein without interfering with the function thereof, which make detection using a readily available reagent possible, which display readily controllable binding properties and which can readily be eluted under*

competitive conditions despite strong binding affinity to surfaces" (see page 5, line 33 to page 6 line 2).

9. The appellant considered document D7 to represent the closest prior art. Document D7 relates to the molecular interaction between the *Strep*-tag affinity peptide and its binding partner streptavidin in the streptavidin/peptide ligand system. The document teaches the mutational engineering of the *Strep*-tag affinity peptide resulting in the *Strep*-tag II affinity peptide (having the sequence -SNWSHPQFEK) with a blocked C-terminus which gives a significantly tighter binding to streptavidin than *Strep*-tag and which does not necessarily need to be fused on the free C-terminal end of the recombinant protein fusion partner for effectively binding to streptavidin (see page 757, right-hand column, line 3, to page 758, right-hand column, line 5). The purpose of the teaching in document D7 is therefore the same as that of the claimed invention, *i.e.* the engineering of a streptavidin-binding affinity peptide in order to improve its binding properties to streptavidin.

10. Rather than the disclosure in document D7, the examining division considered the disclosure in document D4 to represent the closest prior art. Document D4 also discloses the streptavidin-binding S*trep*-tag and *Strep*-tag II affinity tags useful in the isolation and purification of proteins including affinity chromatography (see column 2, lines 7 to 11, and column 5, lines 31 to 64). The aim of the disclosure in document D4 is also to further optimise the binding strength of the streptavidin/peptide ligand systems (see column 2, lines 15 to 17). In an evolutionary research approach, however, the binding affinity for the streptavidin/peptide ligand system was

improved by mutation in a particular region of the streptavidin binding partner, resulting in particular streptavidin muteins (see column 2, lines 33 to 37).

11. The board thus notes that, whereas the streptavidin/peptide ligand system optimisation efforts in document D4 focus on the streptavidin compound, the streptavidin/peptide ligand system optimisation efforts reported on in document D7 relate to the engineering of a streptavidin-binding affinity peptide. Accordingly, the aim of the disclosure in document D7 is very similar to the aim of the claimed invention. The board is therefore satisfied that the teaching in document D7 represents the closest prior art for the assessment of inventive step.

The problem to be solved

12. The technical difference between the subject-matter of claim 1 and the teaching in document D7 is that the claim relates to a bi-modular streptavidin-binding affinity peptide (di-tag) rather than to a mono-modular streptavidin-binding affinity peptide (mono-tag), in particular the *Strep*-tag II affinity tag.

13. The technical effect resulting from the provision of the claimed di-tag peptides is that they have improved streptavidin- and streptavidin mutein-binding capacity properties useful in affinity purification, *i.e.* a strong binding affinity to streptavidin and muteins thereof which can be readily eluted under competitive conditions.

14. Accordingly, the objective technical problem to be solved by the subject-matter of claim 1 is the provision of improved streptavidin- or streptavidin

mutein-binding affinity peptides useful in affinity purification as compared to the mono-tags disclosed in the closest prior art.

15. The board is satisfied that the subject-matter of claim 1 solves the objective technical problem by reference to the experimental results disclosed in examples 1 to 4 of the application and the conclusion on page 34, lines 14 to 17, of the same that "*... these experiments clearly demonstrate that the di-tag approach has great practical use for the immobilization of tagged proteins on solid surfaces and for the purification of small amounts in a batch format*".

16. Further experimental results submitted during the examination proceedings, *i.e.* documents D20 and 21, and the appeal proceedings, *i.e.* document D26, corroborate the results disclosed in the application as filed.

17. The board particularly emphasises the results with the TSD1-tag disclosed in document D26, which demonstrate that binding of cytochrome b562 with a C-terminally attached di-tag of *Strep*-tag II modules, whereby the modules are in direct sequential arrangement, *i.e.* the distance between the two modules is 0 amino acids, to the streptavidin mutein system Strep-Tactin Superflow is equally improved over cytochrome b562 with a C-terminally attached mono-tag of *Strep*-tag II. Di-tags lacking any linker amino acids are therefore functional embodiments of the claimed subject-matter solving the technical problem.

18. The board is accordingly satisfied that the concerns expressed by the examining division in the decision under appeal in this context, *i.e.* that it had not been demonstrated that, in the absence of a linker between

the tag modules, all peptides falling structurally within the scope of claim 1 actually provided a solution to the technical problem, no longer apply.

Obviousness

19. The board notes that document D7 itself does not suggest to the skilled person dimerisation of the disclosed *Strep*-tag affinity peptide and *Strep*-tag II affinity peptide as a further route for possible optimisation of the streptavidin/peptide ligand system. In fact, on the contrary, at the end of the "Discussion" section, document D7 rather suggests on page 763 in lines 8 to 15 that [w]*ith our previously developed efficient recombinant expression system for core streptavidin (...) and the knowledge of the high-resolution crystal structure for the complex between streptavidin and the Strep-tag reported here,* ***the way is open to the engineering of streptavidin itself for improved performance in conjunction with the Strep-tag technology***" (emphasis added by the board). Accordingly, document D7 rather suggests to the skilled person the further optimisation route later published as e.g. document D4.

20. The board is also satisfied that no other cited prior art document suggests such dimerisation.

21. Accordingly, the board judges that for this reason the claimed subject-matter was not obvious in the light of the available prior art.

22. In the decision under appeal the examining division held in an *obiter dictum* relating to the subject-matter of a dependent claim, that at the relevant date of the application as filed the skilled person knew that an

increase in the apparent affinity between binding partners could be achieved by increasing the avidity of the peptide. The division referred in this context to the following disclosure on page 762 of document D7, left-hand column, lines 28 to 50: "*The answer to the question why affinities for streptavidin as low as 3542 M^{-1}* [...] *were picked up, when corresponding peptides were presented by the gene III protein of genetically manipulated phage particles* [...] *or on the surface of plastic beads* [...]*, becomes apparent from a view at the streptavidin tetramer (Figure 1). In the spatially closer pair of binding sites on the oligomer surface, the N-terminal Ala residues of two bound Strep-tag peptides have a C^{α} distance of 9.0 Å, whereas the C-terminal Gly residues are 28.6 Å apart.* ***This feature explains why a strong avidity effect must be expected if corresponding peptides are presented at high local density*** [...]*. In this respect, the low signal variation in our first peptide spot assay during the screening for the Strep-tag II should be noted. The synthetic peptides were immobilized at a high density on the filter support so that it was necessary to apply competitive conditions in order to make the changes in binding strength visible for the different sequences.*" (emphasis added by the board).

23. The examining division held that since document D7 thus disclosed that a strong avidity effect could be expected when *Strep*-tag peptides were presented at high local density to streptavidin, the skilled person would have been motivated to provide, in order to solve the technical problem, a streptavidin-binding affinity peptide comprising several *Strep*-tag peptides in close proximity and would thus have arrived at the claimed subject-matter in an obvious manner.

24. Figure 1 part (a) of document D7 depicts the refined crystal structure of the streptavidin/*Strep*-tag complex. As stated in the passage on page 762 referred to by the examining division (see point 21, above), the N-terminal Alanine residues of the two bound *Strep*-tag peptides indicated in this figure have a C^{α} distance of 9.0 Å, whereas the C-terminal Glycine residues are 28.6 Å apart. Accordingly, the board considers that these data of the crystal structure of the complex disclosed in figure 1 of document D7 teach the skilled person that the spatial orientation of the two bound tags on streptavidin is opposite, *i.e.* "head-on" (the N-terminal "head" residues are 9.0 Å apart, whereas the C-terminal "tail" residues are 28.6 Å apart) and that it is this arrangement of the "head" and "tail" residues which allegedly explains why a strong avidity effect could be expected if corresponding peptides were presented at high local density (see point 21 above).

25. The board notes, however, that in the di-tag of claim 1 the tag modules are defined as being arranged in "tandem", i.e. having the same orientation, whereby the distances between the "head" and "tail" residues would be approximately equal and would need substantial conformational bending of the affinity peptide for a "head-on" arrangement of the modules.

26. The board accordingly considers that the passage referred to by the examining division in the decision under appeal, rather than the claimed sequential ("tandem") arrangement of tag modules, would possibly suggest a "head-on" arrangement of the modules.

27. Accordingly, in this respect too the board is satisfied that there is no motivation in the disclosure in

document D7 to formulate the two sequentially arranged strep-tag modules in the claimed peptide.

28. In view of the above considerations the board is satisfied that the invention forming the subject-matter of claim 1 was not obvious to the skilled person. Accordingly, the requirements of Article 56 EPC are satisfied.

Article 83 EPC - sufficiency of disclosure

29. The examining division did not formulated any objections to the effect that the application did not meet the requirements of Article 83 EPC, and the board has none either. Accordingly, the requirements of Article 83 EPC are considered to be met.

Main request - claims 2 to 23

30. Claims 2 to 23 correspond to claims 16 to 31 of the application as filed and accordingly find a basis therein (Article 123(2) EPC).

31. The board is furthermore satisfied that the findings in relation to claim 1 apply *mutatis mutandis* to the claims 2 to 23.

32. In view of the above considerations the board decided that the main request fulfilled the requirements of the EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent with the following claims and a description to be adapted thereto:

   - claims 1 to 23 of the main request dated 6 November 2017.

The Registrar: The Chairwoman:

P. Cremona G. Alt

Decision electronically authenticated