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Datasheet for the decision of 7 February 2024

Case Number: T 1916/21 - 3.3.04

Application Number: 10747092.4

Publication Number: 2445923

IPC: C07K1/113

Language of the proceedings: EN

Title of invention:
Refolding Proteins using a Chemically Controlled Redox State

Patent Proprietor:
Amgen, Inc

Opponent:
D Young & Co LLP

Headword:
Protein refolding/AMGEN

Relevant legal provisions:
EPC Art. 56

Keyword:
Main request - Inventive step - (yes)

Decisions cited:

Catchword:

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Beschwerdekammern

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Case Number: T 1916/21 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 7 February 2024

Appellant: (Patent Proprietor)	Amgen, Inc One Amgen Center Drive Thousand Oaks, CA 91320-1799 (US)
Representative:	Schiweck Weinzierl Koch Patentanwälte Partnerschaft mbB Ganghoferstraße 68 B 80339 München (DE)
Respondent: (Opponent)	D Young & Co LLP 120 Holborn London EC1N 2DY (GB)
Representative:	D Young & Co LLP 120 Holborn London EC1N 2DY (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 23 August 2021 revoking European patent No. 2445923 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman	A. Chakravarty
Members:	D. Luis Alves
	R. Romandini

Summary of Facts and Submissions

I. European patent EP 2 445 923, entitled "*Refolding proteins using a chemically controlled redox state*", was granted on European patent application No. 10 747 092.4, filed as an international application published as WO 2011/005488 (in the following "application as filed").

II. The patent was opposed by a single opponent under Article 100(a) EPC, on the grounds of lack of inventive step (Article 56 EPC). The opposition division decided to revoke the patent.

III. In the decision under appeal, the opposition division dealt with a main request and five auxiliary requests. As regards auxiliary request 3, the opposition division held that the subject-matter of claim 1 did not extend beyond the content of the application as filed (Article 123(2) EPC), however it did not involve an inventive step (Article 56 EPC).

IV. The patent proprietor (appellant) filed an appeal against that decision. The opponent is respondent to this appeal.

V. With the statement setting out the grounds of appeal, the appellant filed claim sets of a main request and five auxiliary requests. The main request is identical to auxiliary request 3 considered by the opposition division.

VI. The respondent did not file any substantive submissions in the appeal proceedings.

VII. The board appointed oral proceedings and issued a communication pursuant to Article 15(1) RPBA setting out its preliminary opinion on the appeal case.

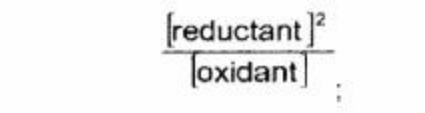
VIII. Oral proceedings were held in absence of the respondent, that had previously informed the board that it would not be attending. At the end of the oral proceedings, the Chair announced the board's decision.

IX. The main request consists of a single independent claims and 13 dependent claims. Claim 1 reads:

"1. A method of refolding a protein expressed in a bacterial expression system and present in a volume at a concentration of 2.0 g/L or greater during refolding, wherein said protein comprises an Fc region, said method comprising:

(a) contacting the protein with a refold buffer comprising a redox component comprising a final thiol-pair ratio having a range of 1.5 to 100 and a thiol-pair buffer strength of 2 mM or greater and:
(i) a denaturant;
(ii) an aggregation suppressor; and
(iii) a protein stabilizer;
to form a refold mixture;

wherein the thiol-pair ratio is calculated according to Equation 1:



wherein the thiol-pair buffer strength is calculated according to Equation 2:

$2[\text{oxidant}]+[\text{reductant}]$;

wherein said thiol-pair ratio and said thiol-pair buffer strength are evaluated in a full or partial factorial matrix; and together with the parameters of incubation time, concentration of said protein and pH, with each component varied over a range of at least 3 concentration or pH levels with all other parameters kept constant; and wherein the thiol-pair ratio value and thiol-pair buffer strength value used in said refold buffer are those respective values resulting in a higher yield of the desired form of said protein than is obtained prior to said varying of said thiol-pair ratio and said thiol-pair buffer strength;

(b) incubating the refold mixture; and

(c) isolating the protein from the refold mixture.

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

D2: WO-A1-95/32216

D4: "Purifying Challenging Proteins: Principles and Methods", GE Healthcare, 2007

D6: Pierce Biotechnology "Instructions Pro-Matrix™ Protein Refolding Kit 89867", 2003

D7: WO-A2-02/068455

D8: WO-A2-2006/047340

D11: WO-A1-99/42486

XI. The appellant's arguments relevant to this decision may be summarised as follows:

It was inherent or implicit in claim 1 that the protein which was the starting point of the method was denatured or fully unfolded.

This was confirmed in the description, which defined refolding as a process of reintroducing secondary and tertiary structure to a protein and referred to protein expression in the form of inclusion bodies (see patent, paragraphs [0034] and [0054]).

This was in contrast to the refolding method disclosed in document D8, which started from a slightly misfolded protein and merely aimed at perturbing the tertiary or quaternary structure of the protein without perturbing the secondary structure (see document D8, page 41, first paragraph).

It was common general knowledge that recombinant protein is expressed in bacteria in inclusion bodies (see document D4, pages 68 and 72, Table 3.2; document D6, page 2; document D11, page 4, lines 16 to 18; and document D2, page 19, first paragraph). Therefore, it was necessary to solubilise proteins present in these inclusion bodies.

Document D8 was not the proper starting point for assessing inventive step of claim 1. The difference between the claimed method and the method disclosed in document D8 was that the protein was expressed in a bacterial expression system. The technical effect of the difference was that a protein expressed by a prokaryotic expression system was refolded at a

concentration of 2 g/l. The objective technical problem could be formulated as the provision of a method for the systematic refolding of a protein with an Fc region, expressed in a bacterial expression system, in a concentration of 2 g/l or more.

Document D8 disclosed the disadvantages of eukaryotic protein expression in prokaryotic systems, such as the need to solubilise inclusion bodies. It further disclosed that the method of refolding proteins expressed in eukaryotic systems did not include denaturation of the protein and that the protein secondary structure was not changed (see page 1, lines 10 to 26, page 3, lines 24 and following, page 24, line 21, page 25, lines 29-31, page 41,first paragraph, page 60, page 71, lines 8 to 9 and page 77, lines 16 to 17).

The skilled person wanting to express Fc-containing proteins would not seriously contemplate or consider starting from a prokaryotic expression system. Refolding at high protein concentrations as claimed presented a danger of aggregation which was higher for proteins expressed in prokaryotic expression systems than for the correctly folded proteins produced by an eukaryotic system. Documents D4 and D11 disclosed refolding of prokaryotic system-expressed eukaryotic proteins at low protein concentrations (see document D4, page 72). The teaching of document D11 was that refolding of prokaryotic system-expressed proteins occurred after diluting 10-fold the protein to final concentrations of 0.25 mg/l (see Figure 2). Therefore, when starting from document D8, the solution in claim 1 was not obvious even when taking into account documents D4 or D11.

XII. The appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of the set of claims of the main request or, alternatively, on the basis of any one of the sets of claims of auxiliary requests 1 to 5, all of which were filed together with the statement of grounds of appeal.

Reasons for the Decision

Main request - all claims

Inventive step (Article 56 EPC)

Claim interpretation

1. Claim 1 is directed to a method of refolding a protein. The starting material of the method, i.e. the protein which is to be refolded, is defined by two features: "*a protein expressed in a bacterial expression system*" and "*said protein comprises an Fc region*". The board disagrees with the appellant's view that claim 1 requires the protein to be in inclusion bodies. While the claim does not define the extent of processing the protein may have undergone after its expression in a bacterial expression system, for example whether it is present in inclusion bodies or has to some extent been isolated, the two features cited above imply that the protein has multiple disulphide bonds, which include incorrect ("scrambled") disulphide bonds, and is in a partially denatured or unfolded state.

This conclusion is based on the characteristics of proteins expressed in bacterial expression systems, in particular the ones which, in their properly folded

state, contain disulphide bonds (see the documents D2 and D4, referred to by the appellant in this context).

Document D2 concerns the production of recombinant proteins in bacterial expression systems (see abstract and claims) and states that the intracellular environment in bacteria leads to the improper folding of proteins that normally form disulphide bonds, resulting in the production of unfolded proteins (see page 19, lines 1 to 12).

Document D4 discloses that recombinant protein expressed in *E.coli* frequently forms insoluble aggregates of misfolded proteins deposited in inclusion bodies, which mostly consist of denatured proteins (see page 68, first and second paragraphs). Document D6 also refers to improperly folded recombinant proteins (see page 2, third paragraph, first sentence).

No documents have been provided in these proceedings to refute the appellant's assertion that the proteins produced by bacterial expression systems will present such a partial or total loss of secondary and tertiary structure.

Closest prior art

2. The opposition division considered the disclosure in documents D7 and D8 to represent equally suitable starting points for the assessment of inventive step. The reason for this was that both documents concern the expression of recombinant proteins comprising an Fc region. Furthermore, document D8 additionally disclosed the recovery of the recombinant proteins and hence this document was the one that was primarily considered in the decision. The board agrees with the opposition

division that document D8 is a suitable starting point for the assessment of inventive step. It is also a more promising starting point than document D7 because it discloses in more detail the optimisation of the refolding buffer.

3. Document D8 concerns refolding of IgG2 produced in mammalian systems (see page 4, lines 4 to 11 and claim 1). Mammalian expression systems were chosen to avoid the need for denaturing and isolating the recombinant protein from inclusion bodies (see page 1, lines 14 to 26 and page 3, line 24 to page 4, line 1). Because mammalian cells produce correctly cross-linked recombinant protein, there was no need for complete protein denaturation when refolding correctly the protein (see page 3, last line to page 4, line 1 and page 4, lines 7 to 9). Nevertheless it was observed that recombinant proteins expressed in eukaryotic systems were also present in several different folded forms. The different forms corresponded to alternative disulphide bonding or unpaired cysteine residues (see page 5, first paragraph). This is the problem addressed by the invention in document D8, which aims at refolding the proteins produced in eukaryotic systems for providing the correct folding and increasing product homogeneity (see page 5, first paragraph, page 24, lines 13 to 17 and page 25, lines 31 to 33). Thus, the aim in document D8 was not to refold proteins which lost all tertiary and quaternary structure but merely to allow for repositioning of the disulphide bonds to obtain the most preferred forms of the protein in detriment of others (see page 41, first paragraph and page 60, lines 5 to 7).

Objective technical problem

4. The opposition division identified the following distinguishing features between the method defined in claim 1 and that disclosed in document D8: in the former, the protein was expressed in a bacterial expression system and the optimal conditions for refolding are identified using a full or partial factorial matrix; in the latter the protein is expressed in a mammalian expression system and several parameters of the refolding buffer are optimised. The board agrees with this assessment.

5. The technical effect due to these differences is the refolding at a protein concentration of 2 g/l of a protein that was expressed in a bacterial system. The patent gives examples of refolding of Fc-containing proteins expressed in bacteria, at a concentration 6 g/l during refolding (see example 4).

6. Hence, the board considers that the objective technical problem is the provision of an alternative method of refolding a protein comprising an Fc region.

7. The claimed method would not have been obvious to the skilled person starting from the disclosure in document D8 for the following reasons. The method disclosed in document D8 is not suitable for refolding of proteins expressed in bacterial cells. Indeed, it is a method of refolding proteins, expressed in eukaryotic cells, that are almost correctly folded (see page 41, first paragraph and page 60, lines 5 to 7). This refolding method avoids denaturing the protein, in contrast to known methods of refolding proteins prepared in prokaryotic expression systems (see page 4, last line to page 4, line 1). The aim of the methods

disclosed in document D8 was to provide methods for refolding proteins produced in mammalian expression systems (see page 4, line 4 and page 25, lines 31 to 33) and to reshuffle only the "scrambled" disulphide bonds (see page 2, lines 1 to 5 in light of the above passages).

8. Since the methods disclosed in document D8 are not suitable for refolding proteins produced in bacterial expression systems, document D8 also contains no indication that its methods would be suitable for refolding partly denatured proteins in the concentrations given in claim 1. As set out above, the board considers that claim 1 refers to a protein to be refolded which is partly denatured and has "scrambled" disulphide bonds. It was known that denatured proteins tend to aggregate and that the presence of even small amounts of aggregates induces accelerated formation of more aggregates (see D4, page 71, "Refolding" and page 74, "Refolding using gel filtration", first paragraph, third sentence).

9. In view of the above considerations, the board concludes that document D8 does not disclose a method of refolding proteins that is generally applicable to all recombinant proteins regardless of the expression system used.

10. The opposition division considered that the claimed method was obvious because document D8 already disclosed that the methods could be applied to any protein and that antibodies could be expressed in bacterial systems. In this context, the decision under appeal referred to the following passages:

"*The invention finds particular use in improving the production of any recombinant proteins that is [sic] produced in e.g., mammalian cells and requires appropriate refolding*" (see document D8, page 33, lines 1 to 2)

and

"*Methods for producing recombinant antibodies in mammalian cells are known. In such methods, the antibody production involves induction of protein expression. Nucleic acids encoding an IgG antibody or an IgG antibody fragment are conveniently rendered expressible by operative association with a promoter, preferably a controllable promoter functional in mammalian cells. Such recombinant constructs are designed for expression of IgG antibody protein in a suitable host (e.g., bacterial, murine, or human)*" (see document D8, page 27, lines 27 to 32).

11. However, the board has a different reading of these passages. As regards the former, the fact that mammalian systems are presented as an example does not imply that the alternative to be considered are bacterial systems. In the board's view, in context, mammalian cells are given as an example of eukaryotic cells, in view of the contrast made in document D8 between prokaryotic and eukaryotic systems (see description of the invention on page 24, first full paragraph). As regards the latter passage this, in the board's view, makes reference to known constructs for protein expression in bacterial, murine or human systems and should not be read out of the context of this sentence, which refers to production in mammalian cells and includes the teaching to use promoters that are functional in mammalian cells. Therefore, this

passage does not teach the skilled person to produce any protein in a bacterial expression system.

12. The opposition division reasoned that the skilled person would consider a protein concentration 2 g/l or more because this concentration was within the range used when refolding proteins from bacterial expression systems. In this context reference was made to document D11, page 1, lines 2 to 8.

13. However, in view of the structural differences between the proteins expressed in bacteria versus eukaryotic systems, the board is not convinced that the skilled person would consider applying the method disclosed in document D8 to proteins expressed in bacterial expression systems in a concentration of 2 g/l or more. The board has not seen any document in the present case disclosing the refolding proteins from prokaryotic expression systems having an Fc portion at concentrations above 1 g/l.

Document D11 does not address refolding of proteins having an Fc portion. Therefore, no conclusion can drawn from it on the refolding of the proteins defined in claim 1. Moreover, the teaching of document D11, taken as a whole, is to refold proteins at concentrations of 0.25 g/l. Despite mentioning a broad range of protein concentrations, 0.1 to 5 mg/ml, as noted by the opposition division, all examples carry out refolding at protein concentrations of 0.25 mg/ml (see Figure 2, page 18, lines 12 to 18, page 22, lines 28 to 32 and p.23, lines 8 to 13). Therefore, the board is of the view that the skilled person reading this document would not have concluded that the method of refolding proteins with an Fc-portion could be carried out at 2 g/l.

14. In view of the above considerations, the board concludes that the subject-matter of claim 1 involves an inventive step (Article 56 EPC). The same conclusion applies to the subject-matter of the dependent claims.

Articles 123, 84, 54 and 83 EPC

15. The main request now pending before the board is identical to auxiliary request 3 considered in the decision under appeal. In said decision, the opposition division held that the subject-matter of this claim request complied with the requirements of Article 123(2) EPC. This finding has not been challenged in the appeal proceedings. The board in turn sees no reason to depart from this assessment.

As regards Article 123(3) EPC, the board is satisfied that claim 1 is restricted in scope vis-à-vis claim 1 as granted: the "non-mammalian expression system" is defined in a more limited way as "bacterial expression system", the range for the final thiol-pair ratio is limited from the range "0.001 to 100" to the narrower range "1.5 to 100", and finally the refold buffer is required to comprise each of the three components listed in claim 1 as (i) to (iii), whereas claim 1 as granted required only that at least one be present.

The assessment of clarity must be restricted to features modified in comparison to claim 1 as granted. The modifications in question do not raise any clarity issues. Novelty was not invoked as a ground for opposition during the opposition proceedings in relation to then auxiliary request 3. The same applies to sufficiency of disclosure.

Conclusion

16. In view of the above considerations, the main request is allowable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent of the basis of the main request filed with the statement of grounds of appeal and description as well as drawings to be amended as necessary.

The Registrar: The Chairman:

I. Aperribay A. Chakravarty

Decision electronically authenticated