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D E C I S I O N
of 16 September 2004

Case Number: T 0870/02 - 3.3.8

Application Number: 96921410.5

Publication Number: 0834074

IPC: G01N 33/50

Language of the proceedings: EN

Title of invention:
Detection of transmembrane potentials by optical methods

Patentee:
The Regents of the University of California

Opponent:
Bayer AG

Headword:
Transmembrane potentials/REGENTS CALIFORNIA

Relevant legal provisions:
EPC Art. 54

Keyword:
"Main request - novelty (no)"
"First auxiliary request - novelty (yes), inventive step (yes)"

Decisions cited:
T 1099/99, T 1070/00, G 0009/91

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0870/02 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 16 September 2004

Appellant: (Opponent)
Bayer AG
Konzernbereich RP
Patente und Lizenzen
D-51368 Leverkusen (DE)

Representative:
-

Respondent: (Proprietor of the patent)
The Regents of the University of California
1111 Franklin Street
12th Floor
Oakland
CA 94607-5200 (US)

Representative:
Grund, Martin Dr.
Dr. Volker Vossius
Patentanwaltskanzlei - Rechtsanwaltskanzlei
Geibelstrasse 6
D-81679 München (DE)

Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 17 June 2002 concerning maintenance of European patent No. 0834074 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julia
V. Di Cerbo

Summary of Facts and Submissions

I. An appeal was lodged by the opponent (appellant) against the decision of the opposition division to maintain the European patent 0 834 074, with the title "Detection of transmembrane potentials by optical methods", on the basis of an auxiliary request filed on 22 February 2002 and which differed from the claims as granted by the deletion of claims 43 and 44 only. The patent had been opposed on the grounds of Article 100(a) EPC (for lack of novelty and of inventive step of all claims) and Article 100(b) EPC (for lack of sufficiency of disclosure of claims 43 and 44). The main request (claims as granted) was considered not to fulfil the requirements of Article 83 EPC because of claims 43 and 44.

II. In the statement of grounds of appeal and with reference to the decision under appeal, the appellant raised issues under Article 83 EPC in relation to the disclosure of the patent specification (cf. Section X *infra*).

III. In its reply, the patentee (respondent) stated that grounds under Article 83 EPC had not been raised against claim 1 in the opposition proceedings but had been introduced in the appeal proceedings for the first time. With reference to decision G 9/91 (OJ EPO 1993, 408), it was requested not to admit them.

IV. The parties were summoned to oral proceedings and, in a communication annexed therein, they were informed of the board's preliminary opinion on the case.

V. Both the appellant and the respondent filed further comments in response to the board's communication. The respondent filed six auxiliary requests.

VI. Oral proceedings took place on 16 September 2004. During the oral proceedings, the respondent withdrew all previous auxiliary requests and filed a new first auxiliary request.

VII. Claim 1 of the **main request** (claims as maintained by the opposition division) was identical to claim 1 as granted, and read as follows:

"*A method of determining the electrical potential across a membrane comprising:*

(a) introducing a first reagent comprising a hydrophobic fluorescent ion capable of redistributing from a first face of the membrane to a second face of the membrane in response to changes in the membrane potential;
(b) introducing a second reagent which labels the first face or the second face of the membrane, which second reagent comprises a chromophore capable of undergoing energy transfer by either (i) donating excited state energy to the fluorescent ion, or (ii) accepting excited state energy from the fluorescent ion;
(c) exposing the membrane to excitation light;
(d) measuring energy transfer between the fluorescent ion and the second reagent; and
(e) relating the energy transfer to the membrane potential."

Dependent claims 2 to 30 related to specific embodiments of the method of claim 1 defining the type of energy transfer (claim 2), type of membrane (claims 3 to 7), the hydrophobic fluorescent ion (claims 8 to 22) and the second reagent (claims 23 to 30). Dependent claim 31 read as follows:

"*The method of claim 1, wherein the first reagent and the second reagent are covalently joined by a linker*."

Dependent claims 32 and 33 further defined the linker of claim 31. Claims 34 to 36 related to a kit for determining the electrical potential across a membrane comprising a first reagent and a second reagent as described in claim 1. Independent claim 37 read as follows:

"*A compound of the formula A-L-B for use in the kit of claim 34 wherein:*

A is independently a polymethine oxonol or a tetraaryl borate linked to a fluorophore;
L is a linker; and
B is a membrane-impermeant fluorophore or a membrane-impermeant conjugate of a fluorophore."

Claim 38, dependent upon claim 37, referred to the formula of the polymethine oxonol. Claims 39 and 42 related, respectively, to a method of identifying a test sample affecting membrane potential in a cell and a method of screening test samples to identify a compound modulating the activity of an ion channel, pump or exchange in a membrane using a first and a second reagent as described in claim 1. Claims 40 to 41

and claims 43 to 48 related to further embodiments of the methods of claims 39 and 42, respectively. Claim 49 was directed to a cell comprising the first and second reagents of claim 1, whereas claims 50 to 54 related to further embodiments of this cell.

VIII. The **first auxiliary request** was as the main request except for the deletion of all subject-matter related to the embodiment wherein the first and second reagents were covalently joined by a linker. Thus, claims 31 to 33 and 37 to 38 of the main request were deleted in this auxiliary request and the remaining claims accordingly renumbered. Independent claims 1, 31 and 44 of this request read, respectively, as claims 1, 34 and 49 of the main request (cf. Section VII *supra*) but with the additional wording at the end:

"*wherein the first reagent and the second reagent are not covalently joined by a linker*."

IX. The following documents are referred to in the present decision:

D1: D. Witzak, "Oxonol-Luminophore mit funktionalisierten Seitenkette". Diplomarbeit, 1994, Heinrich-Heine Universität, Düsseldorf;

D2: S. Bechtel, "Synthese von Styrylfarbstoffen mit Reaktivgruppen". Diplomarbeit, 1994, Heinrich-Heine Universität, Düsseldorf;

D6: PhD Thesis of D. Witzak, "Bichromophore Fluoreszenzfarbstoffe", 2000, Heinrich-Heine Universität, Düsseldorf;

D8: J.E. Gonzalez and R.Y. Tsien, Biophys. J., October 1995, Vol. 69, pages 1272 to 1280.

X. The appellant's arguments which are relevant to the present decision may be summarized as follows:

Main Request
Article 54 EPC

Claim 1 concerned an optical method of measuring the membrane potential covering two different embodiments, namely an embodiment using a first "slow-response" fluorescent reagent (A) and a second "fast-response" fluorescent reagent (B) covalently joined by a linker (L), and a second embodiment wherein no linker was present and both fluorescent reagents were uncoupled. Dependent claim 31 concerned the specific embodiment with the linker, the linker being only generically defined. The patent exemplified the introduction of a linker with functional groups into the first fluorescent reagent only (A-L compounds). However, there was no example showing the actual covalent coupling of those compounds with the second fluorescent reagent B to make the claimed A-L-B compounds. For this coupling reaction, the patent only referred to "*conventional coupling chemistries*" and to "*known methods*". There was no indication of any possible problem or particular requirement nor a guidance for taking specific measures or choosing special reaction conditions. Later document D8 by the inventors referred to this coupling as a theoretical solution only and stated, however, that "*it would pose nontrivial problems in chemical synthesis*". None of these

nontrivial problems were addressed in the patent in suit, which were, however, known to appear by post-published document D6.

Documents D1 and D2 disclosed the same general concept as the patent in suit, namely the use of two fluorescent reagents coupled by a linker for measuring membrane potentials. These reagents were characterized by the same features and properties as the ones of the first and second fluorescent reagents described in the patent. The structure and nature of the linker were also disclosed in these documents, which gave a clear guidance as to how to select an appropriate length. On the one hand, for an effective transfer of energy a minimal distance between the two fluorescent reagents was required, which was indicated in documents D1 and D2 to be similar to the size of the plasma membrane. On the other hand, the efficiency of this transfer diminished by increasing the distance between both reagents in a known proportion. Up to a certain distance the transfer of energy was so inefficient that no significant change could be attained by a further increase in the length of the linker. Other problems had to be taken into account when using a flexible linker with a long length (cf. document D6 as an expert opinion). The figures of documents D1 and D2 were intended to give a schematic representation of the general concept only but not to illustrate it in all detail. These documents disclosed the synthesis of the first or the second fluorescent reagents with a linker having appropriate functional groups (A-L or B-L compounds) so as to react with the other fluorescent reagent and obtain thereby the two fluorescent reagents covalently linked. Although the documents in question

did not report the actual preparation of a covalently coupled compound and made no reference to any possible problems when carrying out the proposed coupling reaction, the patent in suit failed also to provide these coupled compounds as well as a detailed guidance for overcoming the problems associated to the nontrivial chemical synthesis of these compounds.

In accordance with the established case law of the Boards of Appeal, the same standard should be applied when comparing the prior art with the disclosure of the patent specification. Thus, following this approach, documents D1 and D2 anticipated the subject-matter of the main request.

First auxiliary request

No objections of any kind were raised against the subject-matter of the first auxiliary request.

XI. The respondent's arguments which are relevant to the present decision may be summarized as follows:

Main Request
Article 54 EPC

Document D6 referred to the specific fluorescent reagents of documents D1 and D2 and reported that the coupling reaction proposed in these documents could not be successfully carried out even when the experimental conditions for the reaction were optimized. The coupling was only achieved through a very specific reaction ("*Quaternierungsreaktion*") and the use of a particular functional group ("*Iodalkyl-Seitenkette*").

Contrary to documents D1 and D2, both this reaction and the functional group were explicitly disclosed in the patent. Figure 11 showed the synthesis of a first fluorescent reagent with this very specific functional group and its linkage to the second fluorescent reagent. Figures 12 and 13 showed, respectively, linkage points of the first fluorescent reagent to a second fluorescent reagent and the synthesis of this second fluorescent reagent. Figure 14 illustrated the synthesis of a bi-functional linker, whereas Figures 15 and 16 showed the synthesis of a first fluorescent reagent with a built-in linker suitable for attachment to a second fluorescent reagent. The compounds (14) and (15) shown in Figure 16 had an appropriate built-in linker with activated functional groups ready for reacting in a straightforward and easy manner with the second fluorescent reagent. There was no evidence on file showing that this coupling reaction could not be successfully carried out. Whereas the skilled person would have failed in carrying out the teachings of documents D1 and D2, which were thus not enabling, the patent in suit provided detailed instructions and guidance for achieving a coupled compound comprising the first and second fluorescent reagents covalently linked.

Moreover, the patent differed from documents D1 and D2 in that it required the first fluorescent reagent to redistribute from a first face to a second face of the membrane, i.e. to go all the way through the whole membrane, in response to changes in the membrane potential. Consequently, the linker had to be long enough to span the entire membrane. Neither document D1 nor document D2 disclosed this essential feature

required for achieving an advantageous high sensitivity. Figure 5 of document D1 and Figures 5 and 6 of document D2 illustrated the movement of the first fluorescent reagent across the membrane in response to a change in the membrane potential but they did not show the reagent going through the entire membrane. The linker was shorter than the one required in the patent and its structure was also different, as shown in Figure 5 of document D1, wherein part of the linker was hanging outside the membrane. All the linkers built-in the first or second fluorescent reagents, if at all, were very short and extremely close to the fluorescent reagent itself. There was also another important difference between this prior art and the patent specification, namely the type of oxonol used as the first fluorescent reagent. The patent disclosed the advantageous properties of an oxonol with a negative charge delocalized throughout the chromophore with the four equivalent oxygen atoms containing the majority of the charge. These advantages were, however, not taught in documents D1 or D2.

Applying the same standard when comparing documents D1 and D2 with the disclosure of the patent, the latter went much beyond the former since the patent provided guidance and instructions as well as a disclosure of essential structural and functional features that were absent in the teachings of documents D1 or D2. In the absence of all this information, as demonstrated by post-published evidence, the teachings of documents D1 and D2 were not enabling and thus, they could not anticipate the subject-matter of the main request.

XII. The appellant (opponent) requested that the decision under appeal be set aside and that the European patent No. 0 834 074 be revoked or, as auxiliary request, that the patent be maintained on the basis of the first auxiliary request filed at the oral proceedings.

XIII. The respondent (patentee) requested that the appeal be dismissed, or, as auxiliary request, that the decision under appeal be set aside and the patent be maintained on the basis of the first auxiliary request filed at the oral proceedings.

Reasons for the Decision

Main request
Article 54 EPC

The patent specification

1. The patent in suit discloses a method of determining the electrical potential across a membrane with a first and a second fluorescent reagent. The first reagent comprises a hydrophobic permeable ion capable of redistributing from a first face of the membrane to a second face of the membrane, i.e. translocating across the membrane, in response to changes in the membrane potential (cf. page 7, lines 1 to 54). This first fluorescent reagent is related to the known "slow-response" dyes because in order to establish a new equilibrium, ions must diffuse through the membrane (cf. page 2, lines 24 to 32). By contrast, the second fluorescent reagent is immobile or impermeable, asymmetrically bound either to the extracellular or to

the intracellular face of the membrane, i.e. labelling the first or the second face of the membrane (cf. page 15, line 48 to page 16, line 4), and it is related to the known "fast-response" dyes (cf. page 2, lines 33 to 37). This second fluorescent reagent comprises a chromophore capable of undergoing nonradiative fluorescent resonance energy transfer (FRET) by either donating excited state energy to the fluorescent ion, or accepting excited state energy from the fluorescent ion. Upon exposition of the membrane to excitation light, the energy transfer between these two fluorescent reagents is measured and put in relation to the membrane potential (cf. page 21, line 1 to page 22, line 31). The method disclosed in the patent can be carried out according to two different embodiments, namely a first one wherein the two fluorescent reagents are covalently joined by a linker and a second embodiment wherein the two reagents are not covalently linked.

2. The patent discloses several first fluorescent reagents such as polymethine oxonols (cf. page 7, line 56 to page 9, line 34), fluorophore conjugates of tetraaryl borate (cf. page 9, line 36 to page 12, line 53) and fluorophore complexes with transition metals, in particular lanthanide complexes (cf. page 12, line 55 to page 15, line 46). Several second fluorescent reagents are also disclosed, such as fluorescent lectins, lipids, labelled antibodies, cytochromes, carbohydrates, peptides and proteins, wherein the fluorophore is selected from xanthenes, cyanines and coumarins (cf. page 16, line 6 to page 19, line 38). Similarly, references are found to several linker

groups between the first and second fluorescent reagents (cf. page 19, line 40 to page 20, line 58).

3. The teachings of the patent are exemplified by the synthesis of first and second fluorescent reagents, such as for the first reagent: thiobarbiturate derivatives of polymethine oxonols DiBSA-C_4-(3) and DiBSA-C_6-(5) (cf. Example I, pages 23 to 24 and Figure 13), tetraaryl borates (cf. Example VIII, pages 31 to 32 and Figure 10), lanthanide chelates with a single negative charge (cf. Example X, pages 33 to 34); and for the second reagent: fluorescent derivatives of the phospholipid phosphatidylethanolamine (Cou-PE and Cy5-PE) (cf. Example II, pages 24 to 25 and Figure 20). Example III (cf. pages 25 to 27 and Figures 14 to 16) and Example IX (cf. pages 32 to 33 and Figure 11) show the synthesis of linkers and of asymmetric oxonols with these linkers attached, i.e. first fluorescent reagents with built-in linkers. Examples IV to VII (cf. pages 27 to 31 and Figures 6 to 9, 17 to 19 and 22) relate to membrane potential measurement using the first and second fluorescent reagents not covalently joined by a linker - schematically illustrated in Figure 1. There is, however, no example showing the actual preparation and synthesis of a compound comprising the two fluorescent reagents covalently joined by a linker nor an example using this compound in a method of measuring the membrane potential.

The prior art documents D1 and D2

4. Document D1 refers to known "slow-response" and "fast-response" fluorescent dyes (cf. pages 9 to 12) as

well as to their use in a method of measuring the electrical potential of a membrane based on FRET (cf. pages 13 to 17, Figures 4 and 5). It is stated that both reagents should be covalently joined by a linker, which is indispensable for achieving an appropriate distance between the two fluorescent reagents (cf. page 14, first full paragraph). The same disclosure is found in document D2, which also refers to "slow-response" and "fast-response" fluorescent reagents (cf. pages 5 to 9) as well as to their use in a method of measuring membrane potential (cf. pages 10 to 13 and Figures 4 to 6) and to the requirement of a linker that covalently joins both fluorescent reagents (cf. page 11, first paragraph).

5. Documents D1 and D2 originated from the same research group, were written and available at about the same time (October 1993 to June 1994 and September 1993 to April 1994, respectively) and document D1 explicitly refers to document D2 (bibliographic reference (31) in the "Literatur" list of document D1) (cf. page 70). Both documents are complementary and provide together a complete disclosure of the same general teaching.

Document D1 is concerned with the synthesis of first fluorescent reagents, particularly, reagents related to thiobarbiturate derivatives of polymethine oxonols (cf. pages 20 to 43 and 47 to 67), whereas document D2 is more concerned with second fluorescent reagents, in particular, reagents related to styryl derivatives (cf. pages 17 to 37 and 39 to 62). Both types of reagents are also exemplified with built-in linkers with different functional groups and each document refers to the fluorescent reagent – either oxonol or styryl

derivatives – disclosed in the other document. Although two specific coupling reactions (-NCS + HO- and $-SO_2Cl$ + HO-) are schematically illustrated in these documents (cf. pages 18 and 14 in documents D1 and D2, respectively), reference is also made to coupling reactions with other functional groups (cf. page 46, last paragraph in document D1 and *inter alia* page 15, first paragraph in document D2). However, there is no actual preparation and synthesis of any compound comprising a first "slow-response" fluorescent reagent covalently joined by a linker to a second "fast-response" fluorescent reagent nor the use of such a compound in a method of measuring the membrane potential.

The patent in suit vs. the prior art documents D1 and D2

6. It appears from the foregoing, that both the patent in suit and the prior art - represented by documents D1 and D2 - disclose the same general method of determining the membrane potential and refer to the same type of fluorescent reagents covalently joined by linkers, which are also defined in the same manner. However, neither the patent in suit nor this prior art disclose the actual synthesis of a compound comprising both fluorescent reagents covalently joined by a linker and the use of such a compound in the general method of determining the membrane potential. Thus, in accordance with the established case law of the Boards of Appeal, which requires the application of the same standard when assessing the disclosure of the prior art with the teachings of the patent specification (cf. eg T 1070/00 of 23 October 2003, paragraphs 9.4 and 9.5 of the Reasons for the Decision and T 1099/99 of 4 December

2002, paragraph 3.3 of the Reasons for the Decision), documents D1 and D2 anticipate the subject-matter of the main request.

7. Three different arguments have been put forward by the respondent in order to demonstrate the presence of technical differences between the patent in suit and documents D1 and D2. These differences mainly relate to (a) the alleged non-enabling character of this prior art, due to the specific coupling reaction required by the reagents shown in documents D1 and D2, (b) the character and nature of the appropriate linker, and (c) the specific type of first fluorescent reagents used in the patent in suit. These alleged differences are examined hereinafter.

8. It has been first alleged that post-published document D6 (cited as expert evidence) shows that the coupling reactions illustrated in documents D1 and D2 cannot be carried out and thus, that the teachings of these documents are non-enabling (cf. Section XI *supra*).

Document D6 reports that no coupling reaction could be performed between styryl derivatives (second fluorescent reagent) with the functional group –NCS in the built-in linker and first fluorescent reagents with the functional groups –OH or $-NH_2$. No coupling was obtained with several first fluorescent reagents, including the specific polymethine oxonols of document D1, and under different reaction conditions (cf. pages 29 to 30). It was only through a very specific "*Quaternierungsreaktion*" using a first fluorescent reagent with a built-in linker having as a functional

group a "*Iodalkyl-Seitenkette*" that a coupling reaction could be successfully achieved (cf. pages 31 to 32).

9. However, neither the general teachings of document D1 nor the ones of document D2 are limited to any specific first or second fluorescent reagent nor to any type of linker or functional group associated thereto, let alone to a particular coupling reaction. Both documents explicitly refer to several first "slow-response" and second "fast-response" fluorescent reagents (cf. pages 9 to 12 of document D1 and pages 6 and 7 of document D2). Moreover, as stated in paragraph 5 *supra*, apart from the exemplified polymethine oxonols with the built-in linkers having an hydroxyl group (-OH) and the coupling reactions illustrated by formulae (3) and (4) on page 18, document D1 explicitly contemplates the introduction of other functional groups as well as the use of alternative coupling reactions, such as –COOH or a peptide bound (cf. page 46, last paragraph). Document D2 actually exemplifies the introduction of several functional groups in the built-in linker of styryl derivatives, such as *inter alia* the $-SO_2Cl$ of compound 22 (cf. page 32), -NCS of compound 5 (cf. page 27), -OH of compound 20b (cf. page 31), including a -Br in compound 24 (cf. page 28) closely related to the functional group (-I) disclosed in document D6. Although this latter group is disclosed in document D2 among other possible functional groups so is also the reference to the functional group –I in the patent in suit (cf. page 20, lines 19 to 21 and Figure 11), which does not emphasize any particular coupling reaction but only refers to the use of "*conventional coupling chemistries*" (cf. page 20, lines 55 to 56).

10. The board considers that both the patent in suit and the cited prior art are addressed to the person skilled in the field, who is well aware of the particular - bulky, highly hydrophobic and unhandy - nature of these fluorescent reagents. If the manipulation of one of these reagents alone already requires special skills, it becomes immediately apparent that their coupling by a linker will be even more difficult or, as stated in the post-published document D8 (cited as expert opinion), "*nontrivial*" (cf. page 1279, right-hand column, lines 18 to 27). Document D6 only confirms these problems and shows that for each specific combination of first and second fluorescent reagents appropriate attachment points, functional groups and coupling reactions are to be carefully selected, even if all of them are conventional ones, such as the quaternary reaction of document D6 which is based on a method well-established in the early nineteen forties. In this respect, thus, no difference can be found between the general teachings of the patent in suit and the ones of the prior art documents D1 and D2.

11. It has been further argued that the nature of the linker disclosed in the patent in suit differs from the one shown in documents D1 and D2 (cf. Section XI *supra*). In the respondent's view, whereas the patent defines the linker as being "*of an appropriate length to span the plasma membrane*" (cf. page 20, lines 23 to 24) or "*long enough to span the entire membrane*" (cf. page 19, lines 51 to 52) so as to allow "*the hydrophobic ion back and forth across the plasma membrane*" or "*redistributing from a first face of the membrane to a second face of the membrane*" (cf. page 15, line 54 and claims), this essential feature is not disclosed in

documents D1 and D2, which only uses very short linkers in all the examples. In their view, as shown by the figures illustrating the movement of the first reagent across the membrane, this first reagent never reaches the second face of the membrane (cf. Figure 5 of document D1 and Figures 5 and 6 of document D2).

12. An appropriate length of the linker might well be a necessary condition for the first fluorescent reagent to reach the second face of the membrane but it is certainly not a sufficient one. On the one hand, the movement of this first fluorescent reagent across the membrane will depend on further features of the linker, such as its flexibility, the presence of functional groups and/or atoms other than carbon bonds, etc. On the other hand, the intrinsic properties of the membrane, such as its fluidity, phospholipids composition, etc. will significantly influence this movement too, as shown for instance in the problems found with long linkers – formation of micelles - referred to in document D6 (cf. page 111, first paragraph). None of these features nor the problems referred to in document D6 are mentioned in the patent in suit.

13. In fact, the patent defines these long linkers as being the preferred ones only. The specific generic formulae shown in the patent refer to a length "*from about 20 to 40 (preferably 25 and 35)*" and "*from 0 to about 32 ... less than 33*" (cf. page 20, line 38 and line 53, respectively), i.e. open-ended in their lower range and thus, comprising linkers shorter than the whole membrane. In this respect, the wording of the claims "*capable of redistributing from a first face of the*

membrane to a second face of the membrane" cannot be read as requiring the hydrophobic fluorescent ion (first reagent) to necessarily reach the second face but as comprising also those movements wherein the ion only buries itself deeper into the membrane (cf. page 7, lines 14 to 26).

14. Documents D1 and D2 refer to 50 to 100 Armstrong as an appropriate distance for an efficient nonradiative energy transfer between the first and second fluorescent reagents (cf. page 14, lines 1 to 3 and page 10, lines 4 to 5 from the bottom in documents D1 and D2, respectively), which actually corresponds to the 60 to 100 Armstrong of most membranes (25-30 carbon equivalents). Both documents further disclose the specific dependence of the energy transfer on the distance between the first and second fluorescent reagents – R^6 in the generic formulae (2) and (1) of documents D1 and D2, respectively (cf. pages 14 to 15 in document D1 and page 11 in document D2). Document D1 explicitly refers to the length of the linker as influencing the sensitivity of the disclosed method of measuring the membrane potential (cf. page 46, lines 4 to 7). The figures of these two documents only illustrate – in a schematic manner - these general teachings only but they do not describe it in all detail. The skilled person is made aware of an appropriate distance for the energy transfer to take place as well as the change or variation of this transfer – sensitivity - as a direct function of the distance separating the two fluorescent reagents. Thus, the (preferred) length of a linker should be selected according to this information and the type of membrane

used. No difference can be found between these teachings and the ones of the patent in suit.

15. The presence of "*a single negative charge delocalized between the two acidic groups*" in the polymethine oxonols used as the first fluorescent reagents in the patent in suit has been identified also by the respondent as a significant difference over the cited prior art (cf. page 7, line 58 to page 8, line 1 and page 8, lines 9 to 15 of the patent) (cf. Section XI *supra*). This feature is also outlined in the oxonols of document D1 (cf. page 9, lines 3 to 5 from the bottom), which will thus have the same properties as the ones disclosed in the patent. Moreover, none of the independent claims is limited to this particular class of first fluorescent reagents, since this first fluorescent reagent is generically defined in all of them.

16. In view of the above analysis, the board considers that the subject-matter of claims 1 and 31 is anticipated by each of the documents D1 and D2.

17. Thus, the main request does not fulfil the requirements of Article 54 EPC.

First Auxiliary request
Articles 123(2)(3) and 84 EPC

18. The subject-matter of this request has been restricted by the introduction of the feature: "*wherein the first reagent and the second reagent are not covalently joined by a linker*" (cf. Section VIII *supra*) to one of the two possible embodiments described in the granted

patent. No formal objections were raised by the appellant against this request nor does the board have any objections.

19. There is no extension of the scope of protection conferred (Article 123(3) EPC) and the embodiment now claimed is described in the application as filed. Thus, there is a formal basis therein (Article 123(2) EPC). Moreover, no clarity problem arises by the introduction of this feature (Article 84 EPC), which indicates that no linker joins the two reagents.

Substantive matters

20. The appellant did not raise any substantive objection against the subject-matter of this request. As a matter of fact, the appellant had in respect of the first auxiliary request the same request as the respondent, i.e. that the patent be maintained on its basis. The board does not have any objections against this request.

21. In fact, documents D1 and D2 explicitly refer to the linker as being an essential feature of their method of determining the membrane potential (cf. page 14, first full paragraph and page 11, first paragraph in documents D1 and D2, respectively) and none of the other documents cited in these proceedings discloses a method of determining the membrane potential wherein there is no linker joining the two fluorescent reagents. The method claimed has been exemplified in the patent in suit (cf. Examples IV to VII and Figures 6 to 9, 17 to 19 and 22) and shown *inter alia* in later document D8 to work. Thus, the requirements of the EPC are fulfilled.

Adaptation of the description

22. The description has been adapted to the claims limited to the embodiment of the method where no linker is present. No objections have been raised by the appellant to the adapted description. Nor does the board have any objections.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the following documents:

   – Claims of the first auxiliary request filed at the oral proceedings;

   – Amended description;

   – Drawings as granted.

The Registrar: The Chairman:

A. Wolinski L. Galligani