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Date
27-11-2012

Reference	Application No./Patent No.
P36939EP-PCT	09815404.0 - 2404 / 2344891 PCT/US2009057915
Applicant/Proprietor	
University of Washington, et al	

Communication pursuant to Rule 114(2) EPC

Please find enclosed observations by a third party concerning the patentability of the invention of the above-mentioned patent application. That person is not a party to the proceedings before the EPO (Art. 115 EPC).

Under Rule 114(2) EPC you may comment on the observations.

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November 19, 2012

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European Application No. 09815404.0
Applicant(s): University of Washington, The UAB Research Foundation
Title: MSP NANOPORES AND RELATED METHODS

Dear SIRS

Attached hereto are observations under Article 115 EPC concerning the application identified above. The documents cited in the observations follow with a courier version of this letter.

Please contact me if you need any additional information.

Warmest Regards.

Very truly yours,

/ Gregory P. Einhorn/

Gregory P. Einhorn
Attorney at Law

Enclosure

Third party observations submitted in accordance with Art 115 EPC in respect of EP Application No. 09815404.0 (EP regional phase of International Application No. PCT/US2009/057915)

Summary

Claims 1-21 as filed on 11 November 2011 of EP Application No. 09815404.0 (the present application) are considered unpatentable due to a lack of novelty, inventive step and/or sufficiency of disclosure by the application. Furthermore, the subject matter of claims 1, 3 and 13 at least is not entitled to the filing date of the priority application.

Introduction

The present application relates to the area of electrophysiology and related biosensor applications where analytes translocate across an impermeable membrane by means of a membrane-bound pore under the influence of an applied voltage. The area has been the subject of considerable investigation well before the priority date of the present application, particularly the use of α-Hemolysin pores to detect analytes such as divalent metal ions (US6,824,659) and ssDNA (Kasianowicz *et al*, PNAS, vol 93, p13770, 1996).The present application is concerned with the discovery that two mutants of MspA are able to pass DNA across an otherwise impermeable membrane in the same way that α-Hemolysin is known to do.

Prior Art

1) Reference is made to the following prior art documents. K A Ball, REU 2006, Paper and accompanying presentation slides, University of Washington Physics REU Program 2006 June 19 - August 25 2006 (Ball)
2) Faller *et al*, Science vol 303, 20th February 2004, p1189-1192 (Faller)
3) Abstract of NHGRI Grant Application no.1 R21 R21HG04145, 'Engineering MspA for Nanopore Sequencing' J Gundlach, Project Period: 09/26/2006 - 08/31/2008 (Gundlach)
4) Heinz *et al*, J Biol Chem, 2003, vol278, p8678-8685 (Heinz)
5) Bayley and Cremer, Nature 2001, vol 413, p226-230 (Bayley)
6) R Wong, Poster, 'Engineering Mycobacterium smegmatis Porin A (MspA) for DNA analysis' University of Washington Summer Research Poster Session 16th August 2007 (Wong), page 52.
7) Niederweis et al, Molecular Microbiology (1999) 33 (5), 933-945 (Niederweis)

8) L. Jayasinghe and H. Bayley, Protein Science (2005), 14:2550-2561 (Jayasinghe)
9) US 2006/0063171 (Akeson)
10) Butler *et al*, PNAS, Dec 30, 2008, vol. 105, No. 52, p20647-20652 (Butler).
11) Kasianowicz *et al*, PNAS, vol 93, p13770-13773, 1996 (Kasianowicz)
12) Akeson, Branton *et al*, Biophys. J., Vol 77, Issue 6, p3227-3233, 1999 (Branton).

Details of the public availability of the above prior art may be found in Annex 1.

Arguments

Arts 52 (1) and (2), 56 EPC; Novelty and Inventive Step

Claim 1

'A method comprising:
applying an electric field to a Mycobacterium smegmatis porin (Msp) porin having a vestibule and a constriction zone that define a tunnel, wherein the Msp porin is positioned between a first conductive liquid medium and a second conductive liquid medium; and
detecting an analyte present in the first or second conductive liquid media.'

i. Lack of novelty over Ball

Ball discloses a method of applying a voltage to *a Mycobacterium smegmatis* porin A (MspA), which has a vestibule and constriction zone that define a tunnel (see Fig 2). The MspA is placed in a lipid bilayer positioned between first and second liquid conductive media (see Fig 4 and following paragraphs). In the absence of the pore, the bilayer blocked all ion flow across the aperture such that zero current was detected by the amplifier. Following insertion of the protein pore into the bilayer, ions were able to flow through the tunnel of the MspA creating a small detectable current. Ball therefore discloses a method for the detection of ions as analyte. Furthermore from Ball, and specifically the slide entitled "Does DNA interact with MspA?", a comparison of the plots of Pore 1 (no DNA) and Pore 1 (with DNA) shows that the presence of DNA has a measurable effect on the current signal. Therefore the analyte DNA was detected. Claim 1 therefore lacks novelty over Ball.

ii. Lack of novelty over Wong

Wong discloses a method whereby MspA was used to detect the analyte DNA. MspA was inserted into a lipid bilayer and ion current flow through the pore was measured under the application of a voltage difference across the pore. Clear current blockages were observed when single stranded DNA was present. Claim 1 therefore lacks novelty over Wong.

iii. Lack of novelty over Niederweis

Niederweis discloses inserting an Msp porin in a planar lipid bilayer positioned between first and second electrolyte solutions and measuring current flow under the application of an electric field wherein the current flow is indicative of the presence of ions in the first or second electrolyte (see page 942, Lipid bilayer experiments). Niederweis therefore discloses a method for the detection of potassium ions and $Tris^+$ as analytes. Claim 1 therefore lacks novelty over Niederweis.

iv. Lack of novelty over Heinz

Heinz discusses the secondary structure of MspA and discloses "Since a stable pore protein would be of great value as a detection unit in biosensors (17).....a biochemical analysis of purified MspA is needed.....". (page 8678, col 2, 2nd paragraph). Reference 17 of Heinz is Bayley. Bayley gives a general overview of using nanopores to detect analytes such as single-stranded RNA or DNA (page 228, 1st paragraph). The techniques disclosed in Bayley are the same techniques used in the present application and defined in the method of claim 1. Heinz clearly suggests applying the techniques of Bayley to the MspA pore. Thus the subject matter of claim 1 is not novel over Heinz. The subject matter of claim 1 is also not inventive over Heinz in view of Bayley.

v. Lack of inventive step over Gundlach in view of Bayley

Gundlach proposes the use of MspA as a nanopore to detect the passage of DNA. Gundlach neither discloses the application of an electric field, nor the provision of the pore between a first and second conductive liquid. However, it would be readily apparent to a person skilled in the art of nanopore sequencing that the nanopore would be placed between a first and second conductive liquid and that DNA would be caused to translocate the pore under the application of an electric field across the pore, see for example Bayley. Claim 1 therefore lacks an inventive step over Gundlach.

Claim 2

'The method of claim 1, wherein the Msp porin was expressed with a vector comprising an inducible promoter operably linked to an Msp monomer nucleic acid sequence'

i. Lack of inventive step over Ball, Gundlach or Wong in view of Faller

The MspA amino acid sequence was known well before the priority date, for example from Faller. Expression of proteins of known sequence using a vector comprising an inducible promoter was a well-known and predictable technique long before the priority date. As such the skilled person would readily consider applying this method to prepare an Msp porin. Claim 2 therefore lacks inventive step over Ball, Gundlach or Wong in view of Faller.

Claim 3

'The method of claim 1 wherein the Msp porin is a mutant comprising at least a first mutant MspA monomer comprising a mutation at position 93 and a mutation at position 90, 91, or both positions 90 and 91 and optionally comprises one or more mutations at any of the following amino acid positions: 88, 105, 108, 118, 134, or 139.'

i. Lack of inventive step over Ball in view of Faller

Faller clearly shows that residues 90 and 91 are present at the constriction zone of MspA (see Fig 4B) and states at p1190, third column:

> *"the eyelet is fully defined by the carboxylates of Asp90 and Asp91 that cause a rather strong electric field".*

From the MspA sequence shown in Fig. 3 it is clear that residue 93 is also an aspartic acid and thus negatively charged, and is only two residues away from 91. No other negatively charged residues apart from aspartic acids 90, 91 and 93 are present in the constriction zone.

Ball discloses the use of mutant MspA D9091S as a pore for the detection of an analyte wherein the negatively-charged aspartic acids around the constriction zone of the channel were replaced with neutral serine monomers. Ball states "We hope that by replacing these charged amino acids with neutral residues DNA would not be repelled from the narrow channel and would be able to pass through the pore". Ball therefore teaches that it is advantageous for the passage of DNA to remove negatively charged residues from the constriction zone. The skilled person, aware of Ball and in particular Fig 3 of Ball, would appreciate that a further negatively charged aspartic acid is located in the vicinity of the constriction zone, and would consider replacing it. The only other negatively charged residue in the vicinity of the constriction zone is residue 93 and it would have therefore been obvious to the skilled person to select this residue to mutate. Furthermore, the disclosure in Ball that the use of mutant D9091S did not result in a clear interaction with DNA would prompt the skilled person to consider mutating additional negatively charged groups present at the

constriction zone to arrive at the subject matter of claim 3. Thus claim 3 is therefore not inventive over Ball in view of Faller.

Although the negatively-charged aspartic acids around the constriction zone of the channel were replaced with neutral serine monomers in Ball, the skilled person would readily appreciate that other neutral amino-acid residues could be inserted in place of serine and in any further replacement of residues, such as for example position 93. Thus the particular embodiment encompassed by claim 3 wherein the mutant MspA porin is D90N/D91N/D93N is also considered to lack an inventive step over Ball in view of Faller.

ii. Lack of inventive step over Gundlach in view of Ball

Gundlach proposes that in order to tailor MspA for efficient translocation of DNA, excess negative charges will be removed from the rim and vestibule of the pore. Fig 3 of Ball shows a side cut-away and bottom view of MspA illustrating the red negatively charged amino-acids including residues 118 and 134 in the vestibule, and residue139 at the rim. The skilled person aware of Gundlach would therefore consider selecting one or more of residues 118, 134 or 139 without the use of inventive skill. Claim 3 is therefore not inventive over Gundlach in view of Ball.

iii. Lack of inventive step over Wong in view of Ball

Wong discloses that a triple mutant and a sextuplet mutant MspA were engineered with some of the pore's excess negative charges removed. The location of the mutated residues are not disclosed, however Wong teaches that both MspA mutants demonstrate clear current blockages when single strand DNA is present. Ball teaches the replacement of amino-acids having negative charges that face internally into the pore with neutral amino-acids. The skilled person starting from Ball would therefore consider replacing additional negative charges as taught by Wong. Given that Ball discloses that removal of negatively charged residues from the constriction zone is desirable and shows in Fig 3, the negatively charged amino-acids present in the vestibule and rim of MspA, it is highly likely that the skilled person in choosing to prepare a triple mutant MspA would consider selecting residue 93, and in choosing to prepare a sextuplet MspA would additionally consider selecting one or more of residues 118, 134 or 139 having internally facing negative charges, without the use of inventive skill. Therefore claim 3 is not inventive over Wong in view of Ball. .

Claim 4

'The method of any one of claims 1-3, wherein the Msp porin comprises a mutant porin comprising:

a vestibule having a length from about 2 to about 6 nm and a diameter from about 2 to about 6 nm; and

a constriction zone having a length from about 0.3 to about 3 nm and a diameter from about 0.3 to about 3 nm.'

i. Lack of novelty over Ball or Faller

Ball discloses a scale image of the Msp porin MspA (Fig 2) showing that the vestibule has a length of 5.9nm and a diameter of 4.8nm and that the constriction zone has a width of 1nm. The length of the constriction zone is not stated but from the scale drawing it can be seen that it is between 0.3 and 3nm. Faller discloses the crystal structure of wild type MspA from which the dimensions of the vestibule and the constriction zone may be easily determined, see Fig 3 of Faller. Thus claim 4 is not novel over Ball or Faller.

The inventors have described only known pores or mutants of known pores in their methods and systems. These methods and systems cannot be made novel simply by defining a known pore in terms of its dimensions rather than the primary and/or secondary structure known from Faller.

Claim 5

'The method of any one of claims 1-4, wherein the Msp porin is derived from a mutant bacterial strain capable of inducible Msp monomer expression, the bacterial strain comprising:

(a) a deletion of a wild-type MspA;

(b) a deletion of a wild-type MspC;

(c) a deletion of a wild-type MspD;

(d) optionally a deletion of wild-type B; and

(e) a vector comprising an inducible promoter operably linked to an Msp monomer nucleic acid sequence.'

i. Lack of inventive step over Wong or Gundlach in view of Heinz or Niederweis

The use of a mutant bacterial strain capable of inducible monomer expression wherein the bacterial strain comprises a deletion of a wild type gene is a well-known technique, see for example page 937 of Niederweis, 'Expression of the MspA gene in E. coli', or Heinz at page 8679 'Purification of Native and Recombinant DNA'. Heinz also discloses that MspA is known in addition to the other three porins MspB, MspC and MspD. It would be readily obvious to the skilled person to provide bacterial strains comprising deletions of wild type MspB, MspC and MspD. It is considered that the skilled person wishing to obtain a mutant

MspA as disclosed in Ball, Wong or Gundlach would contemplate the derivation of an Msp porin by the methods disclosed in Niederweis or Heinz. Claim 5 is therefore considered to lack an inventive step over Ball, Wong or Gundlach in view of Heinz or Niederweis.

Claim 6

'A system for use in the method of any one of claims 1-5, the system comprising a Mycobacterium smegmatis porin (Msp) having a vestibule and a constriction zone that define a tunnel, wherein the tunnel is positioned between a first liquid medium and a second liquid medium, wherein at least one liquid medium comprises an analyte, and wherein the system is operative to detect a property of the analyte.'

i. Lack of novelty over Ball

Ball discloses a system for use in a method comprising an Msp porin having a vestibule and a constriction zone that define a tunnel, wherein the tunnel is positioned between two reservoirs of aqueous ionic solution (liquid medium). Current due to the flow of ions through the porin may be detected, the magnitude of which would depend upon the concentration of ions or their charge. The system of Ball is therefore operative to detect a property of the ions. Ball also discloses a system whereby DNA is present in at least one reservoir. Due to the narrow width of the constriction of MspA, the system is operative for example to detect whether the DNA is single stranded or double stranded on the basis that double stranded DNA would not pass through the constriction. The system is designed to characterise and sequence DNA (see Introduction and slides). Thus claim 6 lacks novelty over Ball.

ii. Lack of novelty over Niederweis

Niederweis discloses that the channel conductance of the purified Msp porin is dependent upon the specific conductance of different salts in water. (see page 939, Channel properties of the MspA porin). The system of Niederweis is therefore operative to determine a property of the analyte. Claim 6 therefore lacks novelty over Niederweis.

iii. Lack of inventive step over Wong in view of Ball

Wong discloses a system involving a mutant MspA in which clear current blockages were demonstrated when single stranded DNA was present. Wong is therefore operative to detect a property of the analyte DNA. Starting from Wong, it is considered the skilled person would contemplate the system as disclosed in Ball in order to detect a property of the DNA. Claim 6 is therefore not inventive over Wong in view of Ball.

Further the use of a nanopore to identify and/or characterise an analyte was well known before the priority date, see for example Bayley. Thus claim 6 is not inventive over Heinz, Wong or Ball in view of Bayley.

Claim 7

'The system or method of any one of claims 1-6 comprising a mutant MspA porin, wherein the constriction zone of the mutant MspA porin is more positively charged or more negatively charged when compared to the constriction zone of a wild-type MspA porin.'

i. Lack of novelty over Ball

Ball discloses a system or method involving the use of the mutant MspA porin D9091S wherein the negatively charged aspartic acids around the constriction zone of the channel are replaced with neutral serine residues. This provides a constriction zone which is more positively charged when compared to the constriction zone of wild-type MspA. (See Fig 3 and immediately preceding paragraph). Thus claim 7 is not novel over Ball.

ii. Lack of inventive step over Wong in view of Faller

Wong teaches that DNA does not pass through wild type DNA most likely because of electrostatic repulsion between negatively charged amino acids on the surface of the pore and the negative charges on DNA. As a consequence, Wong engineered both a triple mutant and a sextuplet mutant MspA with some of the pore's excess negative charge removed. It is considered that the skilled person, aware from Faller that negative charges exist in the constriction zone of wild type MspA, would consider replacing them in order to allow the detection of DNA and therefore provide a mutant MspA porin according to claim 7. Therefore claim 7 is considered to lack an inventive step over Wong in view of Faller.

Claim 8

'The system or method of any one of claims 1-7, wherein the Msp porin further comprises a molecular motor, wherein the molecular motor is capable of moving an analyte into or through the tunnel with an average translation velocity that is less than the average translocation velocity at which the analyte electrophoretically translocates into or through the tunnel in the absence of the molecular motor.'

i. Lack of inventive step over Ball, Gundlach or Wong in view of Akeson

The use of a molecular motor to modulate or reduce the speed of passage of a DNA analyte through a pore in order to better characterise the analyte was known before the priority date

of the present application, as acknowledged in the application at page 53, lines 13-32. See for example Akeson which isacknowledged in the present application at page 53, lines 22-24. The skilled person starting from Ball, Gundlach or Wong wishing to improve the characterisation of DNA would consider the use of a molecular motor disclosed in Akeson. Claim 8 therefore lacks an inventive step over Ball, Gundlach or Wong in view of Akeson.

Claim 9

'The system or method of claim 8, wherein the molecular motor is selected from one of an enzyme, a polymerase, an exonuclease, a DNA binding protein, or a Klenow fragment.'

i. Lack of inventive step over Ball, Gundlach or Wong in view of Akeson

The molecular motors listed in claim 9 were all known well before the priority date as acknowledged in the present application.See for example claim 3 of Akeson. The skilled person would therefore readily contemplate the use of the specific molecular motors disclosed by Akeson. Thus the subject matter of claim 9 lacks an inventive step.

Claim 10

'The method of any one of claims 1-5 or 7-9, further comprising determining the concentration, size, molecular weight, shape, or orientation of the analyte, or any combination thereof.'

i. Lack of novelty over Ball, Niederweis or Wong

The method of Ball or Niederweis may be used to determine the concentration of ions present. The method disclosed by Wong may also be used to determine whether the DNA analyte was single or double stranded. Claim 10 therefore lacks novelty over Ball, Niederweis or Wong.

ii. Lack of inventive step over Ball, Wong or Gundlach in view of Kasianowicz

It is known from Kasianowicz, see p13773, col. 1, that a pore may be used to determine various properties of the analyte, such as its molecular size. It is considered that a skilled person aware of Kasianowicz would a contemplate using the porin disclosed by Ball, Wong or Gundlach in order determine such properties. Claim 10 therefore lacks an inventive step over Ball, Wong or Gundlach in view of Kasianowicz.

Claim 11

'The method of any one of claims 1-5 or 7-10, wherein the analyte includes a polymer, a nucleotide, or a nucleic acid.'

i. Lack of novelty over Ball, Niederweis or Wong

Ball, Gundlach and Wong disclose a method wherein the analyte is DNA, which is both a polymer and a nucleic acid. Thus claim 11 lacks novelty over Ball, Gundlach or Wong.

Claim 12

'The method of any one of claims 1-5 or 7-10, wherein the analyte includes a peptide, a protein, a polymer, or a combination thereof.'

i. Lack of novelty over Ball, Niederweis or Wong

Ball, Gundlach and Wong disclose a method wherein the analyte is DNA. DNA is a polymer. Thus claim 12 lacks novelty over Ball, Gundlach or Wong.

Claim 13

The method as in claim 11 or claim 12 wherein the analyte is a polymer comprising more than one unit, further comprising:

sequencing the polymer in a method comprising measuring the ion current or optical signals as each unit of the polymer is separately translocated through the tunnel to provide a current pattern comprising a blockade, wherein a blockade is associated with each unit; and

comparing one or more blockades in each current pattern to (i) one or more blockades in the same current pattern or (ii) one or more blockades in a known current pattern of a known unit obtained under the same conditions, such that the polymer is sequenced.'

i. Lack of inventive step over Ball or Wong in view of Branton

Branton carried out pore measurements whereby poly C and poly A caused to translocate a pore resulting in distinguishable current blockades (see Fig 2). Current blockades were also obrtained for the polymer $A_{(30)}C_{(70)}$, see Fig 5, from which current blockades may be compared to the current blockades obtained from that of poly C and poly A alone in order the determine the sequence. Starting from Ball or Wong, it is considered that the skilled person aware of Branton would contemplate measuring current blockades and comparing the blockades of a known current pattern in order to determine the sequence of a polymer, a

desired objective of Ball and Wong.Therefore claim 13 is not inventive over Ball or Wong in view of Branton.

Claim 14

'The method of any one of claims 1-5 or 7-13, wherein the analyte includes a drug, an ion, a pollutant, a nanoscopic agent, or a biological warfare agent.'

i. Lack of novelty over Ball or Niederweis

Ball and Niederweis disclose a method wherein the analyte to be detected may be an ion. Further, the final page of the Ball presentation suggests translocation of antibiotics (final bullet point). Thus Ball discloses a method wherein the analyte is an antibiotic drug. Claim 14 therefore lacks novelty over Ball or Niederweis.

Claim 15

'The method of any one of claims 1-5 or 7-14, wherein detecting the analyte comprises measuring an ion current as the analyte interacts with the tunnel to provide a current pattern, and wherein the appearance of a blockade in the current pattern indicates the presence of the analyte.'

i. Lack of novelty over Ball

The measurement of ion current as the analyte interacts with the tunnel to provide a current pattern, wherein the appearance of a blockade in the current pattern indicates the presence of an analyte is disclosed in Ball, see for example Fig 1 wherein the analyte is an ion. Therefore claim 15 lacks novelty over Ball.

ii. Lack of inventive step over Wong or Gundlach in view of Branton

Branton discloses that the presence of an analyte may be determined by measurement of ion current as the analyte interacts with the tunnel of the pore to provide current blockades. The skilled person starting from Wong or Gundlach would readily apply the teaching in Branton in order to determine an analyte. Thus claim 15 is not inventive over Wong or Gundlach in view of Branton.

Claim 16

'The method of claim 15, further comprising identifying the analyte in a method comprising comparing one or more blockades in the current pattern to (i) one or more blockades in the

same current pattern or (ii) one or more blockades in a known current pattern obtained using a known analyte under the same conditions.'

i. Lack of inventive step over Ball, Wong or Gundlach in view of Branton

Branton discloses that the analyte may be identified by comparing the current blockades obtained for an analyte to one or more blockades in a known current pattern using a known analyte (see the arguments against claim 13). Claim 16 therefore lacks an inventive step over Ball, Wong or Gundlach in light of Branton.

Claim 17

'The system or method of any one of claims 1-16, wherein at least one of the first or second conductive liquid media comprises a plurality of different analytes.'

i. Lack of novelty over Ball

Ball discloses a plurality of analytes in the first or second conductive media, namely ions and DNA. Therefore claim 17 lacks novelty over Ball.

ii. Lack of inventive step over Wong, Gundlach or Heinz in view of Kasianowicz or Branton

It is known from Kasianowicz that a mixture of two different analytes may be determined by addition of said analytes to the cis side of the pore, see for p13771, col. 2, para 4. Branton also discloses addition of a mixture of poly A and poly C to a pore and measuring the pore currents. It is considered that the skilled person aware of Kasianowicz or Branton would appreciate that a mixture of analytes could be applied to one side of the pore in the system or method of Wong, Gundlach or Heinz in order to determine them. Thus the subject matter of claim 17 is not inventive over Wong, Gundlach or Heinz in view of Kasianowicz or Branton.

Claim 18

'The method, system or mutant of any one of claims 1-18, wherein the Msp porin is a mutant single-chain Msp porin encoded by a nucleic acid sequence comprising:

a first and second nucleotide sequence, wherein the first nucleotide sequence encodes a first Msp monomer sequence and the second nucleotide sequence encodes a second Msp monomer sequence; and

the third nucleotide sequence encoding an amino acid linker sequence.'

i. Lack of inventive step over Niederweis, Faller or Ball in view of Jayasinghe.

It is known from Jayasinghe (page 2556) to form dimers of pore monomer units by genetic ligation. The dimers are shown to subsequently assemble to form a pore. Jayasinghe discloses the coupling of full-length LukS and LukF genes through a serine-glycine linker (page 2556, left hand column). LukS and LukF are the two constituent monomers of the octameric leukocidin pore which is a member of the family of β-barrel pore-forming toxins. It is considered that the skilled person would merely combine the teachings of Niederweis, Faller or Ball with Jayasinghe to provide the Msp porins of claim 18. Claim 18 therefore lacks an inventive step.

Claim 19

'The method, system or mutant of any one of claims 1-18, wherein the Msp porin is a mutant single-chain Msp porin, optionally encoded by a nucleic acid sequence comprising:

a first, second, third, fourth, fifth, sixth, seventh and eighth nucleotide sequence, wherein the first, second, third, fourth, fifth, sixth, seventh and eighth nucleotide sequences encode a first, second, third, fourth, fifth, sixth, seventh and eighth Msp monomer sequence, respectively; and

a ninth nucleotide sequence encoding an amino acid linker sequence.'

Methods of genetic ligation are well known in the art. It is obvious, having created the dimer of claim 18, to extend the concept to the expression of an entire pore from a single genetic construct. Claim 19 therefore lacks an inventive step.

Claim 20

'A mutant porin for use in a method according to any one of claims 2-5 or 7-19.'

i. Lack of novelty over Ball, Gundlach or Wong

Ball, Gundlach and Wong disclose a mutant porin for use in a method according to claim 4. Claim 20 therefore lacks novelty over Ball, Gundlach or Wong.

ii. Lack of inventive step Ball in view of Faller, Gundlach in view of Ball or Wong in view of Ball

Claim 20 as dependent upon claim 3 is considered to lack an inventive step over Ball in view of Faller, Gundlach in view of Ball or Wong in view of Ball, see the arguments for claim 3.

Claim 21

'A nucleic acid encoding a mutant porin according to claim 20, wherein said porin is for use in a method according to any one of claims 1-5 or 7-19.'

i. Lack of inventive step over Ball, Gundlach or Wong, in view of Faller

The amino acid sequence of MspA is known from Faller and a double mutant is disclosed in Ball. It would have been a routine task at the priority date to construct a nucleic acid encoding a mutant MspA. As such claim 21 is considered obvious over Ball, Gundlach or Wong, in view of Faller.

Art 83 EPC; Sufficiency of disclosure

Claims 1, 5 and 20 encompass a limitless genus of Msp porins, i.e., a genus which includes all forms of wild-type and mutated Msp porins suitable for the detection of an analyte. The Msp porins encompassed by the claimed method and system are defined for example at page 27, lines 18-22 and 28-31; and at page 28, lines 21-25:

> *'The Msp porin of any embodiment herein may be any Msp porin described herein, such as a wild-type MspA porin, a mutant MspA porin, a wild-type MspA paralog or homolog porin, or a mutant MspA paralog or homolog porin. The Msp porin may be encoded by a nucleic acid sequence encoding a single-chain Msp porin. Any Msp porin here may comprise any Msp monomer described herein, such as a mutant Msp monomer.'*
>
> *'A "mutant MspA porin" is a multimer complex that has at least or at most 70, 75, 80, 85, 90, 95, 98, or 99 percent or more identity, or any range derivable therein, but less than 100%, to its corresponding wild- type MspA porin and retains tunnel-forming capability.'*
>
> *'An Msp porin may comprise two or more Msp monomers. An "Msp monomer" is a protein monomer that is either a wild-type MspA monomer, a mutant MspA monomer, a wild-type MspA paralog or homolog monomer, or a mutant MspA paralog or homolog monomer, and retains tunnel-forming capability when associated with one or more other Msp monomers.'*

Example 2 at page 61 discloses the blockade characteristics of wild type MspA porins with and without analyte and notes:

'Addition of DNA as analyte to the cis compartment did not lead to any noticeable enhancement or alteration of these blockade characteristics.'

Therefore the present application fails to describe a reasonable number of species encompassed by the claimed genus, or the common structural / functional features shared by the members of the claimed genus which result in successful detection of the analyte DNA. Accordingly, the solution for detecting analytes using Msp porins as a class is not solved across the full scope of claim 1 for DNA as analyte, contrary to Article 56 EPC. Moreover, the subject matter of claim 1 is not fully supported by the description, contrary to Art 83 EPC.

Claim 8 is drawn to a method or system for determining an analyte wherein the Msp porin comprises a molecular motor capable of reducing the average translocation speed of an analyte. Claim 8 is dependent upon claim 1 which places no restriction on the type of analyte to be determined (i.e., includes a limitless genus of analytes). Therefore, the specification fails to describe a sufficient number of species encompassed by the claimed genus of analytes. As such the skilled person is not taught how to carry out the method of claim 8 for analytes other than DNA. Therefore the specification fails to contain a written description of the claimed method, and the manner and process of using it. Claim 8 is therefore insufficiently disclosed and lacks an inventive step.

Claim 10 is drawn to a method of determining a characteristic of an analyte. However the present application fails to describe how the claimed characteristics would be determined or which Msp porins would be capable of determining which characteristic. Therefore the specification fails to contain a written description of the claimed method, and the manner and process of using it. As such, the subject matter of claim 10 is insufficiently disclosed and lacks an inventive step.

Claims 11 and 12 are drawn to a method of detecting an analyte wherein the analyte may be a protein, a peptide or a polymer *per se.* The specification fails to provide any teaching with regard to the detection of polymer analytes other than at best, DNA. As such, the subject matter of claim 10 is insufficiently disclosed and lacks an inventive step.

Furthermore claim 11 is dependent upon claim 1. However, as discussed above with respect to claim 1, the genus of Msp porins encompassed by claims 1 and 11 is limitless and not supported by the present specification. Accordingly, claim 11 is not supported or enabled by the specification when, for example, the Msp porin is wild type MspA and where the analyte to be detected is DNA, because the specification fails to show wild type MspA as capable of detecting any DNA.

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Claim 13 is drawn to a method of detecting an analyte wherein the analyte is a polymer comprising one unit comprising the steps of measuring the ion current or optical signal as each unit of the polymer is separately translocated through the tunnel of the Msp porin to provide a current pattern comprising a blockade, wherein each blockade is associated with each unit. As shown in the priority document, the interaction between linear ssDNA 50mers and either M1MspA or M2MspA occurred too rapidly to produce resolvable blockades and that the duplex portion of the hairpin construct is required to produce millisecond time-scale blockades (see page 7, paragraph 2 – page 8, paragraph 1). Therefore, the claimed class of analytes encompassed by claim 13 is not supported by the priority document of the current specification and, in particular, is non-enabling for all types of DNA (as encompassed by claim 13), e.g.,when the Msp porin is mutant M1MspA or M2MspA (see also page 20651, column 1, start of paragraph 3 of Butler).

Claim 14 refers to a method of detecting an analyte, or determining the sequence of an analyte when dependent upon claim 13 whereby the polymer analyte to be detected or sequenced includes a drug, an ion, a pollutant, a nanoscopic agent or a biological warfare agent. The term 'drug' as defined in the application refers to any substance that may alter a biological process of a subject and the term 'biological warfare agent' refers to any organism or any naturally occurring bioengineered or synthesised component of any such microorganism...'. It is highly unlikely that an Msp porin would be capable of detecting analytes or determining the sequence of analytes across such a broad range. The application fails to teach what type of Msp porin would be suitable for the determination of each of the many analytes encompassed by the claim and consequently would leave the skilled person with an undue burden in determining which analytes might be capable of being detected or sequenced by which Msp porin. The subject matter in respect of these claims is therefore insufficiently disclosed and also lacks an inventive step.

Art 88 (3) EPC; Entitlement of the claims of the present application to the date of filing of the priority application.

Butler is effective prior art against claims not entitled to the date of filing of the priority application.

The present application claims priority to US application no. 61/098,938, which was filed on 22 September 2008.

The priority application discloses the following Msp porins only:

Wild type MspA

Mutant D90N/D91N/D93N ('M1MspA')

Mutant D90N/D91N/D93N/D118R/D134R/E139K ('M2MspA')

By contrast the present application discloses a broad range of Msp porins and specifically mentions in Table 7 a list of 140 mutant Msp porins. Claim 1 of the present application refers to an Msp porin *per se*, i.e. a limitless genus of Msp porins. Support for this broad genus is not found within the four corners of the priority document.

Claim 1 is therefore not entitled to the filing date of the priority application.

Claim 3 is drawn to a mutant MspA monomer comprising a mutation at position 93, a mutation at position 90, 91 or both positions 90 and 91. According to claim 3, one or more mutations may optionally be at the following amino-acid positions 88, 105, 108, 118, 134, or 139. Furthermore, claim 3 includes no limitation on the specific type of mutation at each amino-acid position. By contrast the priority document only discloses mutations at the 90, 91 and 93 residues whereby aspartic acid (D) was replaced with asparagine (N). Furthermore the priority document only discloses the mutation D90N/D91N/D93N/D118R/D134R/E139K where three specific mutations are made at the 118, 134 and 139 residues. Moreover, a mutation at the 88 position is not disclosed by the priority document. Therefore support for the broad scope of mutations encompassed by claim 3 (i.e. mutations at positions 93, 91 and 90, as well as the optional mutations at amino-acid positions 88, 105, 108, 118, 134, or 139) is not supported by the disclosure of the priority document. Claim 3 is therefore not entitled to the filing date of the priority application.

Claim 4 is drawn to the Msp porin having a vestibule and a diameter with a length of about 2 to about 6 nm and a constriction zone having a length and a diameter from about 0.3 to about 3nm. By contrast the specific Msp porins disclosed by the priority document have specific dimensions of the constriction zone (having a minimum diameter of about 1nm), and specific dimensions of the vestibule. Therefore the broad range or parameters encompassed by claim 4 finds no support in the priority document and is not unambiguously derivable from the disclosure in the priority document. Therefore claim 4 is not entitled to the filing date of the priority application.

Similarly, support for the subject matter of claims 5, 7-10, 13, 14 and 18-21 is not found within the priority document. These claims are therefore not entitled to the filing date of the priority application.

Annex 1

The subject matter of Ball was publically presented at the University of Washington during the period between June 19 - August 25, 2006.

Ball may be found from the following URL: http://www.int.washington.edu/REU/2006/Projects.html. Links to the paper and accompanying slides are no longer available, however the following cached URL: http://web.archive.org/web/20080829163353/http://www.int.washington.edu/REU/2006/Projects.html, shows that the documents were available to the public as .pdf documents on 29 August 2008, prior to the filing date of the application. Ball is also available from the following URL: http://www.int.washington.edu/REU/Projects.html. The website has a timestamp for modification: 'Last-Modified: 27 Sep 2006 17:02:52 GMT', which indicates that the link to Ball was publically accessible on this date. Ball is therefore prior art against the present application in accordance with Art 54(1) and (2) EPC.

Gundlach is available from the following cached URL: http://webcache.googleusercontent.com/search?q=cache:0leSFOKDov0J:www.experts.scival.com/uwashington/grantDetail.asp%3Ft%3Dep1%26id%3D252746%26o_id%3D91%26+2006+Engineering+MspA+for+Nanopore+Sequencing&cd=1&hl=en&ct=clnk&gl=uk . The cached URL is a snapshot of the following URL: http://www.experts.scival.com/uwashington/grantDetail.asp?t=ep1&id=252746&o_id=91& from which Gundlach is no longer available.

Gundlach is also available from following URL: http://projectreporter.nih.gov/project_info_description.cfm?aid=7192749&icde=14060103 The award notice date is reported as being 25 September 2006, prior to the filing date of the application. Gundlach is therefore prior art against the present application in accordance with Art 54(1) and (2) EPC.

Wong was publically presented on 16th August 2007 at the University of Washington. Wong is also available from the following URL: http://exp.washington.edu/urp/summerstem/archives/2007/2007posterprogram.pdf. Wong is therefore prior art against the present application in accordance with Art 54(1) and (2) EPC.