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28 September 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR PRODUCING SOLUBLE MULTI-MEMBRANE-SPANNING PROTEINS

(57) Abstract: Methods for producing membrane-spanning polypeptides in high yields, with native conformation, and/or in soluble form include solubilizing in non-ionic or zwitterionic detergents as well as use of promoters and expression vectors for expressing high yields of membrane-spanning polypeptides in bacterial cells. Mutated promoters provide tight control of membrane-spanning polypeptides in bacterial cell hosts.

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# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2005/047653

**A. CLASSIFICATION OF SUBJECT MATTER**  
 INV. C12N15/70 C07K14/705 C07K16/18 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  
 EPO-Internal, PAJ, Sequence Search, CHEM ABS Data, EMBASE, BIOSIS, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Further documents are listed in the continuation of Box C.

See patent family annex.

- \* Special categories of cited documents :
- |  |  |
|--|--|
| <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> |
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Date of the actual completion of the international search  <b>18 July 2006</b>	Date of mailing of the international search report  <b>01/08/2006</b>
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  <p style="text-align: center;"><b>Gurdjian, D</b></p>
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# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2005/047653

(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/53033 A (VANDERBILT UNIVERSITY) 21 October 1999 (1999-10-21)  abstract page 6, line 15 - page 8, line 32 page 11, paragraph 1	1-3, 5, 6, 8, 10, 17, 20, 23, 24, 28, 33-36, 55, 56, 81-83, 85, 94, 95, 98-104
Y	page 15, paragraph 4; examples 1,2	4, 7, 9, 11, 18, 19, 21, 22, 25-32, 37, 38, 54, 57-59, 62-68, 70, 73-80, 87, 89-93
X	<p style="text-align: center;">-----</p> XIE XIANG-QUN ET AL: "Expression, purification, and isotope labeling of cannabinoid CB2 receptor fragment, CB2(180-233)." PROTEIN EXPRESSION AND PURIFICATION. NOV 2004, vol. 38, no. 1, November 2004 (2004-11), pages 61-68, XP002379031 ISSN: 1046-5928 abstract page 63, left-hand column; figure 1	1-3, 5, 6, 9, 11-17, 24, 60, 61, 81-84, 86, 94
Y	<p style="text-align: center;">-----</p> WO 01/09189 A (GENENTECH, INC) 8 February 2001 (2001-02-08)  abstract; claims 1-47; example 6; sequence 16	4, 7, 9, 11, 22, 25-32, 57-59, 62, 68, 70, 87, 105-108
	<p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>	

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2005/047653

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/74903 A (AMGEN, INC; WELCHER, ANDREW, A; CALZONE, FRANK, J) 11 October 2001 (2001-10-11)	50, 105-108
Y	abstract; claims 1-71; figures 1-3  page 59	18,19, 21,38, 54, 63-67, 73-80, 89-93
Y	----- WO 01/55178 A (GENENTECH, INC; GODDARD, AUDREY; GURNEY, AUSTIN, L; SMITH, VICTORIA; H) 2 August 2001 (2001-08-02) abstract; claims 1-100; sequence 11	4,25-27, 62,68, 70,87
Y	----- WO 01/98453 A (PIERRE FABRE MEDICAMENT; CHEVALET, LAURENT) 27 December 2001 (2001-12-27)	11,58,59
A	page 2, line 3 - page 6, line 14; sequence 19	1,2,57
A	----- STENSTROM C M ET AL: "INFLUENCES ON TRANSLATION INITIATION AND EARLY ELONGATION BY THE MESSENGER RNA REGION FLANKING THE INITIATION CODON AT THE 3' SIDE" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER, AMSTERDAM, NL, vol. 288, no. 1/2, 2002, pages 1-8, XP008051814 ISSN: 0378-1119	1,2,11, 16,57-59
A	----- DATABASE JPO Proteins [Online] 1 July 1997 (1997-07-01), "Peptide encoding leader peptide trpL." XP002379034 retrieved from EBI accession no. JPOP:E50448 Database accession no. E50448 the whole document -& PATENT ABSTRACTS OF JAPAN vol. 015, no. 324 (C-0859), 19 August 1991 (1991-08-19) & JP 03 123486 A (HITACHI LTD), 27 May 1991 (1991-05-27) abstract	1,11
A	----- US 6 242 177 B1 (SIMMONS LAURA C ET AL) 5 June 2001 (2001-06-05)  abstract; figures 15,18	4,11, 25-27, 62,68, 70,87
	----- -/--	

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2005/047653

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/14407 A (M-PHASYG GMBH; KIEFER, HANS; MAIER, KLAUS) 1 March 2001 (2001-03-01) abstract; claims 1-14 page 7, paragraph 5 - page 9, paragraph 3 -----	33-36
Y	TAYLOR CHRISTOPHER M ET AL: "Enhanced resolution of glycosylphosphatidylinositol-anchored and transmembrane proteins from the lipid-rich myelin membrane by two-dimensional gel electrophoresis." PROTEOMICS. JUL 2003, vol. 3, no. 7, July 2003 (2003-07), pages 1303-1312, XP002390460 ISSN: 1615-9853 abstract page 1304, left-hand column, paragraph 3 page 1306, left-hand column -----	37
X	POLYAK M J ET AL: "Identification of a cytoplasmic region of CD20 required for its redistribution to a detergent-insoluble membrane compartment." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 OCT 1998, vol. 161, no. 7, 1 October 1998 (1998-10-01), pages 3242-3248, XP002390461 ISSN: 0022-1767 abstract page 3246 - page 3247; figure 8 -----	40-43, 45, 46, 102-104
X	WO 97/21829 A (MERCK PATENT GMBH; STRITTMATTER, WOLFGANG; MATZKU, SIEGFRIED; RIESENBE) 19 June 1997 (1997-06-19) abstract; claims 1-14 -----	50, 52
Y	page 6, paragraph 2 -----	51, 105-108
X	EP 0 300 425 A (BOEHRINGER MANNHEIM GMBH) 25 January 1989 (1989-01-25) page 4, paragraph 3; claims 1-16 -----	50
Y	-----	51, 105-108
Y	EP 0 303 925 A (F. HOFFMANN-LA ROCHE & CO. AKTIENGESELLSCHAFT; F. HOFFMANN-LA ROCHE AG) 22 February 1989 (1989-02-22) page 2, line 44 - page 3, line 56; claims 1-7 -----	51
.	----- -/--	

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2005/047653

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ERNST JAMES A ET AL: "Isolation and characterization of the B-cell marker CD20." BIOCHEMISTRY. 22 NOV 2005, vol. 44, no. 46, 22 November 2005 (2005-11-22), pages 15150-15158, XP002390462 ISSN: 0006-2960 the whole document -----	1-108

# INTERNATIONAL SEARCH REPORT

International application No.  
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## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-32,54-94

An expression construct for enhanced expression of membrane spanning polypeptides , comprising a promoter , a positive and a a negative regulatory element that enhances control over the promoter , and a leader sequence comprising a translation initiation enhancer , corresponding method of producing a membrane spanning polypeptide using such constructs

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2. claims: 33-39

A method of isolating an expressed membrane-spanning polypeptide from bacterial cells comprising the use of non-ionic or zwitterionic detergents

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3. claims: 40-49,95-104

Recombinant CD20 related polypeptides corresponding to seq.id.1-4,6 and method of producing a CD20-antibody , method of detecting binding to a ligand , and corresponding surface assay .

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4. claims: 50-53,105-108

A mutant phoA promoter with negative control elements related to seq.id.15,16 and corresponding method for expressing a membrane spanning polypeptide in a bacterial cell .

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**INTERNATIONAL SEARCH REPORT**  
information on patent family members

International application No  
PCT/US2005/047653

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9953033	A	21-10-1999	AU 3644499 A	01-11-1999
WO 0109189	A	08-02-2001	AU 776600 B2 AU 6391300 A CA 2378182 A1 EP 1204682 A2 JP 2003508028 T	16-09-2004 19-02-2001 08-02-2001 15-05-2002 04-03-2003
WO 0174903	A	11-10-2001	AU 5108201 A CA 2401948 A1 EP 1272518 A2 JP 2003529361 T MX PA02009523 A US 2002064823 A1	15-10-2001 11-10-2001 08-01-2003 07-10-2003 12-02-2003 30-05-2002
WO 0155178	A	02-08-2001	AU 3458401 A CA 2395832 A1 EP 1263780 A2 JP 2003523207 T MX PA02007190 A	07-08-2001 02-08-2001 11-12-2002 05-08-2003 12-02-2003
WO 0198453	A	27-12-2001	AU 6922401 A BR 0111907 A CA 2413612 A1 CN 1443242 A EP 1315822 A2 FR 2810675 A1 JP 2004500875 T MX PA02012880 A US 2004260060 A1	02-01-2002 30-12-2003 27-12-2001 17-09-2003 04-06-2003 28-12-2001 15-01-2004 14-05-2003 23-12-2004
JP 03123486	A	27-05-1991	NONE	
US 6242177	B1	05-06-2001	US 2003017592 A1	23-01-2003
WO 0114407	A	01-03-2001	AT 253587 T DE 19939246 A1 DK 1203012 T3 EP 1203012 A2 ES 2208416 T3 JP 2003507490 T US 6939951 B1 US 2006058509 A1	15-11-2003 22-02-2001 02-02-2004 08-05-2002 16-06-2004 25-02-2003 06-09-2005 16-03-2006
WO 9721829	A	19-06-1997	AT 213271 T AU 716612 B2 AU 1032597 A BR 9611937 A CA 2240097 A1 CN 1219203 A CZ 9801652 A3 DE 59608743 D1 DK 866876 T3 ES 2171752 T3 HU 0000317 A2 JP 2000501936 T NO 982672 A PL 327188 A1	15-02-2002 02-03-2000 03-07-1997 02-03-1999 19-06-1997 09-06-1999 12-08-1998 21-03-2002 27-05-2002 16-09-2002 28-06-2000 22-02-2000 10-06-1998 23-11-1998

# INTERNATIONAL SEARCH REPORT

information on patent family members

In  national application No  
PCT/US2005/047653

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9721829	A		PT 866876 T	31-07-2002
			RU 2201455 C2	27-03-2003
			SK 74098 A3	04-11-1998
			UA 61066 C2	17-11-2003
			US 6410270 B1	25-06-2002
			ZA 9610384 A	23-06-1997
EP 0300425	A	25-01-1989	DE 3723992 A1	02-02-1989
			JP 1043195 A	15-02-1989
			JP 2038729 C	28-03-1996
			JP 7073505 B	09-08-1995
EP 0303925	A	22-02-1989	AT 123526 T	15-06-1995
			DE 3853932 D1	13-07-1995
			DK 443288 A	18-02-1989
			JP 1095790 A	13-04-1989
			JP 2624308 B2	25-06-1997