(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 June 2011 (09.06.2011)

PCT

(10) International Publication Number
WO 2011/069156 A3

(51) International Patent Classification:
C12N 5/071 (2010.01) *C40B 30/04* (2006.01)

(21) International Application Number:
PCT/US2010/059093

(22) International Filing Date:
6 December 2010 (06.12.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/266,805 4 December 2009 (04.12.2009) US

(71) Applicant *(for all designated States except US)*: **DUKE UNIVERSITY** [US/US]; 2812 Erwin Road, Suite 306, Office of Science & Technology, Durham, North Carolina 27705 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MATSUNAMI, Hiroaki** [JP/US]; 4606 Sycamore Shoals Road, Durham, North Carolina 27705 (US). **LI, Yun** [CN/US]; 4816 Larchwood Avenue, Philadelphia, Pennsylvania 19143 (US).

(74) Agent: **GOETZ, Robert A.**; Casimir Jones S.C., 2275 Deming Way, Suite 310, Middleton, Wisconsin 53562 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
11 August 2011

(54) Title: COMPOSITIONS AND METHODS FOR ENHANCING ODORANT RECEPTOR ACTIVITY

(57) Abstract: The present invention relates to polypeptides capable of modulating odorant receptor activation. In particular, the present invention provides polypeptides (e.g., type 3 muscarinic actetylcholine receptor M3) capable of enhancing odorant receptor activation. The present invention further provides assays for the detection of ligands specific for various odorant receptors. Additionally, the present invention provides methods of screening for polypeptide polymorphisms and mutations associated with odorant receptor activation (e.g., polymorphisms and mutations associated with muscarinic actetylcholine receptor polypeptides (e.g., M1, M2, M3, M4, M5)), as well as methods of screening for therapeutic agents, ligands, and modulators of such proteins.

WO 2011/069156 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/59093

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 5/071; C40B 30/04 (2011.01)
USPC - 435/366; 506/9
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)
USPC: 435/366; 506/9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/375 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PubWEST (PGPB, EPAB, JPAB, USPT); Google Scholar: Odorant receptor, olfactory,olfaction, odorant receptor modulation, muscarinic receptor (M1, M2, M3, M4, M5), G-protein coupled receptors (GPCRs), coexpression.
GenCore 6.3: SEQ ID NO: 1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BUSH et al. Specificity of olfactory receptor interactions with other G protein-coupled receptors. J Biol Chem 2007, 282(26):19042-19051; pg 19043 right col para 1, pg 19045 fig 1	1-4
Y	GenBank Direct Submission AK138282.1. Mus musculus adult male hypothalamus cDNA, RIKEN full-length enriched library, clone:A230054O16 product:cholinergic receptor, muscarinic 1, CNS, full insert sequence. 19 September 2008. [Retrieved from the internet 26.04.2011: <URL: http://www.ncbi.nlm.nih.gov/nuccore/74209797?sat=OLD05&satkey=3046464 >]; pg 3, nucleotides 527-1905	1-4
Y	COOK et al. Large-scale production and study of a synthetic G protein-coupled receptor: human olfactory receptor 17-4. Proc Nat Acad Sci 21 July 2009, 106(29):11925-11930; pg 11925 right col para 3	4

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2011 (26.04.2011)	16 JUN 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/59093

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore 6.3: SEQ ID NO: 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/59093

Box No. 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 No. 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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-5, drawn to a cell line expressing an odorant receptor, wherein said expression is localized to the cell surface, wherein said cell line comprises a heterologous gene, wherein said heterologous gene encodes a Muscarinic Acetylcholine Receptor polypeptide. The first invention is restricted to a SEQ ID NO:1. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. The exact claims searched will depend on the specifically elected SEQ ID NO(s).
[NOTE: claim 5 was not searched, because it is drawn to a non-elected subject matter.]

----------continued on Extra 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 additional fees, this Authority did not invite payment of additional fees.

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.:
Claims 1-4 restricted to SEQ ID NO: 1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

********** Supplemental Box **********

continuation of Box III (Lack of Unity of Invention)

Group II, claims 6-29, drawn to a method for identifying an odorant receptor ligand, comprising:
a) providing
i) a cell comprising an odorant receptor, wherein said cell further comprises a heterologous nucleic acid encoding a polypeptide comprising an amino acid sequence that is at least 80% identical to SEQ ID No.3, wherein said polypeptide is capable of promoting odorant receptor activity;
ii) at least one test compound;
b) exposing said test compound to said cell; and
c) detecting the activity of said odorant receptor.

Group III, claims 30-53, drawn to a method for enhancing the activation of an odorant receptor in an olfactory sensory neuron of a patient comprising co-expressing a non-OR GPCR and an odorant receptor in said neuron.

Group IV, claims 54-59, drawn to a method for screening novel receptors for cognate ligands in a heterologous cell system comprising contacting said cells with a ligand of interest, wherein said cell co-expresses a non-OR GPCR and an odorant receptor; and measuring the activity of said odorant receptor.

The inventions listed as Groups I+ through IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups I+ and II do not include the inventive concept of a method for enhancing the activation of an odorant receptor in an olfactory sensory neuron of a patient comprising co-expressing a non-OR GPCR and an odorant receptor in said neuron, as required by Groups III-IV.

The inventions of Group I+ do not include the inventive concept of a method for identifying an odorant receptor ligand, as required by Group II.

The inventions of Groups I+ and II share the technical feature of a cell line expressing an odorant receptor, wherein said expression is localized to the cell surface, wherein said cell line comprises a heterologous gene, wherein said heterologous gene encodes a Muscarinic Acetylcholine Receptor polypeptide. However, this shared technical feature does not represent a contribution over prior art as being anticipated by the publication titled "Specificity of olfactory receptor interactions with other G protein-coupled receptors" by BUSH et al. (hereinafter "Bush") (J Biol Chem. 2007, 282(26):19042-51) that teaches a cell line expressing an odorant receptor , wherein said expression is localized to the cell surface (abstract; " functional expression of the OR M71 [i.e. odorant receptor M71] at the plasma membrane of HEK-293 cells. In the present study, we examined the specificity of M71 interactions with other G protein-coupled receptors (GPCRs)"; pg 19045 fig 1 ordinate--cell surface expression level of M71), wherein said cell line comprises a heterologous gene that encodes a muscarinic acetylcholine receptor polypeptide (pg 19045 fig 1; FLAG-M71-GFP was expressed alone or co-expressed with 42 other GPCRs in HEK-293 cells including the muscarinic M1 receptor [numbered in figure as 18]). As said cell line was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another technical feature of the inventions listed as Group I+ is the specific nucleic acid sequence recited therein. The inventions do not share a special technical feature, because 1) Bush teaches that the heterologous gene is the muscarinic M1 receptor (pg 19043 right col para 1; "M1-5 acetylcholine receptor constructs were kindly provided by Allan Levey (Emory University School of Medicine)"), and 2) US 2009/0178153 A1 to Gaitanaris, et al. (09 July 2009) discloses a nucleic acid sequence 99.0% identical to the claimed SEQ UD NO: 1 (Gaitanaris, et al., SEQ ID NO 1236). Without a shared special technical feature, the inventions lack unity with one another.

The inventions of Groups III and IV share the technical feature of a method for enhancing the activation of an odorant receptor in an olfactory sensory neuron of a patient comprising co-expressing a non-OR GPCR and an odorant receptor in said neuron. However, this shared technical feature does not represent a contribution over prior art as as being obvious over Bush that teaches co-expressing a non-OR GPCR and an odorant receptor in said neuron (abstract; "functional expression of the OR M71 [i.e. odorant receptor M71] at the plasma membrane of HEK-293 cells. In the present study, we examined the specificity of M71 interactions with other G protein-coupled receptors (GPCRs). M71 was co-expressed in HEK-293 cells with 42 distinct GPCRs"; pg 19045 fig 1--coexpression of GPCRs numbered 8 [beta 2 adrenergic receptor] and 25 [Purigenic receptor P2Y1] resulted in at least 5x greater expression of M71 over control cells expressing M71 alone; pg 19047 fig 6A--"M71 co-expressed with G alpha0 in addition to P2Y1R exhibits increased phospho-ERK1/2signalingwhenstimulated with acetophenone"). As said method would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I+ through IV therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.