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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
24 July 2008

(54) Title: MODIFIED TRAFFICKING PATTERNS FOR ARRESTIN AND G-PROTEIN-COUPLED RECEPTORS VIA ARRESTIN-UBIQUITIN CHIMERA

(57) Abstract: The present invention relates to a modified arrestin which includes an arrestin molecule and a ubiquitin molecule. This modified arrestin has an increased affinity for a GPCR, and traffics with the GPCR into endosomes. The present invention further relates to a method of screening compounds and sample solutions for a GPCR agonist, antagonist, inverse agonist, or desensitization active compound. The modified arrestin is useful in the methods of the present invention.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/02029

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C07K 19/00**(2006.01);**C07K 14/47**(2006.01)
C07H 21/04(2006.01)

USPC: 530/350;536/23.4
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)
U.S. : 530/350; 536/23.4

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 practicable, search terms used)
STN, MEDLINE, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHENOY, S. K. Regulation of Receptor Fate by Ubiquitination of Activated Beta2-Adrenergic Receptor and Beta-Arrestin. 9 November 2001. 294: 1307-1313, especially page 1310.	1-6 and 18-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* 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 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
25 April 2008 (25.04.2008)

Date of mailing of the international search report
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Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/02029

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.: 7 and 9-11
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:
Please See Continuation Sheet

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:
Please See Continuation 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 any 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.: 1-6 and 18-22, first species in each category

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

Continuation of Box II Reason 2:
Claim 7 is unsearchable because it recites "The modified arrestin of cliam 1, wherein the modified arrestin comprises the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3", but the Sequence Listing file don 5/6/2004 indicates that SEQ ID NO: 2 is a nucleic acid sequence.

Claim 9 is unsearchable because it recites a "nucleic acid comprising the nucleic acid of SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6", bu the Sequence Listing filed on 5/6/2004 indicates that SEQ ID NO: 5 is an amino acid sequence.

Claims 10 and 11 are unsearchable because they each depend from claim 9.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
Group I, claims 1-6 and 18-22, drawn to a modified arrestin comprising an arrestin and an ubiquitin moiety.

Group II, claims 8, 12-17, 23 and 24, drawn to a nucleic acid encoding a modified arrestin, expression vectors and host cells comprising said nucleic acid, and substrates with a plurality of said cells.

Group III, claims 25-31, drawn to a method of screening compounds comprising exposing a cell expressing a modified arrestin to a test compound.

Group IV, claim 32, drawn to a modulator compound.

Group V, claim 33, drawn to a method of treating a subject comprising administering a modulator compound.

Group VI, claims 34 and 35, drawn to a method of treating a subject comprising administering a nucleic acid encoding a modified arrestin (gene therapy).

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

A) The 1st species of label is radioisotope and the 2nd-6th species of label are epitope tag, affinity label, enzyme, fluorescent group or chemiluminescent group.

The claims correspond to the species listed above in the following manner:
Claims 6 and 29 correspond to each species (recited as a Markush-type group).
The following claim(s) are generic: 1-5, 8, 12-28 and 30-35.

B) The 1st species of arrestin is a visual arrestin and the 2nd-4th species of arrestin are a cone arrestin, a beta-arrestin-1, and a beta-arrestin-2.

The claims correspond to the species listed above in the following manner:
Claim 22 corresponds to each species (recited as a Markush-type group).
The following claim(s) are generic: 1-6, 8, 12-21 and 23-35.

~~C) The 1st species of GPCR is mu opioid and 2nd-5th species of GPCR are β1AR, β2AR, dopamine receptor or Class B GPCR.~~

The claims correspond to the species listed above in the following manner:
Claims 20 and 28 correspond to each species of class A receptor (first four species only), with claim 28 reciting each of the first four species as a Markush-type group.
Claim 27 correspond to the fifth species (Class B GPCR).
The following claim(s) are generic: 1-6, 8, 12-19, 21-26 and 29-35.

The fees previously paid cover the first group (I) and the first species of each category (label, arrestin and GPCR). In order for more than one group or more than one species of each category to be examined, the appropriate additional fees must be paid. Each additional species counts as an additional group. There are 5 additional groups of inventions (II-VI above), 5 additional species of label, 3 additional species of arrestin and 4 additional species of GPCR. Therefore, for all of the groups and species to be searched, search fees for an additional 17 groups must be paid.

The inventions listed as Groups I-VI do not relate to a single general inventive concept under PCT rule 12.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. 1.475 (B-D), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto, unless such a group lacks a special technical feature. In the instant case, the technical feature linking groups I-VI appears to be that they all relate to a "modified arrestin". The specification defines the term "modified arrestin" as "an arrestin that has one or more ubiquitin moieties and a label molecule associated or attached to the arrestin" (pg 20). Thus claim 1 encompasses modified arrestin that encompasses an arrestin and a ubiquitin molecule with the following functional characteristic: "increased affinity for a GPCR, as compared to the affinity of a wild-type increased affinity for a GPCR, and wherein increased affinity means that the arrestin remains associated with the GPCR and traffics with the GPCR into endosomes, and wherein the arrestin does not dissociate at or near the plasma membrane". However, the specification further indicates that the addition of ubiquitin to an arrestin results in this functional characteristic: "the addition of ubiquitin moieties to arrestin increases the affinity of arrestin to binding to a GPCR" (pg 26). Therefore, the special technical feature appears to be any ubiquitinated arrestin that associates with a GPCR. Shenoy et al (2001. Science. 294: 1307-1313) teach a modified arrestin that meets the limitations of claim 1. Shenoy et al teach ubiquitinated β-arrestin that associates with the β2-adrenergic receptor (which is a GPCR), wherein said ubiquitination is required for receptor internalization. Therefore, the technical feature linking the inventions of group I-VI does not constitute a special technical feature as defined by PCT rule 13.2, as it does not define a contribution over the prior art.

The species of label listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each label is a structurally different molecule, and each is detected using different means.

The species of arrestin listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each arrestin is a different protein (which each has a discrete amino acid sequence) and which is therefore a structurally and functionally different molecule.

The species of GPCR listed above do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: each GPCR is a different protein (which each has a discrete amino acid sequence) and which is therefore a structurally and functionally different molecule.

The Authority therefore considers that the seventeen inventions do not share a special technical feature within the meaning of PCT Rule 13.2 and thus do not relate to a single general inventive concept within the meaning of PCT Rule 13.1.