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(54) Title: COMPOSITIONS AND METHODS FOR CRISPR MODULATION

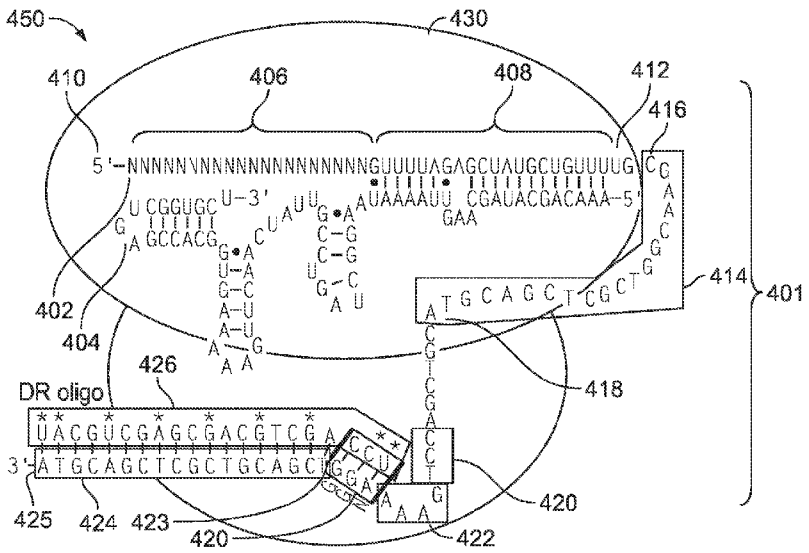

FIG. 4B

(57) Abstract: A composition, a method for selectively altering expression of a gene, and a method of selectively modulating activity of a Cas protein is provided. The composition includes a nucleotide regulator wherein the regulator is an oligonucleotide sequence at least 95% identical to a sequence selected from the group consisting of SEQ ID NOs. 1-6 and 8-19 or a functional fragment thereof and may comprise a derepressor wherein the derepressor has an oligonucleotide sequence at least 95% identical to SEQ. ID. NO. 20 or a functional fragment thereof. The method of selectively altering gene expression includes administering the composition containing a nucleotide regulator. The method of selectively modulating activity of a Cas protein includes administering a composition of a repressor and a derepressor to a subject, wherein the repressor competes with or disrupts the PM module structure of the Cas protein, and the derepressor reverses the disruption of the repressor.

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[Continued on next page]

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06 June 2024 (06.06.2024)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/75654

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/11 (2024.01)
ADD. C12N 9/22 (2024.01)

CPC - INV. C12N 15/11

ADD. C12N 2310/113, C12N 2310/20, C12N 2310/3519, C12N 9/22

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)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2021/0355488 A1 (BOARD OF TRUSTEES OF SOUTHERN ILLINOIS UNIVERSITY) 18 November 2021 (18.11.2021) abstract; para [0075]	1-8
A	GenBank Accession No CP082273, Nocardioides coralli strain SCSIO 67246 chromosome, complete genome. 05 September 2021 [online]. [Retrieved on 04 March 2024]. Retrieved from the internet: <URL: https://www.ncbi.nlm.nih.gov/nuccore/CP082273> full document, especially DNA sequence nts 1799249-1799202	1-8
A	CN 114457103 A (SHANGHAI TRAFFIC UNIVERSITY) 10 May 2022 (10.05.2022) para [0006]; SEQ ID NO: 3	1-8
A	US 2022/0073912 A1 (SYNTHEGO CORPORATION) 10 March 2022 (10.03.2022) full document	1-8

☐ 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

"D" document cited by the applicant in the international application

"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
05 March 2024	APR 09 2024
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/75654

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:

   a. [X] forming part of the international application as fi led.

   b. [ ] furnished subsequent to the international fi ling date for the purposes of international search (Rule 13ter.1(a)),

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/75654

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. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+, claims 1-8, directed to a composition comprising a nucleotide regulator. The composition will be searched to the extent that the nucleotide regulator sequence encompasses SEQ ID NO: 1. The first named invention was determined based on this being the first listed nucleotide regulator sequence (claim 1). This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-8 encompass this first named invention, and thus these claims will be searched without fee to the extent that the nucleotide regulator sequence is SEQ ID NO: 1. Additional nucleotide regulator sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected nucleotide regulator sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be where the nucleotide regulator sequence is SEQ ID NO: 2, (claims 1-8). --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.:
1-8, limited to nucleotide regulator sequence 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.

--continued from: Box No. III Observations where unity of invention is lacking--

Group II, claims 9-16, directed to a method for selectively altering expression of at least one gene product.

Group III+, claims 17-20, directed to a method for selectively altering expression/activity of at least one gene product (Cas protein) in a subject. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to encompass nucleotide regulator sequence is SEQ ID NO: 1 for an additional fee and election as such. It is believed that claims 17-20 read on this exemplary invention. Additional nucleotide regulator sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected nucleotide regulator sequence(s). Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be where the nucleotide regulator sequence is SEQ ID NO: 2, (claims 17-20).

The inventions listed as Groups I+, II, and III+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I+ has the special technical feature of a composition comprising or consisting of a nucleotide regulator, that is not required by Group II or III+.

Group II has the special technical feature of a method for selectively altering expression of at least one gene product, that is not required by Group I+ or III+.

Group III+ has the special technical feature of a method for selectively modulating the activity of a Cas protein, that is not required by Group I+ or II.

The inventions of Group I+ and the inventions of Group III+ each include the special technical feature of a unique nucleotide regulator sequence, and is considered a distinct technical feature.

Common technical features

The inventions of Groups I+, II and III+ share the common technical feature of a composition comprising a nucleotide regulator.

No technical features are shared between the nucleotide regulator sequences of Group I+ and accordingly these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to share the technical features of including: a composition comprising a nucleotide regulator, these shared technical features are previously disclosed by the prior art, as further discussed below.

No technical features are shared between the nucleotide regulator sequences of Group III+ and accordingly these groups lack unity a priori.

Additionally, even if the inventions listed as Group III+ were considered to share the technical features of including: a method of selectively modulating the activity of a Cas protein comprising administering a composition comprising a repressor and a derepressor to a subject, wherein the repressor competes with or disrupts the PM module structure of the Cas protein, and wherein the derepressor reverses the disruption of the repressor, these shared technical features are previously disclosed by the prior art, as further discussed below.

The feature shared by Groups I+, II and III+ and the feature shared by the inventions listed as Group I+ and III+ are taught by US 2021/0355488 A1 to Board of Trustees of Southern Illinois University, (hereinafter "SIU"), and WO 2021/108442 A2 to the Regents of the University of California, (hereinafter "UC").

SIU teaches a composition comprising a nucleotide regulator (abstract "A CRISPR inhibitor molecule is provided, comprising an artificial nucleic acid construct having a first polynucleotide, the inhibitor molecule capable of establishing several points of contact with a CRISPR protein and high binding affinity thereto").

UC teaches a method of selectively modulating the activity of a Cas protein comprising administering a composition comprising a repressor and a derepressor to a subject, wherein the repressor competes with or disrupts the PM module structure of the Cas protein, and wherein the derepressor reverses the disruption of the repressor (para [0005] "polypeptides that inhibit activity of a CRISPR/Cas effector polypeptide, nucleic acids encoding the polypeptides"; [0181] "An implantable device suitable for use in delivering a polypeptide and/or a nucleic acid (e.g., one or more of: a) recombinant expression vector comprising a nucleotide sequence encoding an Acr polypeptide of the present disclosure...(where the Cas9 polypeptide is one whose activity can be inhibited by the Acr polypeptide)"; [0398] "A number of reported AcrIIA inhibit their cognate Cas9 by competing with target DNA through PAM mimicry (Yang and Patel 2017; Jiang et al.2019). It was noted that SinCas9 was susceptible to inhibition by AcrIIA4 (FIG.18A) and AcrIIA2 (FIG.17D), both PAM mimics that inhibit PAM recognition by SpyCas9"; [0118] "In some cases, the nucleotide sequence encoding an Acr polypeptide of the present disclosure, or encoding an Acr fusion polypeptide of the present disclosure, is operably linked to one or more of a promoter"; [0121] "A suitable promoter may be an inducible promoter (i.e., a promoter whose state, active/"ON" or inactive/"OFF", is controlled by an external stimulus, e.g., the presence of a particular temperature, compound, or protein)").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I+, II and III+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.