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(54) Title: RIBOZYME-ACTIVATED RNA CONSTRUCTS AND USES THEREOF

In vitro transcribed RNA delivery

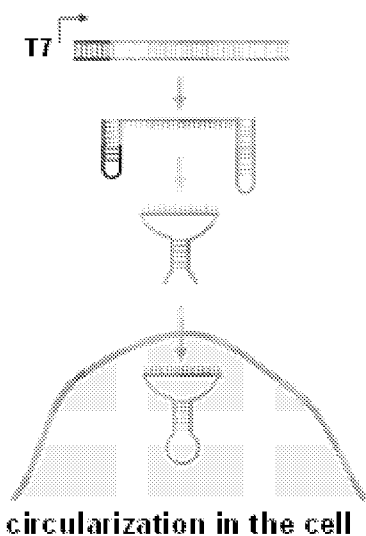


FIG. 15

(57) Abstract: The disclosure provides for ribozyme-mediated fusion constructs and systems and methods thereof, for use in a variety of applications, including for inducible gene expression systems, gene therapy, and combinatorial screening.



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))*

(88) Date of publication of the international search report:

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/012004

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/09; C12N 15/11; C12N 15/113; C12Q 1/68 (2022.01)

CPC - C12N 15/113; C12N 2310/11; C12N 2310/12; C12N 2310/53 (2022.05)

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.
X	WO 2018/237372 A1 (CORNELL UNIVERSITY) 27 December 2018 (27.12.2018) entire document	1, 2, 9-12
A	WO 2020/209803 A1 (AGENCY FOR SCIENCE TECHNOLOGY AND RESEARCH) 15 October 2020 (15.10.2020) entire document	1, 2, 9-12
A	LITKE et al. "Highly efficient expression of circular RNA aptamers in cells using autocatalytic transcripts," Nat Biotechnol, June 2019, Vol. 37, Pgs. 667-675. entire document	1, 2, 9-12
A	US 2020/0032253 A1 (MEIRAGTX UK II LIMITED) 30 January 2020 (30.01.2020) entire document	1, 2, 9-12
A	US 2015/0056174 A1 (MULLIGAN et al) 26 February 2015 (26.02.2015) entire document	1, 2, 9-12

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	"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
"D" document cited by the applicant in the international application	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search 26 May 2022	Date of mailing of the international search report JUN 16 2022
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Taina Matos Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/012004

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. forming part of the international application as filed:

in the form of an Annex C/ST.25 text file.

on paper or in the form of an image file.

b. furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. furnished subsequent to the international filing date for the purposes of international search only:

in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1, 1354, 1356, and 1358 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/012004

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.: 17
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:

See extra sheet(s).

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, 2, 9-12

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

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 examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-16, 18-30, and 32-67 are drawn to ribozyme activated RNA-constructs comprising: one or more ribozymes; and one or more RNA coding sequences for at least one polypeptide of interest; wherein the production of a translatable transcript of the one or more RNA coding sequences for at least one polypeptide of interest is activated by or dependent upon the activity of the one or more ribozymes.

The first invention of Group I+ is restricted to a ribozyme activated RNA-construct, further comprising: a first engineered RNA element, an RNA coding sequence for a polypeptide of interest and a complementary sequence to a sequence of a second engineered RNA element, and a first self-cleaving ribozyme; a second engineered RNA element comprising an optional primer region, an optional barcode region, an RNA coding sequence for a polypeptide of interest and a complementary sequence to a sequence of the first engineered RNA element, and a second self-cleaving ribozyme; wherein cleavage of the first and second engineered RNA elements by the first and second self-cleaving ribozymes, respectively, provides for a hybridization construct that comprises a region of dsRNA from the complementary sequences being hybridized together, wherein the hybridization construct can be further ligated by an RNA ligase to form an RNA-fusion construct, and wherein expression from the RNA-fusion construct produces the at least one polypeptide of interest. It is believed that claims 1, 2, and 9-12 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional construct(s) and/or construct element(s) and/or embodiment(s) associated with said construct(s) and/or construct element(s) for each additional embodiment to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected embodiment requires the selection of a single definition for each additional construct or construct element, including, where applicable, specifying individual sequence(s) of the elements. An exemplary election would be a primer region. Additional embodiment(s) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on 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/examined.

Groups I+ share the technical features of: a ribozyme activated RNA-construct comprising: one or more ribozymes; and one or more RNA coding sequences for at least one polypeptide of interest; wherein the production of a translatable transcript of the one or more RNA coding sequences for at least one polypeptide of interest is activated by or dependent upon the activity of the one or more ribozymes. However, these shared technical features do not represent a contribution over the prior art, as disclosed by US 2020/0032253 to MeiraGTx UK II Limited (hereinafter, "Meiragtx").

Specifically, Meiragtx discloses a ribozyme activated RNA-construct comprising: one or more ribozymes (constructs for the regulation of gene expression by aptamer-based modulation of self-cleaving ribozymes, Abstract; a polynucleotide cassette for the regulation of a target gene comprising a riboswitch that comprises a twister ribozyme, Para. [0016]); and one or more RNA coding sequences for at least one polypeptide of interest (making and using riboswitches that ...increase target gene expression in response to an aptamer ligand, Abstract; polynucleotide cassette for the regulation of the expression of a target gene, Para. [0016]); wherein the production of a translatable transcript of the one or more RNA coding sequences for at least one polypeptide of interest is activated by or dependent upon the activity of the one or more ribozymes (the riboswitch is an "on riboswitch" because aptamer/ligand binding inhibits the ribonuclease function of the twister ribozyme decreasing cleavage of the target gene RNA...and thereby increasing expression, Para. [0016]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.