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(54) Title: VIRUS-LIKE PARTICLES COMPRISING CIRCULAR MRNA EXPRESSION SYSTEMS AND METHODS OF USE THEREOF

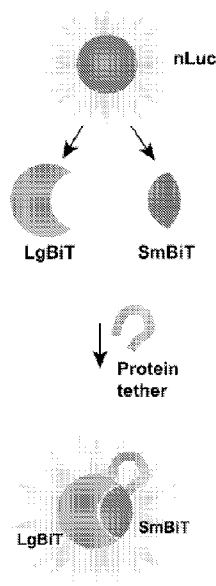


FIG. 1A

(57) Abstract: The present invention relates to a virus-like particle (VLP). The virus-like particle comprising a circular RNA molecule comprising: a first ligation sequence; an internal ribosomal entry site (IRES) coupled to an RNA molecule encoding one or more peptide(s), where the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s) is positioned 3' to the first ligation sequence; a second ligation sequence positioned 3' to the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s); and a plurality of one or more proteins that can self-assemble into a nanoparticle. Also disclosed are compositions and methods of making and using such virus-like particles.

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

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:

13 February 2025 (13.02.2025)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033132

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.
 - b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.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:

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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.: **5-40, 45-111, 116-127, 132-139**
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 1-4, 41-44, 112-115, and 128-131, directed to a virus-like particle (VLP) comprising: a circular RNA molecule. The VLP will be searched to the extent that the VLP encompasses SEQ ID NO: 1. The first named invention was determined based on first claim IRES sequence (claims 1-2). 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 1-4, 41-44, 112-115, and 128-131, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a VLP comprising SEQ ID NO: 1. Additional VLPs comprising additional IRES sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected VLPs. 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 a virus-like particle (VLP) comprising: a circular RNA molecule comprising SEQ ID NO: 2 (claims 1-4, 41-44, 112-115, 128-131).

The inventions listed as Groups I+ and II+ 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:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is a virus-like particle (VLP) comprising: a circular RNA molecule with the specific IRES sequence recited therein. Each invention requires a specific IRES sequence not required by any of the other inventions.

Common Technical Features

The inventions of Group I+ share the technical feature of a vector encoding a translation system comprising: a promoter and a nucleic acid sequence encoding an RNA molecule comprising:

a first ribozyme; a first ligation sequence positioned 3' to the first ribozyme; an internal ribosomal entry site (IRES) coupled to an RNA molecule encoding one or more peptide(s), wherein the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s) is positioned 3' to the first ligation sequence; a second ligation sequence positioned 3' to the internal ribosomal entry a second ribozyme positioned 3' to the second ligation sequence.

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

Cornell Univ discloses a vector (para [0011] - "Vectors and cells comprising the RNA molecule of the present application are also disclosed") encoding a translation system (para [0062] - "The vector contains the necessary elements for the transcription and/or translation of the nucleic acid sequence encoding the effector molecule(s) of the present invention"; para [0137] - "This version of the circular RNA expression system was named "Tornado" because it contains two Twister ribozymes") comprising:

a promoter (para [0012] - "This method involves providing one or more vectors comprising a nucleic acid sequence encoding a 5' polymerase promoter sequence") and a nucleic acid sequence encoding an RNA molecule comprising:

a first ribozyme (para [0012] - "a nucleic acid sequence encoding a first ribozyme");

a first ligation sequence positioned 3' to the first ribozyme (para [0012] - "a nucleic acid sequence encoding a first ligation sequence"; para [0086] - "The nucleic acid sequence encoding the effector molecule may be positioned downstream of the nucleic acid sequence encoding the first ligation sequence and upstream of the nucleic acid sequence encoding the second ligation sequence to produce a vector encoding the effector molecule");

an internal ribosomal entry site (IRES) coupled to an RNA molecule encoding one or more peptide(s), wherein the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s) is positioned 3' to the first ligation sequence (para [0060] - "the IRES is operatively coupled to the RNA molecule encoding the peptide sequence"; para [0086] - "The nucleic acid sequence encoding the effector molecule may be positioned downstream of the nucleic acid sequence encoding the first ligation sequence and upstream of the nucleic acid sequence encoding the second ligation sequence to produce a vector encoding the effector molecule");

a second ligation sequence positioned 3' to the internal ribosomal entry a second ribozyme positioned 3' to the second ligation sequence (para [0076] - "a nucleic acid sequence encoding a second ligation sequence"; para [0086] - "The nucleic acid sequence encoding the effector molecule may be positioned downstream of the nucleic acid sequence encoding the first ligation sequence and upstream of the nucleic acid sequence encoding the second ligation sequence to produce a vector encoding the effector molecule").

The inventions of Group I+ further share the technical features of a virus-like particle (VLP) comprising: a circular RNA molecule comprising: a first ligation sequence; an internal ribosomal entry site (IRES) coupled to an RNA molecule encoding one or more peptide(s), wherein the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s) is positioned 3' to the first ligation sequence; a second ligation sequence positioned 3' to the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s); and a plurality of one or more proteins that can self-assemble into a nanoparticle.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are made obvious over Cornell Univ in view of US 2023/0125935 A1 to the Board of Trustees of the Leland Stanford Junior University (hereinafter "Leland Stanford Junior Univ").

Cornell Univ discloses a circular RNA molecule (para [0055] - ("the RNA molecules of the present invention are further processed to form circular RNA") comprising:

a first ligation sequence (para [0076] - "a nucleic acid sequence encoding a first ligation sequence");

an internal ribosomal entry site (IRES) coupled to an RNA molecule encoding one or more peptide(s), wherein the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s) is positioned 3' to the first ligation sequence (para [0060] - "the IRES is operatively coupled to the RNA molecule encoding the peptide sequence"; para [0086] - "The nucleic acid sequence encoding the effector molecule may be positioned downstream of the nucleic acid sequence encoding the first ligation sequence and upstream of the nucleic acid sequence encoding the second ligation sequence to produce a vector encoding the effector molecule");

a second ligation sequence positioned 3' to the internal ribosomal entry site coupled to the RNA molecule encoding the one or more peptide(s) (para [0076] - "a nucleic acid sequence encoding a second ligation sequence"; para [0086] - "The nucleic acid sequence encoding the effector molecule may be positioned downstream of the nucleic acid sequence encoding the first ligation sequence and upstream of the nucleic acid sequence encoding the second ligation sequence to produce a vector encoding the effector molecule").

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Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

Cornell Univ does not disclose a virus-like particle (VLP) comprising: a plurality of one or more proteins that can self-assemble into a nanoparticle.

Leland Stanford Junior Univ discloses recombinant circular RNA (circRNA) molecules comprising an internal ribosome entry site (IRES) operably linked to a protein-coding nucleic acid sequence (abstract), comprising a virus-like particle (VLP) (para [0116] - ("VLPs may be derived from the, AAVs, retrovirus, Flaviviridae, paramyxoviridae, or bacteriophages") comprising: a plurality of one or more proteins that can self-assemble into a nanoparticle (para [0116] - "They can be naturally occurring or synthesized through the individual expression of viral structural proteins, which can then self-assemble into a virus-like structure").

Since Cornell Univ teaches methods of producing circular RNA molecules (abstract), and Leland Stanford Junior Univ discloses a virus-like particle (VLP) for use in the formulation of recombinant circRNA molecules (para [0116], [0138]); it would have been obvious to one of ordinary skill in the art that the VLPs of Leland Stanford Junior Univ could have been combined with the method of Cornell Univ to provide a method for delivering the circular RNA to a cell.

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

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

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.: **1-4, 41-44, 112-115, 128-131, limited 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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033132

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12N 15/115* (2023.01); *C12N 15/63* (2023.01); *C12N 15/67* (2023.01); *C12N 15/113* (2023.01); *C12N 15/85* (2023.01); *A61K 31/7115* (2023.01)

CPC: *C12N 15/115*; *C12N 15/63*; *C12N 15/67*; *C12N 15/113*; *C12N 15/85*; *A61K 31/7115*; *C12N 2310/16*; *C12N 2310/3519*; *C12N 2310/532*; *C12N 2310/128*; *C12N 2840/203*; *C12N 2790/14023*

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	US 2021/0340542 A1 (CORNELL UNIVERSITY) 04 November 2021 (04.11.2021) para [0011]-[0012], [0055], [0060], [0062], [0076], [0086], [0137]	41
Y	para [0011]-[0012], [0055], [0060], [0062], [0076], [0086], [0137]	1-4, 42-44, 112-115, 128-131
Y	US 2023/0125935 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 27 April 2023 (27.04.2023) abstract; para [0116], [0138]	1-4
Y	WO 2019/236673 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 12 December 2019 (12.12.2019) abstract; para [0017], [0025], [00139]; claim 1	2-4, 42-44, 112-115, 128-131
Y	WO 2023/024500 A1 (PEKING UNIVERSITY) 02 March 2023 (02.03.2023) abstract; para [0076]	115, 131



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

30 December 2024 (30.12.2024)

Date of mailing of the international search report

13 January 2025 (13.01.2025)

Name and mailing address of the ISA/US

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