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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))*

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:
27 June 2024 (27.06.2024)

(54) Title: EFFECTOR PROTEINS, COMPOSITIONS, SYSTEMS AND METHODS OF USE THEREOF

(57) Abstract: Provided herein are compositions, systems, devices, kits, and methods comprising effector proteins, and uses thereof. These effector proteins may be characterized as CRISPR-associated (Cas) proteins. Various compositions, systems, devices, kits, and methods of the present disclosure may leverage the activities of these effector proteins for the editing, detecting and/or engineering of nucleic acids.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/79916

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/87, C12N 9/22, C12N 15/11, C12N 15/113 (2024.01)
ADD. C12N 15/10, C12N 15/90, G01N 33/50 (2024.01)

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ADD. C12N 15/102, C12N 15/907, G01N 33/5002, C12N 2310/20

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 2018/0003696 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 04 January 2018 (04.01.2018) para [0007], [0010], [0038], [0210], Claim 1, Claim 9	1-3, 5-7, 51-53 56-58
A	WO 2022/140572 A1 (MAMMOTH BIOSCIENCES, INC.) 30 June 2022 (30.06.2022) Claim 1, Claim 69	1-3, 5-7, 51-53 56-58

☐ 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
20 March 2024	APR 23 2024
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. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 23/79916

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 23/79916

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.: 8-50, 54-55, 59-60
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 for Box No. III Observations where unity of invention is lacking -

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.:
1-3, 5-7, 51-53 and 56-58 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/US 23/79916

Continuation of:
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 searched, the appropriate additional search fees must be paid.

Groups I+: Claims 1-7, 51-53 and 56-58, drawn to a system that comprises: (i) a polypeptide, (ii) a donor nucleic acid; and (iii) an engineered guide nucleic acid. The system will be searched to the extent that the polypeptide encompasses the first named amino acid sequence in set forth in Table 1, SEQ ID NO: 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 1-3, 5-7, 51-53 and 56-58 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1. Additional polypeptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected polypeptide(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 a polypeptide comprising SEQ ID NO: 22 (Claims 1-2, 4-7, 51-53, 56-58).

The inventions listed as Groups I+ 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

No technical features are shared between the amino acid sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Common Technical Features

The inventions of Groups I+ share the technical feature of a system that comprises: (i) a polypeptide, (ii) a donor nucleic acid; and (iii) an engineered guide nucleic acid.

However, these shared technical features do not represent a contribution over prior art in view of US 2018/0003696 A1 to Massachusetts Institute of Technology (hereinafter "MIT").

MIT teaches a system (para [0038], FIGS. 1A and 1B are schematic diagrams of a microfluidic system in which cells are exposed to the delivery material (payload) after passing through the constriction.; Claim 1, A method for delivering a protein and a nucleic acid into a cell, the method comprising: providing a cell in a solution.....and contacting the cell with the protein and the nucleic acid before, during, and/or after the cell passes through the constriction.) that comprises:
(i) a polypeptide, or a recombinant nucleic acid encoding the polypeptide (Claim 9, (a) the protein is a Cas protein or a Cpf1 protein; and (b) the nucleic acid is a single guide RNA (sgRNA) or a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA).); and
(ii) a donor nucleic acid (Claim 19, wherein the solution further comprises donor DNA before, during, and/or after the cell passes through the constriction.); and
(iii) an engineered guide nucleic acid or a nucleic acid that encodes the engineered guide nucleic acid (Claim 9, (a) the protein is a Cas protein or a Cpf1 protein; and (b) the nucleic acid is a single guide RNA (sgRNA) or a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA).).

MIT teaches a microfluidic device (para [0038], FIGS. 1A and 1B are schematic diagrams of a microfluidic system in which cells are exposed to the delivery material (payload) after passing through the constriction.) comprising:
(a) a sample interface configured to receive a sample comprising nucleic acids;
(b) a chamber fluidically connected to the sample interface (para [0010], The microfluidic system may include a plurality of microfluidic channels. Each of the microfluidic channels of the plurality defines a lumen and is configured such that a cell suspended in a buffer can pass through the lumen. In some embodiments, microfluidic channels include one or more cell-deforming constrictions.);
wherein the chamber comprises a polypeptide and an engineered guide nucleic acid (para [0007], An exemplary protein-nucleic acid complex comprises gene editing components. For example, the protein-nucleic acid complex comprises a Cas protein (such as a Cas9 protein) and a guide RNA (gRNA) or donor DNA.).

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

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

Item 4 (continued):
Claims 8-50, 54-55 and 59-60 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).