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(54) Title: DEEP MUTATIONAL EVOLUTION OF BIOMOLECULES

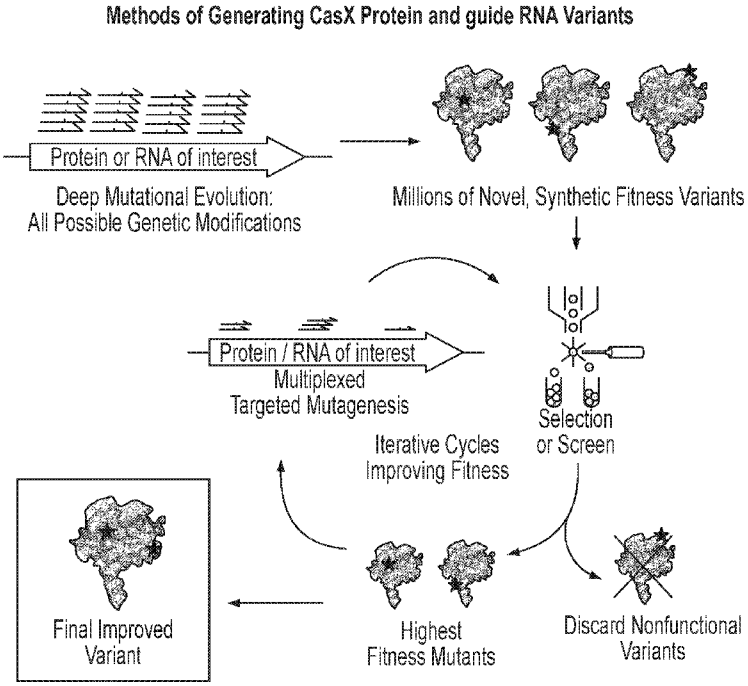

FIG. 1

(57) Abstract: Provided herein are methods of developing biomolecule variants (such as proteins, RNA, or DNA) with improved characteristics, for example by developing libraries of variants with alterations to one or more specific monomer locations and screening said libraries for characteristics of interest. These alterations can include deletion, substitution, and insertion, and variants may comprise one alteration or a combination of alterations. Said methods may include further iterative cycles of library construction and evaluation to develop, for example, a biomolecule variant with improved characteristics compared to a reference biomolecule. The methods can also provide information that may be used in the rational design of variants.

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[Continued on next page]

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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))*

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07 January 2021 (07.01.2021)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/36506

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/00, C12N 15/09, C12N 15/63, C12N 15/70, C12N 15/85, C12N 9/22 (2020.01)

CPC - C12N 15/746, C12N 15/113, C12N 15/907, C12N 15/8509, C12N 15/74, C12N 15/102, C12N 15/1082, C12N 2310/20, C12N 2310/3519, C12N 2800/101

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 2010/054007 A1 (FABRUS LLC) 14 May 2010 (14.05.2010) abstract; pg 15, ln 31-32; pg 34, ln 23-28; pg 43, ln 4-7; pg 47, ln 6-11, 32-33; pg 57, ln 17-18; pg 68, ln 3-4; pg 70, ln 9-10; pg 106, ln 3; pg 119, ln 8-10; pg 287, ln 33-34; pg 305, ln 14-16; pg 339, ln 25-27; pg 342, ln 5-6	1-7, 31-34
A	US 6,979,538 B2 (LADNER et al.) 27 December 2005 (27.12.2005) entire document	1-7, 31-34

☐ 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
06 November 2020	24 NOV 2020
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 Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/36506

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 13*ter*.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 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/36506

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.: 8-30, 35-64, 69-110, 116-120, 124-131, 135
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 Supplemental Box ---

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.:
1-7, 31-34

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
Information on patent family members

International application No.
PCT/US 20/36506

--- 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 searched, the appropriate additional search fees must be paid.

Group I, claims 1-7, 31-34, directed to a method of selecting an improved biomolecule variant, wherein the biomolecule variant is a protein, RNA, or DNA, and constructing a library of polynucleotide variants of a reference biomolecule.

Group II, claims 65-68, 111-115, 132-134, directed to a polynucleotide variant library.

Group III, claims 121-123, directed to a library comprising a plurality of protein variants.

The inventions listed as Groups I-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 method of constructing a library and selecting an improved biomolecule variant, that is not required by Groups II-III.

Group II has the special technical feature of a polynucleotide variant library, that is not required by Groups I and III.

Group III has the special technical feature of library comprising a plurality of protein variants, that is not required by Groups I-II.

Common technical features:

Groups I-III share the common technical feature of:
a library comprising a plurality of biomolecule variants, wherein each variant is independently a variant of the same reference biomolecule, and each variant comprises an alteration of one or more monomer locations of the reference biomolecule, wherein the monomer is an amino acid of the protein or a ribonucleotide of the RNA or a deoxyribonucleotide of the DNA,
wherein each alteration of a monomer location is independently selected from the group consisting of substitution of the monomer, deletion of one or more consecutive monomers beginning at the location, and insertion of one or more consecutive monomers adjacent to the location;
wherein the library represents variants comprising the single alteration of a single location, for at least 1% of the amino acids of the reference protein sequence. However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is anticipated by WO 2010/054007 A1 to Fabrus, Inc. (hereinafter 'Fabrus').

Fabrus teaches a method (pg 15, ln 31 - 'the method') of selecting (pg 15, ln 32 - 'of selecting') an improved biomolecule variant (pg 43, ln 4-5 - 'optimized antibody refers to an antibody, or portion thereof, that has an improved binding affinity'; pg 43, ln6-7 - 'the antibody is optimized by virtue of one or more amino acid modifications'), wherein the biomolecule variant is a protein (pg 43, ln 4-5 - 'optimized antibody refers to an antibody, or portion thereof, that has an improved binding affinity), RNA, or DNA, comprising: i. constructing a library comprising a plurality of biomolecule variants (abstract - 'Methods for making a combinatorial antibody library'; pg 43, ln6-7 - 'the antibody is optimized by virtue of one or more amino acid modifications'); wherein each variant is independently a variant of the same reference biomolecule (pg 34, ln 23-28 - 'modified form with reference to a germline segment refers to a sequence of nucleotides that is substantially the same as the sequence of nucleotides of a human germline segment, except that the sequence of nucleotides contains one or a few nucleotide differences, for example, 2, 3, 4, 5, 6, 7 , 8, 9 or 10 nucleotide differences compared to the corresponding sequence in a germline segment sequence'), wherein each variant comprises an alteration of one or more monomer locations of the reference biomolecule (pg 34, ln 23-28 - 'modified form with reference to a germline segment refers to a sequence of nucleotides that is substantially the same as the sequence of nucleotides of a human germline segment, except that the sequence of nucleotides contains one or a few nucleotide differences, for example, 2, 3, 4, 5, 6, 7 , 8, 9 or 10 nucleotide differences compared to the corresponding sequence in a germline segment sequence'), wherein the monomer is an amino acid of the protein or a ribonucleotide of the RNA or a deoxyribonucleotide of the DNA (pg 34, ln 23-28 - 'modified form with reference to a germline segment refers to a sequence of nucleotides that is substantially the same as the sequence of nucleotides of a human germline segment, except that the sequence of nucleotides contains one or a few nucleotide differences, for example, 2, 3, 4, 5, 6, 7 , 8, 9 or 10 nucleotide differences compared to the corresponding sequence in a germline segment sequence'), wherein each alteration of a monomer location is independently selected from the group consisting of substitution of the monomer, deletion of one or more consecutive monomers beginning at the location, and insertion of one or more consecutive monomers adjacent to the location (pg 43, ln 7 - 'amino acid deletion, replacement or insertion'); and wherein the library represents variants comprising alteration of one or more locations for at least 1% of the monomer locations of the reference biomolecule (pg 57, ln 17-18 - 'No more than 10%, i.e., 10 out of 100, of the amino acids in the test polypeptide differs from that of the reference polypeptide'); ii. screening the library of i. (abstract - 'Methods for screening antibody libraries'); iii. identifying at least a portion of the library of i. (abstract - 'Methods for screening antibody libraries against a target protein antigen, and the identified or selected') that exhibits one or more improved characteristics compared to the reference biomolecule (pg 43, ln 4-5 - 'optimized antibody refers to an antibody, or portion thereof, that has an improved binding affinity'); and iv. selecting the improved biomolecule variant from the at least a portion of the library (pg 47, ln 32-33 - 'identified, recognized or selected as having an activity in a screening assay'), wherein the improved biomolecule variant exhibits one or more improved characteristics compared to the reference biomolecule (pg 43, ln 4-5 - 'optimized antibody refers to an antibody, or portion thereof, that has an improved binding affinity').

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, Group I-III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.