



(51) International Patent Classification:

C40B 40/10 (2006.01) C40B 40/02 (2006.01)

C40B 40/08 (2006.01) C12N 15/10 (2006.01)

(21) International Application Number:

PCT/NZ2021/050086

(22) International Filing Date:

27 May 2021 (27.05.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

16/911,398 25 June 2020 (25.06.2020) US

(72) Inventor; and

(71) Applicant: RODLEY, Philip [NZ/JP]; Minami Rokugo 2-22-2-505, Ota-ku, Tokyo (JP).

(81) Designated States (unless otherwise indicated, for every kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW,

SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

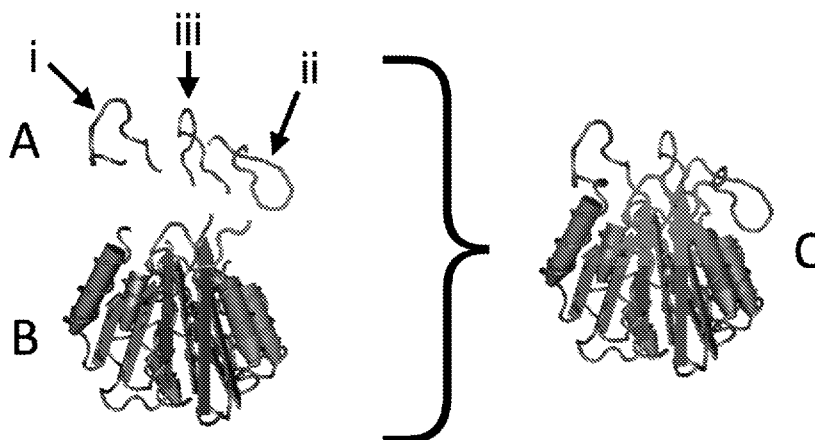
ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

(54) Title: PROTEIN SCAFFOLD

Fig. 3



(57) Abstract: The invention provides a protein scaffold and methods of preparing, screening, engineering and using the protein scaffold.



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))*
- *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

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

03 February 2022 (03.02.2022)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ21/50086

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C40B 40/10; C40B 40/08; C40B 40/02; C12N 15/10 (2021.01)

CPC - C40B 40/10; C40B 40/08; C40B 40/02; C12N 15/1037; C12N 15/1041; C12N 15/1044; C12N 15/102; C07K 14/195; C07K 14/001

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.
Y	***-1st Invention-***	1, 9-15
Y	WO 2009/058379 A2 (MEDIMMUNE, LLC) 7 May 2009; paragraphs [0010], [0011], [0015], [0017], [0018], [0095], [00133], [00156], [00246], [00247], [00435]; embodiments 51 and 77; claims 1, 38, and 43-46	1, 9-15
A	(CHO, KH et al.) An insight into the interaction mode between CheB and chemoreceptor from two crystal structures of CheB methylesterase catalytic domain. Biochemical and Biophysical Research Communications. 22 July 2011, Epub 21 June 2011, Vol. 411, No. 1; pages 1-17; page 2, 5th paragraph; page 4, 4th paragraph; pages 16-17; fig. 1; DOI: 10.1016/j.bbrc.2011.06.090	1, 9-15
	US 2016/0090400 A1 (LONGO, M et al.) 31 March 2016; entire document	
Y	***-2nd Invention-***	1-4, 9-15
A	WO 2009/058379 A2 (MEDIMMUNE, LLC) 7 May 2009; paragraphs [0010], [0011], [0015], [0017], [0018], [0094], [0095], [0098], [00100], [00101], [00133], [00156], [00222], [00246], [00247], [00435]; embodiments 51 and 77; claims 1, 38, and 43-46; figs. 7A, 7B, and 8	5-8
Y		1-4, 9-15
A	(CHO, KH et al.) An insight into the interaction mode between CheB and chemoreceptor from two crystal structures of CheB methylesterase catalytic domain. Biochemical and Biophysical Research Communications. 22 July 2011, Epub 21 June 2011, Vol. 411, No. 1; pages 1-17; page 2, 5th paragraph; page 4, 4th paragraph; pages 16-17; fig. 1; DOI: 10.1016/j.bbrc.2011.06.090	5-8
A		5-8

☒ 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

08 November 2021 (08.11.2021)

Date of mailing of the international search report

DEC 14 2021

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

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ21/50086

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2007/0281006 A1 (NICOLAU, YC et al.) 6 December 2007; paragraph [0126]	5-8
A	US 2017/0088602 A1 US 2017/0088602 A1 (BRISTOL-MYERS SQUIBB COMPANY) 30 March 2017; abstract; paragraphs [0009], [0021], [0099]	1-15
	US 20160090400 A1 (LONGO, M et al.) 31 March 2016; entire document	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ21/50086

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:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ21/50086

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:
-***Please See Supplemental Page-***

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.:
1st Invention: Groups I+, Claims 1-15 and a recombinant CheBc, wherein loop region (a) is a non-naturally occurring variant of the sequence for residues 16 to 18 [inclusive] of SEQ ID NO: 1 (recombinant CheBc)
2nd Invention: Groups I+, Claims 1-15 and a recombinant CheBc, wherein loop regions (c), (h), and (j) are non-naturally occurring variants of the sequences for residues 43 to 47 [inclusive], residues 103 to 107 [inclusive], and residues 135 to 137 [inclusive] respectively of SEQ ID NO: 1 (recombinant CheBc).
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.:

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 must be paid.

Groups I+, Claims 1-15 and a recombinant CheBc, wherein loop region (a) is a non-naturally occurring variant of the sequence for residues 16 to 18 [inclusive] of SEQ ID NO: 1 (recombinant CheBc) are directed toward a display library, isolated nucleic acids molecules, vectors and cells associated therewith, and methods associated therewith.

The library, nucleic acids, vectors, cells, and methods will be searched to the extent they encompass a recombinant CheBc, wherein loop region (a) is a non-naturally occurring variant of the sequence for residues 16 to 18 [inclusive] of SEQ ID NO: 1 (first exemplary recombinant CheBc). Applicant is invited to elect additional recombinant CheBc(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO: such that the each elected species is fully specified, to be searched. Additional CheBc(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), and 9-15 (each in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a recombinant CheBc, wherein loop region (a) is a non-naturally occurring variant of the sequence for residues 16 to 18 [inclusive] of SEQ ID NO: 1 (recombinant CheBc). Applicants must specify the searchable claims that encompass any additionally elected CheBc(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/examined. An exemplary election would be a recombinant CheBc, wherein loop region (a) is a non-naturally occurring variant of the sequence for residues 29 to 37 [inclusive] of SEQ ID NO: 1 (recombinant CheBc).

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

Groups I+ share the technical features including: a polypeptide display library comprising a recombinant polypeptide scaffold comprising a recombinant CheBc domain comprising: (i) fourteen loop regions corresponding to the cognate loop regions of SEQ ID NO: 1, the cognate loop regions of SEQ ID NO: 1 consisting of residues: (a) from 16 to 18 inclusive; (b) from 29 to 37 inclusive; (c) from 43 to 47 inclusive; (d) from 60 to 61 inclusive; (e) from 66 to 75 inclusive; (f) from 80 to 84 inclusive; (g) from 92 to 93 inclusive; (h) from 103 to 107 inclusive; (i) from 124 to 125 inclusive; (j) from 135 to 137 inclusive; (k) from 149 to 150 inclusive; (l) from 160 to 162 inclusive; (m) from 173 to 176 inclusive; (n) from 180 to 181 inclusive, (ii) linked to secondary structural elements corresponding to the non-loop regions of SEQ ID NO: 1; wherein at least one of said loop regions is a non-naturally occurring variant of the cognate loop region of SEQ ID NO: 1; a collection of isolated nucleic acid molecules encoding the library; an expression vector operably linked to the nucleic acid molecules; a cell that has been genetically engineered to express the nucleic acid molecules; and a method of obtaining a polypeptide scaffold that binds to a target, said method comprising (a) contacting a target ligand with the library, under conditions that allow a scaffold:target ligand complex to form and, (b) obtaining from the complex, the scaffold that binds the target ligand.

However, these shared technical features are previously disclosed by WO 2020/089235 A1 to ETH Zurich (hereinafter 'Zurich'), in view of the article 'Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*' by Nelson, et al. (hereinafter 'Nelson').

Zurich discloses a polypeptide display library (a polypeptide display library; page 5, first paragraph - second paragraph) comprising a recombinant polypeptide scaffold comprising a recombinant CheBc domain (comprising a recombinant polypeptide scaffold comprising a recombinant esterase, including CheB (CheBc domain); page 3, third paragraph; page 9, fourth paragraph; page 23, second paragraph - page 24, first paragraph); wherein at least one region is a non-naturally occurring variant of the cognate (wherein mutational variants of the catalytic domain are formed (wherein at least one region is a non-naturally occurring variant of the cognate); page 23, third paragraph); a collection of isolated nucleic acid molecules encoding the library (a collection of isolated nucleic acid molecules encoding the library; page 3, third paragraph; page 5, first paragraph - second paragraph); an expression vector operably linked to the nucleic acid molecules (an expression vector operably linked to the nucleic acid molecules; page 3, third paragraph; page 5, first paragraph - second paragraph; page 23, third paragraph); a cell that has been genetically engineered to express the nucleic acid molecules (a cell that has been genetically engineered to express the nucleic acid molecules; page 3, third paragraph); and a method of obtaining a polypeptide scaffold that binds to a target (a method of obtaining an esterase with improved properties (a method of obtaining a polypeptide scaffold that binds to a target); page 23, second paragraph - third paragraph; wherein the esterase binds to a target as a part of its enzymatic activity), said method comprising (a) contacting a target ligand with the library, under conditions that allow a scaffold:target ligand complex to form (said method comprising: screening expressed variants for enhanced esterase activity (contacting a target ligand with the library, under conditions that allow a scaffold:target ligand complex to form); page 23, second paragraph - page 24, first paragraph) and, (b) obtaining from the complex, the scaffold that binds the target ligand (isolating from the screening, clones that produce the variants with enhanced activity (obtaining from the complex, the scaffold that binds the target ligand); page 23, second paragraph - page 24, first paragraph; wherein the obtaining from the complex is accomplished by selecting the variants with enhanced activity, which formed said complex as a part of the enhanced activity).

Zurich does not disclose the CheBc domain comprising: (i) fourteen loop regions corresponding to the cognate loop regions of SEQ ID NO: 1, the cognate loop regions of SEQ ID NO: 1 consisting of residues: (a) from 16 to 18 inclusive; (b) from 29 to 37 inclusive; (c) from 43 to 47 inclusive; (d) from 60 to 61 inclusive; (e) from 66 to 75 inclusive; (f) from 80 to 84 inclusive; (g) from 92 to 93 inclusive; (h) from 103 to 107 inclusive; (i) from 124 to 125 inclusive; (j) from 135 to 137 inclusive; (k) from 149 to 150 inclusive; (l) from 160 to 162 inclusive; (m) from 173 to 176 inclusive; (n) from 180 to 181 inclusive, (ii) linked to secondary structural elements corresponding to the non-loop regions of SEQ ID NO: 1, wherein at least one of said loop regions is a non-naturally occurring variant of the cognate loop region of SEQ ID NO: 1.

Nelson discloses a *T. maritima* CheB comprising a sequence that is 96.1% identical to SEQ ID NO: 1 (*T. maritima* CheB comprising a sequence that is 96.15% identical to SEQ ID NO: 1; page 325, second column, first paragraph; appended sequence, page 4/4; wherein the sequence is 96.1% identical to Applicant's SEQ ID NO: 1).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of

---Continued Within the Next Supplemental Box---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ21/50086

-Continued from previous Supplemental Box-

Zurich to have used a thermophilic esterase, such as the CheB methylesterase disclosed by Nelson, as an initial wild-type sequence for the screening of a display library of mutants with enhanced activity, as disclosed by Zurich, including wherein the mutations would have been present in loop regions present in the catalytic domain, in order to identify useful, thermostable methylesterase mutants via directed evolution.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Zurich and Nelson references, unity of invention is lacking.