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(71) Applicant: **THE UNIVERSITY OF CHICAGO**
[US/US]; 5801 South Ellis Avenue, Chicago, Illinois 60637
(US).

(72) Inventors: **MOELLERING, Raymond E.**; Polsky Center
for Entrepreneurship and Innovation, University of Chicago,
5113 S. Harper Ave., Suite 2C, Chicago, Illinois 60615
(US). **LI, Gang**; Polsky Center for Entrepreneurship and In-

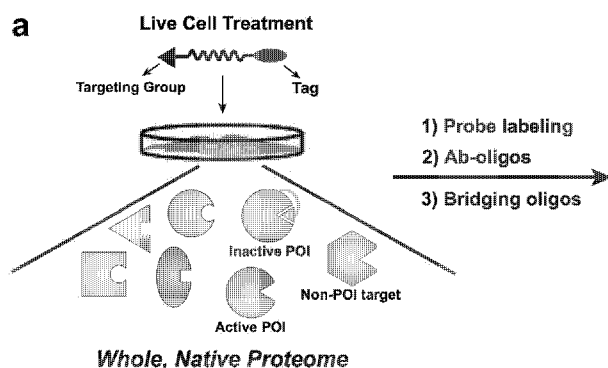
novation, University of Chicago, 5113 S. Harper Ave., Suite
2C, Chicago, Illinois 60615 (US).

(74) Agent: **STELLMAN, Laurie Friesenhahn**; NORTON
ROSE FULBRIGHT US LLP, 98 San Jacinto Blvd., Suite
1100, Austin, Texas 78701 (US).

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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, 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, SM, ST, SV, SY, TH, TJ, TM, TN,
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(54) Title: CHEMICAL PROBE-DEPENDENT EVALUATION OF PROTEIN ACTIVITY AND USES THEREOF



(57) Abstract: Aspects of the disclosure relate to a method for evaluating two or more target proteins of interest from the same family in a specified functional form, the method comprising: (i) contacting a composition comprising or suspected of comprising the two or more proteins of interest with a molecular construct comprising: a targeting group operatively linked to a retrieval tag; wherein the targeting group specifically binds to the specialized functional form of the two or more target proteins of interest; (ii) contacting the composition with at least two antibody-oligo constructs, wherein at least one of the constructs comprises a first antibody operatively linked to a first oligo and at least a second construct comprises a second antibody operatively linked to a second oligo; wherein the first antibody specifically binds to one of the two or more target proteins of interest and the second antibody specifically binds to the other of the two or more target proteins of interest; (iii) contacting the composition with a second molecular construct comprising a retrieval tag binder operatively linked to a retrieval oligo; (iv) incubating the composition under conditions sufficient for the ligation or annealing of the first oligo to the retrieval oligo when the first and retrieval oligos are in close proximity to each other and ligation or annealing of the second oligo to the retrieval oligo when the second and retrieval oligos are in close proximity to each other; and (v) detecting the ligated or annealed first and retrieval oligo and the ligated or annealed second and retrieval oligo.

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 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))*

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- *with international search report (Art. 21(3))*

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02 April 2020 (02.04.2020)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/062231

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.: 5-42, 48-53, 57-60
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:

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.:

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/US2018/062231

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/10; C40B 40/10; C40B 70/00 (2019.01)

CPC - C12N 15/1068; C40B 30/04; C40B 40/14; C40B 70/00 (2019.02)

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

USPC - 506/4; 506/9; 506/15 (keyword delimited)

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 2012/006556 A2 (LIFE TECHNOLOGIES CORPORATION et al) 12 January 2012 (12.01.2012) entire document	1, 54, 56
X	THOMPSON et al. "SH2-PLA: a sensitive in-solution approach for quantification of modular domain binding by proximity ligation and real-time PCR," BMC Biotechnology, 26 June 2015 (26.06.2015), Vol. 15, No. 60, Pgs. 1-13. entire document	54-56
X	NOMURA et al. "Activity-based protein profiling for biochemical pathway discovery in cancer," Nat Rev Cancer, 12 August 2010 (12.08.2010), Vol. 10, No. 9, Pgs. 630-638. entire document	54-56
X	WO 2017/192633 A1 (PROCURE LIFE SCIENCES INC.) 09 November 2017 (09.11.2017) entire document	54
P, X	LI et al. "An activity-dependent proximity ligation platform for spatially resolved quantification of active enzymes in single cells," Nature Communications, 24 November 2017 (24.11.2017), Vol. 8, No. 1775, Pgs. 1-12. entire document	1, 54-56
A	SCHEDIN-WEISS et al. "Visualizing Active Enzyme Complexes Using a Photoreactive Inhibitor for Proximity Ligation - Application on γ -Secretase," PLoS One, 29 November 2012 (29.11.2012), Vol. 8, Iss. 5, e63962, Pgs. 1-10. entire document	1-4, 43-47, 54-56
A	US 8,940,497 B2 (CRAVATT et al) 27 January 2015 (27.01.2015) entire document	1-4, 43-47, 54-56
A	GREENWOOD et al. "Proximity assays for sensitive quantification of proteins," Biomolecular Detection and Quantification, 20 May 2015 (20.05.2015), Vol. 4, Pgs. 10-16. entire document	1-4, 43-47, 54-56



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

"E" earlier application or patent but published on or after the international filing date

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"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

12 April 2019

Date of mailing of the international search report

16 APR 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/062231

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIM et al. "A DNA-assisted immunoassay for enzyme activity via a DNA-linked, activity-based probe," Chem. Commun., 01 August 2017 (01.08.2017), Vol. 53, Pgs. 9474-9477. entire document	1-4, 43-47, 54-56