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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM,

(54) Title: SYNTHETIC DEGRADER SYSTEM FOR TARGETED PROTEIN DEGRADATION

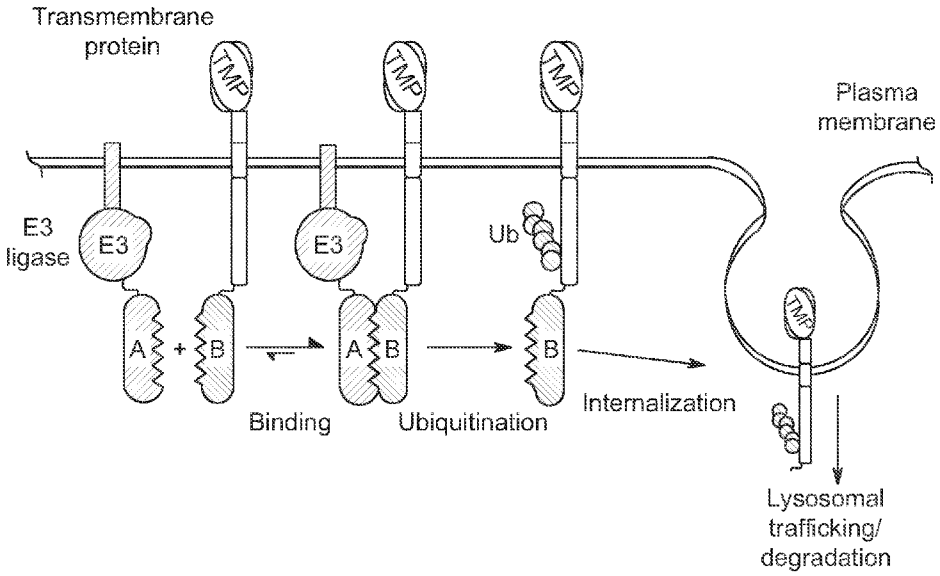


FIG. 4A

(57) Abstract: A fusion protein is provided having a binding element and a degradation initiator, where the binding element selectively binds a target molecule, and the degradation initiator has a sequence isolated or derived from an E3 ligase. A composition is provided comprising: (a) a first fusion protein comprising a first binding element; and (b) a second fusion protein comprising a second binding element; wherein: (1) the first fusion protein further comprises a degradation initiator or a functional variant thereof and the second fusion protein further comprises a target molecule; or (2) the first fusion protein further comprises a target molecule and the second fusion protein further comprises a degradation initiator or a functional variant thereof. The fusion proteins and compositions may be used for the targeted degradation of endogenous and exogenous proteins, optionally, in a cell or in vivo, for the treatment or prevention of a disease or disorder.

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*[Continued on next page]*

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.

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**Published:**

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— *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:**
29 September 2022 (29.09.2022)

# INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/10 C12N15/52 C12N15/62 A61P35/00 C07K14/705
C07K14/725
ADD.
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)
C12N C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DENG WEN ET AL: "Tunable light and drug induced depletion of target proteins", NATURE COMMUNICATIONS, vol. 11, no. 1, 16 January 2020 (2020-01-16), XP055933138, DOI: 10.1038/s41467-019-14160-8 Whole document, especially figure 1, and page 2, right column, top paragraph. -/-- | 1-143 |

[x] Further documents are listed in the continuation of Box C.
[x] 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
"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 |
|---|---|
| 28 June 2022 | 02/09/2022 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Kools, Patrick |

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| | -& Deng Wen ET AL: "Supplementary information Tunable light and drug induced depletion of target proteins", Nature communications, 16 January 2020 (2020-01-16), pages 1-24, XP055933148, Retrieved from the Internet: URL:https://static-content.springer.com/esm/art:10.1038/s41467-019-14160-8/MediaObjects/41467_2019_14160_MOESM1_ESM.pdf [retrieved on 2022-06-20] whole document, especially figure 1a, b | |
| A | EMMANUEL CAUSSINUS ET AL: "Fluorescent fusion protein knockout mediated by anti-GFP nanobody", NATURE STRUCTURAL & MOLECULAR BIOLOGY, vol. 19, no. 1, 1 January 2012 (2012-01-01), pages 117-121, XP055613880, New York ISSN: 1545-9993, DOI: 10.1038/nsmb.2180 whole document, especially figure 1. | 1-143 |
| A | CHEN ZIBO ET AL: "Programmable design of orthogonal protein heterodimers", NATURE, NATURE PUBLISHING GROUP UK, LONDON, vol. 565, no. 7737, 19 December 2018 (2018-12-19), pages 106-111, XP036664328, ISSN: 0028-0836, DOI: 10.1038/S41586-018-0802-Y [retrieved on 2018-12-19] the whole document | 1-143 |
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | FOIGHT GLENNA WINK ET AL: "Multi-input chemical control of protein dimerization for programming graded cellular responses", NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP US, NEW YORK, vol. 37, no. 10, 9 September 2019 (2019-09-09), pages 1209-1216, XP036897234, ISSN: 1087-0156, DOI: 10.1038/S41587-019-0242-8 [retrieved on 2019-09-09] the whole document<br>-----<br>SCHNEEKLOTH A R ET AL: "Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 18, no. 22, 15 November 2008 (2008-11-15), pages 5904-5908, XP025627166, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2008.07.114 [retrieved on 2008-07-31] the whole document<br>-----<br>Baltz Morgan R ET AL: "Design and functional characterization of synthetic E3 ubiquitin ligases for targeted protein depletion: Selective protein knockout using engineered ubiquibodies", Current protocols in chemical biology, 1 March 2018 (2018-03-01), pages 72-90, XP055920080, DOI: 10.1002/cpch.37 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6062852/pdf/nihms920139.pdf [retrieved on 2022-05-11] abstract<br>-----<br>ALYSE D. PORTNOFF ET AL: "Ubiquibodies, Synthetic E3 Ubiquitin Ligases Endowed with Unnatural Substrate Specificity for Targeted Protein Silencing", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 289, no. 11, 28 January 2014 (2014-01-28), pages 7844-7855, XP055198417, ISSN: 0021-9258, DOI: 10.1074/jbc.M113.544825 abstract<br>-----<br>-/-- | 1-143<br>1-143<br>1-143<br>1-143 |

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/014998

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | BUCKLEY DENNIS L ET AL: "HaloPROTACS: Use of Small Molecule PROTACs to Induce Degradation of HaloTag Fusion Proteins", ACS CHEMICAL BIOLOGY, ACS PUBLICATIONS, USA, vol. 10, no. 8, 21 August 2015 (2015-08-21), pages 1831-1837, XP002762672, ISSN: 1554-8937 the whole document | 1-143 |
| A | WO 2020/093043 A1 (CHEN ZIBO [US]; BOYKEN SCOTT [US] ET AL.) 7 May 2020 (2020-05-07) Whole document, especially the claims | 1-143 |
| A | NAYAK DIGANT ET AL: "Structure of LNX1:Ubc13 ~ Ubiquitin Complex Reveals the Role of Additional Motifs for the E3 Ligase Activity of LNX1", JOURNAL OF MOLECULAR BIOLOGY, vol. 430, no. 8, 1 April 2018 (2018-04-01), pages 1173-1188, XP055933104, United Kingdom ISSN: 0022-2836, DOI: 10.1016/j.jmb.2018.02.016 the whole document | 1-143 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/014998

**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. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] 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/US2022/014998

**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:

see additional sheet

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 an 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.:

1-143(partially)

**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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-143(partially)

Fusion protein comprising a binding element and a degradation initiator, wherein the binding element selectively binds a target molecule and wherein the degradation initiator comprises a sequence isolated or derived from an E3 ligase comprises LNX1 or functional fragment thereof. A composition comprising said fusion protein. Nucleic acid encoding said fusion protein. Vector comprising said nucleic acid. Cells expressing said fusion protein. Composition comprising said nucleic acids, vectors, or cells. Pharmaceutical composition comprising any of these products. Use of any of these products in the manufacture of a medicament. Method of treatment using any of these products.

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2-16. claims: 1-143(partially)

As for subject 1, now for E3 ligase comprising RNF4, RNF43, RNF128, XNRF3, MARCH8, LRG1, NEDD4, SOCS2, CHIP, SPOP, FBXW7, FBXW1A, ELOC, TRAF6, VHL.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/014998

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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| | | CA 3117841 A1 | 07-05-2020 |
| | | CN 113272317 A | 17-08-2021 |
| | | EP 3873919 A1 | 08-09-2021 |
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| | | US 2022017579 A1 | 20-01-2022 |
| | | US 2022098250 A1 | 31-03-2022 |
| | | WO 2020093043 A1 | 07-05-2020 |