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(54) Title: DEGRON FUSION CONSTRUCTS AND METHODS FOR CONTROLLING PROTEIN PRODUCTION

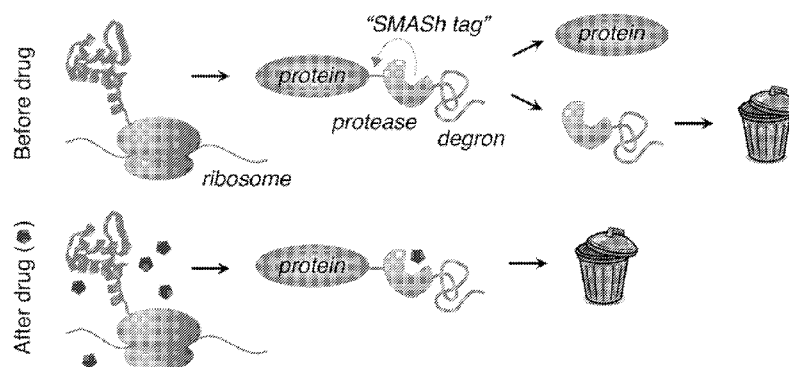


FIG. 1A

(57) Abstract: Engineered fusion proteins comprising a self-excising degron for controlling protein production are disclosed. In particular, the inventors have constructed fusion proteins comprising a degron connected to a protein of interest through a cleavable linker comprising a hepatitis C virus (HCV) protease site. The degron can be removed from the protein of interest by a cys-encoded HCV protease such that the protein of interest can be produced with minimal structural modification. Clinically available HCV protease inhibitors can be used to block protease cleavage such that the degron is retained after inhibitor addition on subsequently synthesized protein copies. The degron when attached causes rapid degradation of the linked protein. Such fusions of a degron to a protein of interest will be especially useful when control over protein production with minimal structural modification is desired.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/39792

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/035, 14/005; C12N 15/62, 15/79 (2016.01)

CPC - C07K 14/035, 14/005; C12N 15/62, 15/79

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)

IPC: C07K 14/035, 14/005; C12N 15/62, 15/79 (2016.01)

CPC: C07K 14/035, 14/005, 2319/00; C12N 15/62, 15/79

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)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; Lens.org; NCBI blast.

Search terms used: degron, fusion protein, SEQ ID 1, SEQ ID 7, SEQ ID 8

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,210,962 B1 (BRECHOT, C et al.) 3 April 2001; Claim 2; SEQ ID NO: 7	1-41, 67-69
A	US 2010/0041092 A1 (LIN, MZ et al.) 18 February 2010; paragraph [0016]	1-41, 67-69
A	GB 2 212 511 A (HOUGHTON, M et al.) 26 July 1989; Figure 5	1-41, 67-69
A	WO 89/04669 (CHIRON CORPORATION) 1 June 1989; Figure 9	1-41, 67-69
A	(HOUSE, CM et al.) Elucidation of the Substrate Binding Site of Siah Ubiquitin Ligase. Structure. April 2006, Vol. 14, No. 4; pages 695-701	1-41, 67-69

☐ 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

"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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/39792

Box No. 1 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/US16/39792

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.:
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.:
1-41 and 67-69

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.

Group I, Claims 1-44 and 67-69 are directed toward a degron comprising an amino acid sequence having at least 80% identity to an amino acid sequence of SEQ ID NO: 1, wherein the degron is capable of promoting degradation of a polypeptide; and fusion proteins, cells, organoids, and methods and kits associated therewith.

Group II, Claims 45-66 and 70 are directed toward a conditionally replicating viral vector comprising a modified genome of a virus such that production of a polypeptide required for efficient replication of the virus is controllable, wherein the viral vector comprises a nucleic acid encoding a fusion protein comprising: i) the polypeptide required for efficient replication of the virus; ii) a degron; and a method and kit associated therewith.

The inventions listed as Groups I and II 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: the special technical features of Group I include SEQ ID NO: 7, not present in Group II; the special technical features of Group II include SEQ ID NO: 9, not present in Group I.

Groups I and II share the technical features including: an amino acid sequence of SEQ ID NO: 1; controllable production of a polypeptide; a nucleic acid encoding a fusion protein comprising: i) a polypeptide; and ii) a degron, wherein the degron is operably linked to the polypeptide when the fusion protein is in an uncleaved state, such that the degron promotes degradation of the polypeptide in a cell; iii) a protease, wherein the protease can be inhibited by contacting said fusion protein with a protease inhibitor; and iv) a cleavable linker that is located between the polypeptide and the degron, wherein the cleavable linker comprises a cleavage site recognized by the protease, wherein cleavage of the cleavable linker by the protease releases the polypeptide from the fusion protein, such that when the fusion protein is in a cleaved state, the degron no longer controls degradation of the polypeptide; a host cell comprising a recombinant polynucleotide; culturing the host cell under conditions suitable for producing; and c) contacting the host cell with a protease inhibitor, such that the polypeptide is degraded when production is no longer desired; and a kit comprising a recombinant polynucleotide.

However, these shared technical features are previously disclosed by US 2010/0041092 A1 to Lin et al. (hereinafter 'Lin') in view of the article 'Elucidation of the Substrate Binding Site of Siah Ubiquitin Ligase' by House et al. (hereinafter 'House').

Lin discloses controllable production of a polypeptide (controllable production of a polypeptide; paragraph [0007]); a nucleic acid encoding (a nucleic acid encoding; paragraph [0010]) a fusion protein (a fusion protein; paragraph [0008]) comprising: i) a polypeptide (comprising: i) a polypeptide; paragraphs [0008], [0016]); and ii) a degron (and ii) a degron; paragraph [0016]), wherein the degron is operably linked to the polypeptide when the fusion protein is in an uncleaved state (wherein the degron is operably linked to the polypeptide when the fusion protein is in an uncleaved state; paragraph [0016]), such that the degron promotes degradation of the polypeptide in a tissue (cell) (such that the degron promotes degradation of the polypeptide in a cell; paragraphs [0007], [0016]); iii) a protease (iii) a protease; paragraph [0008]), wherein the protease can be inhibited by contacting said fusion protein with a protease inhibitor (wherein the protease can be inhibited by contacting said fusion protein with a protease inhibitor; paragraph [0016]); and iv) a cleavable linker that is located between the polypeptide and the degron (and iv) a cleavable linker that is located between the polypeptide and the tag (degron); paragraphs [0008], [0016]), wherein the cleavable linker comprises a cleavage site recognized by the protease (wherein the cleavable linker comprises a cleavage site recognized by the protease; paragraph [0008]), wherein cleavage of the cleavable linker by the protease releases the polypeptide from the fusion protein, such that when the fusion protein is in a cleaved state, the degron no longer controls degradation of the polypeptide (wherein cleavage of the cleavable linker by the protease releases the polypeptide from the fusion protein, such that when the fusion protein is in a cleaved state, the degron no longer controls degradation of the polypeptide; paragraph [0016]); a host cell comprising a recombinant polynucleotide (a host cell comprising a recombinant polynucleotide; paragraph [0012]); culturing the host cell under conditions suitable for producing (culturing the host cell under conditions suitable for producing; paragraphs [0104], [0112]); and c) contacting the host cell with a protease inhibitor (contacting the host cell with a protease inhibitor; paragraphs [0012], [0016]), such that the polypeptide is degraded when production is no longer desired (such that the polypeptide is degraded when production is no longer desired; paragraph [0016]); and a kit comprising a recombinant polynucleotide (a kit comprising a recombinant polynucleotide; paragraph [0015]).

Lin does not disclose an amino acid sequence of SEQ ID NO: 1.

House discloses the structure of a degron motif bound to a ubiquitin ligase (the structure of a degron motif bound to a ubiquitin ligase; abstract), wherein the degron motif comprises an amino acid sequence of SEQ ID NO: 1 (wherein the degron motif comprises the sequence from residues 107-130 of PHYL: LQQERTKLRPVAMVRPTVRVQPQL (wherein the degron motif comprises an amino acid sequence of SEQ ID NO: 1); page 700, first column, second paragraph; wherein Thr112 - Lys113 of the PHYL peptide is identical to thr3 - lys4 of SEQ ID NO: 1), and models interaction of Lys113 with the ligase (models interaction of Lys113 with the ligase; page 699, first column, first paragraph).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Lin to have specified a particular degron sequence, such as the degron provided in the crystal structure disclosed by House, including the residues thr-lys, as disclosed by House, wherein at least one of the residues interacts with a processing enzyme that binds the degron, as disclosed by House, in order to enable a person of ordinary skill in the art at the time the invention was made to produce the fusion molecule disclosed by Lin using a degron having known structure and activity.

Since none of the special technical features of the Groups I and II 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 Lin and House references, unity of invention is lacking.