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29 October 2015

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Previous Correction:
see Notice of 23 July 2015

(54) Title: 18F LABELING OF PROTEINS USING SORTASES

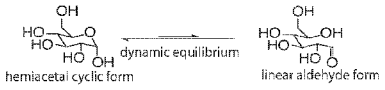

Fig. 1A

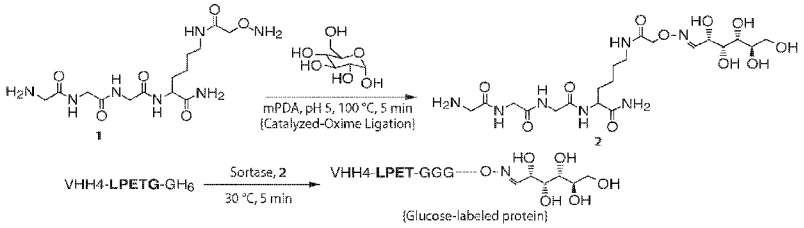

Fig. 1B

(57) Abstract: The present invention, in some aspects, provides methods, reagents, compositions, and kits for the radiolabeling of proteins, for example, of proteins useful for positron emission tomography (PET) or single-photon emission computed tomography (SPECT) (e.g., for diagnostic and therapeutic applications), using sortase-mediated transpeptidation reactions. Some aspects of this invention provide methods for the conjugation of an agent, for example, a radioactive agent or molecule to diagnostic or therapeutic peptides or proteins. Compositions comprising sortagged, radiolabeled proteins as well as reagents for generating radiolalebed proteins are also provided. Kits comprising reagents useful for the generation of radiolabeled proteins are provided, as are precursor proteins that comprise a sortase recognition motif.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/65574

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 16/00, C12N 9/50, C12P 21/06 (2015.01)
CPC - A61K 49/14, A61K 49/16, A61K 49/085, A61K 51/0406, A61K 51/08, A61K 51/088, A61K 51/10
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)
CPC- A61K 49/14, A61K 49/16, A61K 49/085, A61K 51/0406, A61K 51/08, A61K 51/088, A61K 51/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12N 9/54
USPC- 435/68.1, 530/391.5, 435/219

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); PatBase, Google/Scholar: sortase recognition motif, LPXTG, Leu-Pro-any-Thr-Gly, transpeptidation, sortagging, GGG, Cu64, copper-64, radiolabeled, radioconjugate, radioactive, scintigraphic, NMR, imagnetic resonance imaging, MRI, positron emission tomography, PET, 18F...

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Wu. F-18 Labeled Diabody-Luciferase Fusion Proteins for Optical-ImmunoPET. 18 January 2013 [Retrieved from the Internet 23 February 2015: <http://www.osti.gov/scitech/biblio/1060194>]; in entirety	1-3, A1, A2
Y	WO 2013/003555 A1 (PLOEGH, et al.) 03 January 2013 (03.01.2013) Abstract; claims 13, 44, 58; Fig 2B, 2nd col, 1st reaction; Fig 2C; para [37] [00202]-[00220]	1-4, 28-38, and A1-A6
Y	Cooper, et al. Comparison of (64)Cu-complexing bifunctional chelators for radioimmunoconjugation: labeling efficiency, specific activity, and in vitro/in vivo stability. Bioconjug Chem. 2012, 23(5):1029-39; Abstract, pg 1030, col 1 and Fig 1 and its legend	1, 2, 4, 28, 29, 33, 34, A3 -A6
Y	Namavari, et al. A novel method for direct site-specific radiolabeling of peptides using [18F]FDG. Bioconjug Chem. 2009, 20(3):432-436; Abstract, pg 11 [according to the posted document], Scheme 1 and its legend	28-38
X,P	Paterson, et al. Enzyme-mediated site-specific bioconjugation of metal complexes to proteins: sortase-mediated coupling of copper-64 to a single-chain antibody. Angew Chem Int Ed Engl. Epub 28 April 2014, 53(24):6115-9; in entirety	1-4, 28-38, and A1-A6

☐ Further documents are listed in the continuation of Box C. ☐

* 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
07 May 2015 (07.05.2015)	04 JUN 2015
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 W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/65574

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.: 5-23,23(a-c),24-27,39-57,62-79, 84-94,105,109-111,115-120,A7-A76,B25-B35,B39-B44,B58,B59,
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:
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.

Group I: claims 1-4, 28-38, and A1-A6, drawn to a method for site-specifically radio labeling a protein; and a composition comprising a radiolabeled sortase substrate polypeptide, a sortase, and a protein comprising a sortase recognition motif.

Group II: claims 58-61, 95-104, 106-108, 112-114, drawn to a method for radiolabeling a sortase substrate peptide (58-61); composition/kit comprising a sortase substrate polypeptide, a radiolabeled agent (95-104, 106-108, 112-114).

Group III claims 80-83, drawn to a sortase substrate polypeptide comprising a radiolabeled agent.

********** See Supplemental Sheet to continue **********

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.:
1-4, 28-38, and A1-A6

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.

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking:

Group IV claims B1-B20, drawn to a modified protein of Formula (I), wherein L1 is a linker comprising at least four amino acids formed by enzymatic conjugation between two enzyme recognition sequences; and R1 is a reactive group capable of undergoing a click chemistry reaction.

Group V claims B21-B24, B36-B38, B45-B57, B60-B73, drawn to a radioactive protein of Formula (II), Formula (III) or Formula (IV), wherein L1 is a linker comprising at least four amino acids formed by enzymatic conjugation between two enzyme recognition sequences.

The inventions listed as Groups I-V 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:

Special Technical Features
The inventions of Groups I-IV do not include the shared or common technical feature of a radioactive protein of Formula (II), Formula (III) or Formula (IV), as required by Group V.

The inventions of Group V do not include the shared or common technical feature of a method for site-specifically radio labeling a protein or a composition comprising a radiolabeled sortase substrate polypeptide, a sortase, and a protein comprising a sortase recognition motif, as required by Group I; or a method for radiolabeling a sortase substrate peptide, as required by Group II, or a modified protein of Formula (I), as required by Group IV.

The inventions of Group IV do not include the shared or common technical feature of a method for site-specifically radio labeling a protein, or a composition comprising a radiolabeled sortase substrate polypeptide, a sortase, and a protein comprising a sortase recognition motif , as required by Group I; or a method for radiolabeling a sortase substrate peptide, as required by Group II.

The inventions of Group I do not include the shared or common technical feature of a method for radiolabeling a sortase substrate peptide by reacting nucleophilic group with electrophilic group or forming an oxime linkage, as required by Group II.

The inventions of Group II do not include the shared or common technical feature of a method for site-specifically radio labeling a protein or a composition comprising a radiolabeled sortase substrate polypeptide, a sortase, and a protein comprising a sortase recognition motif via click chemistry, as required by Group I.

Common Technical Features
Some inventions of Group I and the inventions of Groups II, III, and V share the technical feature of a radiolabeled sortase substrate peptide. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2002/0142297 A1 to Bogdanov, et al. (03 October 2002) (hereinafter "Bogdanov").

Per Applicant, "the sortase substrate polypeptide comprises an N-terminal sortase recognition motif" (instant application, claim 29), "wherein the N-terminal sortase recognition motif comprises the sequence GGG" (instant application, claim 32).
As such, Bogdanov discloses a sortase substrate peptide (para [0020], FIG. 2, GGG-C; SEQ ID NO: 2) comprising a radiolabeled agent (para [0020], "FIG. 2 is a schematic illustration of a proposed model of the binding of an RPC (GGG-C; SEQ ID NO: 2) to a metal compound"; para [0035], "FIG. 2 illustrates the binding interaction of a proposed model of another RPC core, GGGC (SEQ ID NO: 2), which shows coordination of Tc(V)O by the GGGC motif. The high affinity of oxotechnetate interaction with GGGC leads to re-chelation of oxotechnetate from a complex with other ligands, such as glucoheptanoic or glucaric acids, allowing visualization of the presence of these motifs in recombinant peptides either in situ or in vivo. The latter is more attractive for the purpose of non-invasive detection of spatial distribution and levels of foreign gene expression in vivo, e.g., to monitor gene expression during gene therapy", wherein Technetium in Tc(V)O is a radioactive Technetium-99m (claim 6, " the metal of the metal compound is a radioisotope"; claim 7, "the metal compound is selected from the group consisting of .sup.99mTcO.sub.4.sup.-, .sup.99mTcO.sup.2+").
Bogdanov does not specifically disclose that the GGG-C peptide is a sortase substrate peptide. However, said limitation is met/inherently present in the disclosure of Bogdanov, because the GGG-C peptide of Bogdanov comprises GGG, i.e. the sortase recognition motif (instant application, claim 32, "the N-terminal sortase recognition motif comprises the sequence GGG"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

The inventions of Groups IV and V share the technical feature of a modified protein comprising a linker comprising at least four amino acids formed by enzymatic conjugation between two enzyme recognition sequences. However, this shared technical feature does not represent a contribution over prior art as being anticipated by a publication titled "Production of unnaturally linked chimeric proteins using a combination of sortase-catalyzed transpeptidation and click chemistry" by Witte, et al. (Nat Protoc. Epub 29 August 2013, 8(9):1808-19) (hereinafter "Witte").
'Witte discloses a modified protein comprising a linker comprising at least four amino acids formed by enzymatic conjugation between two enzyme recognition sequences (pg 1809, Figure 1, and its legend, "... unnaturally linked C-to-C and N-to-N chimeric proteins. (a) C-to-C chimeric proteins. (b) N-to-N chimeric proteins. By using sortase A, the first protein is equipped with a click handle (i.e., azide). The second protein is equipped with a complementary click handle (i.e. cyclooctyne) in a similar manner. Combining the purified proteins produces the unnaturally linked chimeric protein", wherein the linker is LPXTGGG and the protein is modified either with an zido groups N3 or cyclooctyne). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the groups.

********** See the Following Supplemental Sheet to continue **********

In Continuation of Box III. Observations where unity of invention is lacking and the Preceding Supplemental Sheet:

In addition, a method for site-specifically radio labeling a protein would have been obvious to one of ordinary skill in the art at the time of the invention over a publication "F-18 Labeled Diabody-Luciferase Fusion Proteins for Optical-ImmunoPET" by Wu (December 2012) [Retrieved from the Internet 23 February 2015: <http://www.osti.gov/scitech/biblio/1060194>] (hereinafter "Wu") in view of WO 2013/003555 A1 to PLOEGH, et al. (3 January 2013) (hereinafter "Ploegh").

Wu suggest s site-specifically radiolabeling a protein by "(1) using sortase mediated ligation (SML) for introduction of azido groups site-specifically into LPXTG-tagged diabody-luciferase fusion proteins. It can be later radiolabeled with a universal cyclooctyne 18F-tag by a strain-promoted, copper-free click reaction" (pg 3), and further discloses that "[s]everal amino ligands posessing tetrazine or azadibenzocyclooctyne moieties were synthesized. [18F]-SFB was produced by one-pot synthesis and coupled to produce five different tags for bioorthogonal labeling. [18F]TBFB proved most stable and reproducible" (pg 3).
Wu does not specifically disclose a specific embodiment of a method for site-specific sortase mediated radiolabeling of a LPXTG-tagged protein.
Ploegh discloses a method for "[p]roduction of N-to-N and C-to-C protein fusions created by combining click chemistry with a sortase-catalyzed transacylation" (para [00202]-[00210]), including radioactively labeled protein fusions (claim 44, "the detectable label comprises... a radioisotope"). It would have been obvious to one of ordinary skill in the art to combine, in the course of routine experimentation and with a reasonable expectation of success, Wu and Ploegh, by using the method for "[p]roduction of N-to-N and C-to-C protein fusions created by combining click chemistry with a sortase-catalyzed transacylation" disclosed by Ploegh (Ploegh, para [00202]-[00210]; claim 44) to obtain a site-specifically radiolabeled diabody-luciferase by "(1) using sortase mediated ligation (SML) for introduction of azido groups site-specifically into LPXTG-tagged diabody-luciferase fusion proteins" (pg 3) for use in nuclear medicine imaging (Wu, pg 2, "c)... Development of these combined optical/nuclear imaging probes will have direct applications to observation and quantitation of biological targets. In particular, these multimodality probes will enhance nuclear medicine imaging, especially in PET, by enabling simultaneous detection of more than one molecular target").

Groups I-V therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note re item 4:
Claims 5-23, 23a, 23b, 23c, 24-27, 39-57, 62-79, 84-94, 105, 109-111, 115-120, A7-A76, B25-B35, B39-B44, B58, B59, B74-B78 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

Note re claims 23a, 23b, 25, 38, B72, and B73:

Claim 23a is objected to as self-referring. For the purpose of this Lack of Unity argument, this ISA has construed claim 23a as being dependent from claim 23.

Claim 23b is objected to as self-referring. For the purpose of this Lack of Unity argument, this ISA has construed claim 23b as being dependent from claim 23 or claim 23a.

Claim 25 is objected to as unclear, because is depends on claim 23d that is not present in the application. For the purpose of this Lack of Unity argument, this ISA has construed claim 25 as being dependent from any one of claims 23-23c.

Claim 38 is objected to as self-referring. For the purpose of this Lack of Unity argument, this ISA has construed claim 38 as being dependent from claim 34:

Claim B72 and B73 are objected to as unclear, because their parent claim 66 does not provide a sufficient antecedent basis for the 'radioactive protein" limitation. For the purpose of this Lack of Unity argument, this ISA has construed claim B72 and B73 as being dependent from claim B66.