(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 December 2016 (08.12.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/196846 A3

(51) International Patent Classification:
C07K 14/005 (2006.01)

(21) International Application Number:
PCT/US2016/035594

(22) International Filing Date:
2 June 2016 (02.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/169,814 2 June 2015 (02.06.2015) US
62/344,862 2 June 2016 (02.06.2016) US

(71) Applicant: **SANOFI PASTEUR INC.** [US/US]; 1 Discovery Drive, Swiftwater, PA 18730 (US).

(72) Inventors: **STRUGNELL, Tod**; Sanofi Pasteur Inc., 38 Sidney Street, Cambridge, MA 02139 (US). **OLOO, Eliud**; Sanofi Pasteur Inc., 38 Sidney Street, Cambridge, MA 02139 (US). **OOMEN, Raymond**; Sanofi Pasteur Inc., 38 Sidney Street, Cambridge, MA 02139 (US).

(74) Agent: **CHEN, Fangli**; Proskauer Rose LLP, One International Place, Boston, MA 02110 (US).

(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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, 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, TR, TT, TZ, UA, UG, US, UZ, VC, VN, 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).

Published:

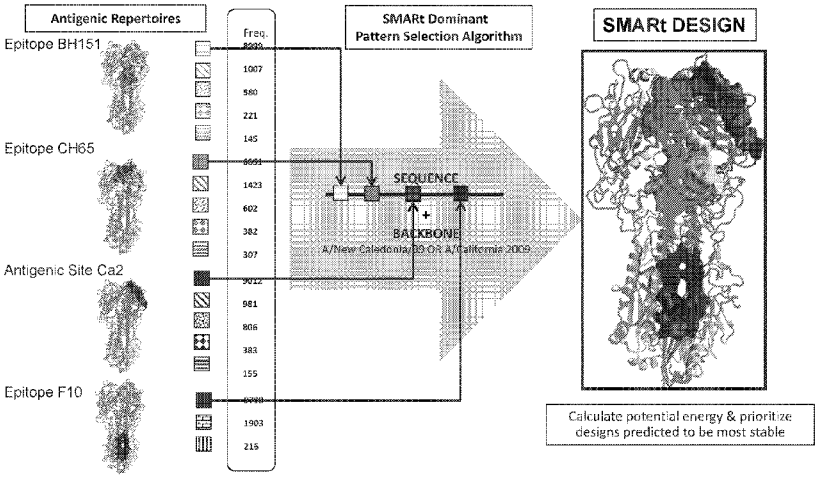
— *with international search report (Art. 21(3))*

— *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))*

(88) Date of publication of the international search report:
12 January 2017

(54) Title: ENGINEERED INFLUENZA ANTIGENIC POLYPEPTIDES AND IMMUNOGENIC COMPOSITIONS THEREOF

Figure 16



(57) Abstract: The present invention provides, among other things, a novel and improved method for generating "mosaic" influenza antigenic polypeptides including hemagglutinin (HA) and neuraminidase (NA) polypeptides based on unique combination of epitope patterns that maximize exposure to epitopes present across multiple HA or NA sequences and therefore improved influenza strain coverage. In particular, the present invention provides engineered H1N1 influenza hemagglutinin (HA) polypeptides that are comprised of novel combinations of protective epitopes and antigenic regions from multiple H1N1 viral strains. Such engineered HA polypeptides have improved properties over HA polypeptides developed through conventional approaches that rely on consensus alignments of viral sequences.

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/005
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

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 Y	WO 2009/026397 A2 (FRAUNHOFER USA INC [US]; YUSIBOV VIDADI [US]) 26 February 2009 (2009-02-26) page 72, line 1 - line 4; claims 1-3,13, 21, 23-33, 39-42, 44; sequence 31 -----	1,2, 26-30, 36,39-45 1-6, 26-45
X Y	WO 2013/044203 A2 (US OF AMERICA AS REPRESENTED BY THE SECRETARY DEPTARTMENT OF HEALTH &) 28 March 2013 (2013-03-28) claims 1,11-13,17,19,31-34,38,43,44,45,49,57-59,63,70-78; sequence 8 ----- -/--	1,2,26, 28,29, 31,36, 37,39,43 1-6,26, 28,29, 31-37, 39,43

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
30 November 2016	06/12/2016

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

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

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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.:

6(completely); 1-5, 26-45(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.

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/003225 A1 (MEDICAGO INC [CA]; D AOUST MARC-ANDRE [CA]; COUTURE MANON [CA]; ORS FR) 14 January 2010 (2010-01-14)	1,2, 26-29, 32,33, 38,39, 43,44
Y	claims; sequence 77	1-6, 26-29, 32-35, 38,39, 43,44

X	WO 2009/076778 A1 (MEDICAGO INC [CA]; D AOUST MARC-ANDRE [CA]; COUTURE MANON [CA]; ORS FR) 25 June 2009 (2009-06-25)	1,2, 26-29, 32,33, 36,38-45
Y	paragraph [0277]; claims 1,4-9, 12, 16-27; sequence 75	1-6, 26-29, 32-36, 38-45

Y	Harry Kleanthous, Sanofi Pasteur: "Re-eingeneering HA as a strategy to develop universal influenza vaccines", , 24 January 2013 (2013-01-24), XP002762245, Retrieved from the Internet: URL:http://apps.who.int/vaccine_research/meetings/Harold_Kleanthous.pdf [retrieved on 2016-09-22] the whole document	1-6, 26-45

Y	WO 2013/148164 A1 (UNIV PITTSBURGH [US]) 3 October 2013 (2013-10-03) claims; figures	1-6, 26-33, 36-45

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Y	WO 2008/094200 A2 (LIGOCYTE PHARMACEUTICALS INC [US]; HAYNES JOEL R [US]) 7 August 2008 (2008-08-07) paragraphs [0027] - [0031]; claims 6,11-15,18-25,39-56; figures 2-4 paragraphs [0044] - [0048] paragraphs [0078] - [0088]	34,35

Y,P	WO 2015/184272 A2 (SANOFI PASTEUR BIOLOGICS LLC [US]) 3 December 2015 (2015-12-03) claims; figures	1-6, 26-45

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/035594

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2016/100926 A1 (UNIV OREGON HEALTH & SCIENCE [US]; SANOFI PASTEURE INC [US]) 23 June 2016 (2016-06-23) claims; figures	1,2
X,P	CARTER DONALD M ET AL: "Design and Characterization of a Computationally Optimized Broadly Reactive Hemagglutinin Vaccine for H1N1 Influenza Viruses", JOURNAL OF VIROLOGY (ONLINE), AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 90, no. 9, 14 April 2016 (2016-04-14) , pages 4720-4734, XP009189781, ISSN: 1098-5514 the whole document	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 6(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to an engineered influenza HA polypeptide that appears in Table 1; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 1; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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2. claims: 7(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 2; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 2; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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3. claims: 8(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 3; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 3; an isolated nucleic acid molecule encoding said engineered HA

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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4. claims: 9(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 4; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 4; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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5. claims: 10(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 5; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 5; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said

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influenza HA polypeptide, said fusion protein, or said influenza VLP;

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6. claims: 11(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 6; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 6; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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7. claims: 12(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 7; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 7; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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8. claims: 13(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 8; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 8; an isolated nucleic acid molecule encoding said engineered HA

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polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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9. claims: 14(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 9; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 9; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said influenza HA polypeptide, said fusion protein, or said influenza VLP;

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10. claims: 15(completely); 1-5, 26-45(partially)

An engineered influenza hemagglutinin (HA) polypeptide comprising an amino acid sequence that is at least 90% identical to to SEQ ID NO 10; said HA polypeptide wherein the amino acid sequence is identical to SEQ ID NO 10; an isolated nucleic acid molecule encoding said engineered HA polypeptide; a vector comprising said nucleic acid sequence; an isolated cell comprising said vector; a fusion protein comprising said influenza HA polypeptide; an influenza virus-like particle (VLP) comprising said influenza HA polypeptide; a method for producing an influenza VLP comprising said influenza HA polypeptide; a pharmaceutical composition comprising saidinfluenza HA polypeptide; a method of immunizing a subject against influenza virus, comprising administering to the subject said pharmaceutical composition; a method of inducing an immune response in a subject, comprising administering to the subject said

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

influenza HA polypeptide, said fusion protein, or said influenza VLP;

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11. claims: 16-25(completely); 26-45(partially)

an engineered influenza hemagglutinin (HA) polypeptide comprising an antigenic region that is associated with, adjacent to and/or encompasses a receptor binding site, wherein the antigenic region comprises an amino acid sequence, or subset thereof, that is at least 90% identical to an antigenic region sequence, or subset thereof, that appears in Table 2 (Seq ID NO 11 to 39) ; An isolated nucleic acid molecule encoding such an engineered HA polypeptide; A vector comprising such nucleic acid sequence; An isolated cell comprising said vector; A fusion protein comprising such an influenza HA polypeptide; An influenza virus-like particle (VLP) comprisingsuch an influenza HA polypeptide; A method for producing an influenza VLP comprising said influenza HA polypeptide; A pharmaceutical composition comprising such an influenza HA polypeptide; A method of immunizing a subject against influenza virus, comprising administering to the subject such a pharmaceutical composition; A method of inducing an immune response in a subject, comprising administering to the subject such an influenza HA polypeptide, such a fusion protein, or such an influenza VLP;

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12. claims: 46(completely); 48-78(partially)

A method of engineering a mosaic influenza hemagglutinin (HA) polypeptide, comprising obtaining HA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus; aligning the HA amino acid sequences to generate an alignment; identifying the positions of amino acids comprising known epitopes and antigenic regions; compiling the amino acid residues across the alignment at the identified positions for each epitope and antigenic region;defining a set of amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino acid sequence pattern in the set is represented only once; selecting a sequence from the set for each epitope or antigenic region; and inserting selected sequences into corresponding locations in a structural backbone of HA to generate a mosaic influenza HA polypeptide;

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13. claims: 47(completely); 48-78(partially)

A method of engineering a mosaic influenza hemagglutinin (HA) polypeptide, comprisingobtaining HA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus;aligning the HA amino

acid sequences to generate an alignment;identifying the positions of amino acids comprising known epitopes and antigenic regions across the alignment;compiling the amino acid residues at the identified positions for each epitope and antigenic region;defining a set amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is represented only once;generating a consensus sequence from each set for each epitope or antigenic region; andinserting the consensus sequences into corresponding locations in a structural backbone of HA to generate a mosaic influenza HA polypeptide.

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14. claims: 79(completely); 81-99(partially)

A method of engineering a mosaic influenza neuraminidase (NA) polypeptide, comprising:
obtaining NA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus;
aligning the NA amino acid sequences to generate an alignment;
identifying the positions of amino acids comprising known epitopes and antigenic regions;
compiling the amino acid residues across the alignment at the identified positions for each epitope and antigenic region;
defining a set of amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is represented only once;
selecting a sequence from the set for each epitope or antigenic region; and
inserting selected sequences into corresponding locations in a structural backbone of NA to generate a mosaic influenza NA polypeptide ;

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15. claims: 80(completely); 81-99(partially)

A method of engineering a mosaic influenza neuraminidase (NA) polypeptide, comprising:
obtaining NA amino acid sequences from multiple circulating strains of a particular type and/or subtype of influenza virus;
aligning the NA amino acid sequences to generate an alignment;
identifying the positions of amino acids comprising known epitopes and antigenic regions across the alignment;
compiling the amino acid residues at the identified positions for each epitope and antigenic region;
defining a set amino acid sequence patterns within the compiled sequences for each epitope and antigenic region, wherein each amino sequence pattern in the set is

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

represented only once;
generating a consensus sequence from each set for each epitope or antigenic region; and
inserting the consensus sequences into corresponding locations in a structural backbone of NA to generate a mosaic influenza NA polypeptide.
