UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
62/191,028	07/10/2015		130	00742-257001		

CONFIRMATION NO. 3822

FILING RECEIPT

21559
CLARK & ELBING LLP
101 FEDERAL STREET
BOSTON, MA 02110

Date Mailed: 07/27/2015

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

John J. MEKALANOS, Brighton, MA;

Applicant(s)

President and Fellows of Harvard College, Cambridge, MA;

Power of Attorney:
Kristina Bieker-Brady--39109

If Required, Foreign Filing License Granted: 07/24/2015

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 62/191,028**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent

in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE

FIELD OF THE INVENTION

5

The invention relates to immunogenic polysaccharide-protein conjugates, a novel sortase-mediated method of making immunogenic polysaccharide-protein conjugates, and methods of administering immunogenic polysaccharide-protein conjugates.

10
BACKGROUND OF THE INVENTION

Many antigens, particularly those associated with a pathogen's capsule layer stimulate little or no immune response and complicate efforts to create effective immunogenic conjugates against those antigens. Capsules are surface components of microbes that are typically composed of polymers of
15 organic compounds such as carbohydrates, amino acids, or alcohols. Capsules are quite diverse chemically. The monomeric units that make up capsules (e.g., carbohydrates) can be linked together in various molecular configurations and can be further substituted with phosphate, nitrogen, sulfate, and other chemical modifications. These chemical variations allow capsules to present numerous antigenic targets on the microbial surface, thus allowing escape from the host immune response directed at these
20 targets. Capsules can also be virulence factors which prevent microbes from being phagocytosed and killed by host macrophages and polymorphonuclear leukocytes. Antibodies against capsules provide a potent defense against encapsulated organisms by fixing complement to the microbial surface, which can result in their lysis or their opsonization, uptake, and killing by phagocytic host immune cells. The most potent antibodies against capsules are IgG antibodies. Capsules that fail to induce significant levels of
25 IgG are called T-independent antigens. Covalent coupling of a protein to capsules renders them "T-dependent" and such antigens can elicit an IgG response.

There is a need for safe, synthetically accessible, cost-effective immunogenic conjugates directed to capsules and other T-independent antigens that do not evoke strong immune responses or IgG
30 antibodies. Such immunogenic conjugates are needed to protect against various infectious diseases such as infection by anthrax, pneumococcus, influenzae Type B, meningococcus, and streptococcus.

SUMMARY OF THE INVENTION

35 The present invention relates to immunogenic compositions containing a polysaccharide antigen conjugated with a carrier protein in a complex, methods of making such immunogenic compositions, and methods of administering such immunogenic compositions.

Petitioner Merck Ex-1013, 0004

In a first aspect, the invention features an immunogenic composition including a polysaccharide-sortase conjugate, wherein the polysaccharide is an antigen and the sortase is a carrier protein, and wherein the sortase is covalently linked to the polysaccharide antigen by a sortase recognition sequence.

5 In a second aspect, the invention features an immunogenic composition including a polysaccharide-protein conjugate, the conjugate including a polysaccharide antigen and a carrier protein, wherein the polysaccharide antigen is covalently linked to the carrier protein by a sortase recognition sequence and a polyglycine motif present at the N-terminus of the carrier protein.

10 In some embodiments, the immunogenic composition includes a sortase selected from sortase A, sortase B, sortase C, and sortase D. The immunogenic composition may include a sortase A, or a fragment thereof. The immunogenic composition may include sortase A having the amino acid sequence of SEQ ID NO: 1. The immunogenic composition may include a sortase having an amino acid sequence that has at least 90% identity to the amino acid sequence of SEQ ID NO: 1. The immunogenic
15 composition may include a sortase having an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO: 1. The immunogenic composition may include a sortase having an amino acid sequence that has at least 99% identity to the amino acid sequence of SEQ ID NO: 1. The immunogenic composition may include a sortase B, or a fragment thereof. The immunogenic composition may include a sortase C, or a fragment thereof. The immunogenic composition may include
20 a sortase D, or a fragment thereof.

In additional embodiments, the immunogenic composition may include a sortase including at least one mutation. The immunogenic composition may include a sortase that includes a substitution. The immunogenic composition may include the sortase recognition sequence having the formula $X_1PX_2X_3$ X_4,
25 where X_1-X_4 are any amino acid. The immunogenic composition may further include the sortase recognition sequence having the formula $X_1PX_2X_3G$ for a sortase A substrate, where X_1 is Leu, Ile, Val or Met, X_2 is any amino acid, and X_3 is Ser, Thr or Ala. The immunogenic composition may include the sortase recognition sequence LPX_1TG for a sortase A substrate, where X_1 is any amino acid. The immunogenic composition may include the sortase recognition sequence NPX_1TX_2 for a sortase B
30 substrate, where X_1 is Lys or Gln and X_2 is Asn, Asp, or Gly. The immunogenic composition may include the sortase recognition sequence LPX_1TX_2 for a sortase C substrate, where X_1 and X_2 are any amino acid. The immunogenic composition may include the sortase recognition sequence LPX_1TA for a sortase D substrate, where X_1 is any amino acid. The immunogenic composition may include the sortase recognition sequence, LAX_1TG for a sortase D substrate, where X_1 is any amino acid.

35

In other embodiments, the invention features an immunogenic composition where the polyglycine motif includes an amino acid sequence selected from GG, GGG, GGGG, and GGGGG. The invention

Petitioner Merck Ex-1013, 0005

further includes an immunogenic composition where the polyglycine motif includes the amino acid sequence GGGGG.

In another embodiment, the immunogenic composition of the invention includes carrier protein molecules that are selected from diphtheria toxin, diphtheria toxoid, tetanus toxin, tetanus toxoid, *Pseudomonas aeruginosa* exotoxin A, cholera toxin B subunit, tetanus toxin fragment C, bacterial flagellin, pneumolysin, an outer membrane protein of *Neisseria menningitidis*, *Pseudomonas aeruginosa* Hcp1 protein, *Escherichia coli* heat labile enterotoxin, shiga-like toxin, human LTB protein, pneumolysin, listeriolysin O, a protein extract from whole bacterial cells, the dominant negative mutant (DNI) of the protective antigen of *Bacillus anthracis*, and *Escherichia coli* beta-galactosidase. The invention also features an immunogenic composition where the whole bacterial cells are *Pseudomonas aeruginosa* or *Streptococcal* cells. The invention includes an immunogenic composition where the bacterial flagellin is the *Vibrio cholerae* flagellin protein. The invention includes an immunogenic composition where the shiga-like toxin is the *Shigella* SltB2 protein.

In additional embodiments, the immunogenic composition includes an antigen of interest where the antigen of interest is a polysaccharide, a polyalcohol, or a poly amino acid. The polysaccharide of the immunogenic composition may include at least 18 residues. The polysaccharide of the immunogenic composition may include a *Streptococcus pneumoniae* polysaccharide, *Francisella tularensis* polysaccharide, *Bacillus anthracis* polysaccharide, *Haemophilus influenzae* polysaccharide, *Salmonella typhi* polysaccharide, *Salmonella* species polysaccharide, *Shigella* polysaccharide, or *Neisseria meningitidis* polysaccharide. The *Streptococcus pneumoniae* polysaccharide of the immunogenic composition may be a capsular type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, or 48. The immunogenic composition of the invention may further include a *Francisella tularensis* polysaccharide that is an O antigen.

In further embodiments, the antigen of interest of the immunogenic composition is a microbial capsular polymer. The microbial capsular polymer of the immunogenic composition may include a poly-gamma-D-glutamic acid from *Bacillus anthracis*. The antigen of interest of the immunogenic composition may also include an organic polymer consisting of monomers having at least three atoms where each of the atoms is independently selected from carbon, oxygen, hydrogen, phosphate, nitrogen, and sulfate. The antigen of interest may include an organic polymer that is obtained from a microbe and/or does not occur in nature.

The immunogenic composition may further include, e.g., a second antigen of interest or a third antigen of interest. The immunogenic composition may include an antigen of interest including whole cell pathogens where the whole cell pathogens include heat inactivated whole cell pathogens; chemically inactivated whole cell pathogens; and/or *Pseudomonas aeruginosa* or *Streptococcal* cells, wherein the
5 *Streptococcal* cells include *Streptococcus pneumonia*. The immunogenic composition may further include whole cell pathogens from *Streptococcus pneumonia* type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40,
10 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, or 48. The antigen of interest of the immunogenic composition may include at least one whole cell pathogen selected from the group consisting of *Streptococcus pneumonia* type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A,
15 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48. The antigen of interest may also include two or more whole cell pathogens selected from the group consisting of *Streptococcus pneumonia* type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F,
20 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

The invention features an immunogenic composition that, when administered to a mammal, elicits a T-cell dependent immune response in the mammal. In the immunogenic composition of the invention,
25 the molar ratio of the antigen to the carrier protein molecules may be 1 to 1.

The invention further features a pharmaceutical composition in unit dosage form including (i) the immunogenic composition of the invention and (ii) a pharmaceutically acceptable excipient.

30 In another aspect, the invention features a method of making an immunogenic composition including a polysaccharide-protein conjugate, the method includes mixing a polysaccharide antigen including a sortase recognition sequence and a sortase where the mixing results in formation of a thioester bond between the polysaccharide antigen and the sortase, yielding the polysaccharide-protein conjugate. The method of making an immunogenic composition may further include mixing the
35 polysaccharide-protein conjugate and a carrier protein including a polyglycine motif at its N-terminus where the mixing results in formation of a peptide bond between a terminal carboxyl group of a sortase recognition sequence attached to the polysaccharide antigen and a terminal amino group of the

Petitioner Merck Ex-1013, 0007

polyglycine motif of the carrier protein. The method of making an immunogenic composition may include the polysaccharide antigen that is a whole cell pathogen.

In additional embodiments, the invention features the use of the immunogenic composition of the invention to generate an immune response in a subject including administering the pharmaceutical composition of the invention to a subject where the immunogenic composition elicits a T-cell dependent immune response in the subject. The invention also features a method of generating an immune response in a subject including administering the pharmaceutical composition of the invention to a subject where the immunogenic composition elicits a T-cell dependent immune response in the subject. The subject may be an infant, a child, or an adolescent.

DEFINITIONS

By "administering" as used herein in conjunction with an immunogenic conjugate, is meant providing to a subject an immunogenic conjugate in a dose sufficient to induce an immune response in the subject, where the immune response results in the production of antibodies that specifically bind an antigen contained in the immunogenic conjugate. Administering desirably includes parenteral administration (for instance, by subcutaneous, intramuscular, intravenous, or intradermal injection). While administering by a means that physically penetrates the dermal layer is desirable (e.g., a needle, airgun, or abrasion), the immunogenic conjugates of the invention can also be administered by transdermal absorption. Desirably, administration involves inclusion of the appropriate immune adjuvants. Administering also includes enterally (for instance, by oral administration) by ingestion of an immunogenic conjugate in the form of a e.g., liquid, powder, capsule, or tablet. Administering may involve a single administration of an immunogenic conjugate or administering an immunogenic conjugate in multiple doses. Desirably, a second administration is designed to boost production of antibodies in a subject to reduce the likelihood of infection by an infectious agent. The frequency and quantity of the dosage of immunogenic conjugate depends on the specific activity of the immunogenic conjugate and can be readily determined by routine experimentation.

By “amino acid” is meant a residue in a polypeptide sequence that can be naturally occurring or synthetic. A naturally occurring amino acid is one encoded by the genetic code. A synthetic amino acid is one that is analogous in chemical structure to a naturally occurring amino acid; or one that has a different chemical structure from a naturally occurring amino acid yet functions similarly to a naturally occurring amino acid. Amino acids may be referred to herein by their single or three letter abbreviations. The single letter abbreviation for a particular amino acid, its corresponding amino acid, and three letter abbreviation are as follows: A, alanine (Ala); C, cysteine (Cys); D, aspartic acid (Asp); E, glutamic acid (Glu); F, phenylalanine (Phe); G, glycine (Gly); H, histidine (His); I, isoleucine (Ile); K, lysine (Lys); L,

Petitioner Merck Ex-1013, 0008

leucine (Leu); M, methionine (Met); N, asparagine (Asn); P, proline (Pro); Q, glutamine (Gln); R, arginine (Arg); S, serine (Ser); T, threonine (Thr); V, valine (Val); W, tryptophan (Trp); and Y, tyrosine (Tyr).

By "antigen" as used herein is meant any molecule or combination of molecules that is
5 specifically bound by an antibody or an antibody fragment.

By "boost the production of antibodies" is meant the activation of memory B-cells that occurs during a second exposure to an antigen, called a "booster response," and is indicative of a long lived "secondary" memory immune response, resulting in the long lived production of antibodies.

10

By "carrier protein" is meant a protein used in an immunogenic composition that invokes an immune response to itself and/or to an antigenic polysaccharide covalently linked with a carrier protein. Desirably, the carrier protein contains an epitope recognized by a T-cell. Also encompassed by the definition of a carrier protein are enzymes of the sortase family of proteins. Desirably, a carrier protein
15 includes multi-antigenic peptides (MAPs), which are branched peptides and include lysine. Exemplary desirable carrier proteins include toxins and toxoids (chemical or genetic), which may be mutant. Desirably, a carrier protein is diphtheria toxin or a mutant thereof, diphtheria toxoid, tetanus toxin or a mutant thereof, tetanus toxoid, *Pseudomonas aeruginosa* exotoxin A or a mutant thereof, cholera toxin B subunit, tetanus toxin fragment C, bacterial flagellin, pneumolysin, listeriolysin O (and related molecules),
20 an outer membrane protein of *Neisseria menningitidis*, *Pseudomonas aeruginosa* Hcp1 protein, *Escherichia coli* heat labile enterotoxin, shiga-like toxin, human LTB protein, a protein extract from whole bacterial cells, the dominant negative mutant (DNI) of the protective antigen of *Bacillus anthracis*, or *Escherichia coli* beta-galactosidase, or any other protein that can be conjugated by a peptide linker.

25 By "conjugated" or "conjugation" is meant an association of two entities, for example, of two molecules such as a polysaccharide and a protein. The association can be, for example, via an indirect (e.g., via a peptide linker) covalent linkage connecting both molecules. Exemplary desirable conjugations include a polysaccharide and a carrier protein conjugated to each other to form a polysaccharide-protein fusion, where the polysaccharide and carrier protein are conjugated via a peptide linker, e.g., an amino
30 acid sequence connecting a polysaccharide side chain to an amino acid residue of the carrier protein. Desirably, conjugation of a polysaccharide to a carrier protein is achieved by transpeptidation, where the carrier protein is a sortase enzyme. Desirably, conjugation of a polysaccharide to a carrier protein is achieved by transamidation, where the carrier protein replaces a sortase covalently linked to a polysaccharide to form a new polysaccharide-carrier protein conjugate.

35

Petitioner Merck Ex-1013, 0009

By "covalently linked" is meant the formation of a covalent bond between two molecules, macromolecules, or combination of molecules, e.g., between a polysaccharide and a carrier protein.

By "DNI" is meant the dominant negative mutant (DNI) protein, which is a mutated form of
5 protective antigen (PA) of *B. anthracis*, as described by Benson et al. (*Biochemistry* 37:3941-3948, 1998).

By "infection" is meant the invasion of a subject by a microbe, e.g., a bacterium, fungus, parasite, or virus. The infection may include, for example, the excessive multiplication of microbes that are normally present in or on the body of a subject or multiplication of microbes that are not normally present
10 in or on a subject. A subject is suffering from a microbial infection when an excessive amount of a microbial population is present in or on the subject's body or when the presence of a microbial population(s) is damaging the cells or causing pathological symptoms to a tissue of the subject.

By "infectious agent" is meant a microbe, such as a bacterium, fungus, parasite, or virus that is
15 capable of causing an infection in a subject.

By "immunogenic" is meant a compound that induces an immune response in a subject. Desirably, the immune response is a T-cell dependent immune response that involves the production of IgG antibodies.

20

By "microbial capsular polymer" is meant a polymer present in or on the capsule coating of a microbe. Desirably, a microbial capsular polymer is an organic polymer such as a polysaccharide, phosphopolysaccharide, polysaccharide with an amino sugar with a N-acetyl substitution, polysaccharide containing a sulfonylated sugar, another sulfate-modified sugar, or phosphate-modified sugar,
25 polyalcohol, poly amino acid, teichoic acid, and an O side chain of a lipopolysaccharide.

By "monomer" is meant a molecular structure capable of forming two or more bonds with like monomers, often yielding a chain or a series of branched, connected chains of repeating monomer substructures, when part of a "polymer."

30

By "organic polymer" is meant a polymer composed of covalently linked monomers each having three or more of the following atoms: carbon, oxygen, hydrogen, phosphate, nitrogen, and sulfate. Desirably, an organic polymer is a polysaccharide, phosphopolysaccharide, polysaccharide with an amino sugar with a N-acetyl substitution, polysaccharide containing a sulfonylated sugar, another sulfate-
35 modified sugar, or phosphate-modified sugar, sugar, polyalcohol, polyamino acid, teichoic acid, and an O side chain of lipopolysaccharide.

By "polyalcohol" is meant a hydrogenated form of a carbohydrate where a carbonyl group has been reduced to a primary or secondary hydroxyl group. Exemplary polyalcohols are a polyalkylene oxide (PAO), such as a polyalkylene glycols (PAG), including polymethylene glycols, polyethylene glycols (PEG), methoxypolyethylene glycols (MPEG) and polypropylene glycols; poly-vinyl alcohol (PVA); polyethylene-co-maleic acid anhydride; polystyrene-co-maleic acid anhydride; dextrans including carboxymethyl-dextrans; celluloses, including methylcellulose, carboxymethylcellulose, ethylcellulose, hydroxyethylcellulose carboxyethylcellulose, and hydroxypropylcellulose; hydrolysates of chitosan; starches such as hydroxyethyl-starches and hydroxy propyl-starches; glycogen; agaroses and derivates thereof; guar gum; pullulan; insulin; xanthan gum; carrageenan; pectin; alginic acid hydrolysates; sorbitol; an alcohol of glucose, mannose, galactose, arabinose, gulose, xylose, threose, sorbose, fructose, glycerol, maltose cellobiose, sucrose, amylose, amylopectin; or mono propylene glycol (MPG).

By "polyglycine" is meant a $(Gly)_n$ sequence. Desirably n is between 2 and 20, or more desirably between 2 and 5, and even more desirably 5 glycine residues.

By “polysaccharide antigen” is meant a polymer of saccharides (sugars) derived from capsules of encapsulated bacterial pathogens such as *Streptococcus pneumoniae*, *Francisella tularensis*, *Bacillus anthracis*, *Haemophilus influenzae*, *Salmonella typhi*, *Salmonella* species, *Shigella*, or *Neisseria meningitidis* that is specifically bound by an antibody or an antibody fragment.

By “sortase” is meant a protein having a catalytic domain with activity capable of i) selectively cleaving a backbone amide bond of a polypeptide (peptidase activity) at a "sortase recognition sequence," and ii) selectively catalyzing the formation of an amide bond between the terminal carboxyl group created by the cleavage and the free primary amino ($R\text{-}CH_2\text{-}NH_2\text{-}R'$) group of a glycine (transamidase activity). Sortases may be derived from enzymes expressed on the surface of Gram-positive bacteria which cleave cell surface proteins and link them to cell wall proteoglycans. Sortases may desirably be derived from one of four families of sortase enzymes including sortase A, sortase B, sortase C, and sortase D.

By "sortase ligation sequence" is meant an amino acid sequence that is capable of being selectively ligated to a second amino acid sequence by a sortase. A sortase ligation sequence can be either a sortase recognition sequence or a polyglycine sequence.

By “sortase recognition sequence” is meant the consensus sequence for a sortase enzyme substrate. Desirably, the consensus sequence is $X_1PX_2X_3G$ for a sortase A substrate, where X_1 is Leu, Ile, Val or Met, P is Pro, X_2 is any amino acid, X_3 is Ser, Thr or Ala, and G is Gly. Desirably, the sortase recognition sequence for sortase A is LP X_1TG. Desirably the consensus sequence is NPX_1TX_2 for a

Petitioner Merck Ex-1013, 0011

sortase B substrate, where N is Asn, P is Pro, X_1 is Lys or Gln, T is Thr, and X_2 is Asn, Asp, or Gly. Desirably, the sortase recognition sequence for sortase C is LPX_1TX_2, where L is Leu, P is Pro, X_1 and X_2 are any amino acid residue, and T is Thr. Desirably the consensus sequences for sortase D are LPX_1TA or LAX_1TG, where L is Leu, P is Pro, X_1 is any amino acid, T is Thr, A is Ala, and G is Gly.

5

By "subject" is meant an animal that can be infected by a microbe. Desirably, a subject is a mammal such as a human, monkey, dog, cat, mouse, rat, cow, sheep, goat, or horse. In a desirable embodiment, the subject is a human, such as a human child. Desirably, the subject is a human infant, toddler, pre-pubescent child, pubescent child, young adult, or adult under the age of 55 years old.

10

By "T-cell independent antigen" is meant an antigen which results in the generation of antibodies without the cooperation of T lymphocytes. The T-cell independent antigen desirably directly stimulates B lymphocytes without the cooperation of T lymphocytes. Exemplary desirable T-cell independent antigens include capsular antigen poly-gamma-D-glutamic acid (PGA), alginic acid (algenate), dextran,

15 polysaccharides (PS), poly amino acids, polyalcohols, and nucleic acids.

The term “percent (%) identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if

20 necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent identity to a polypeptide sequence can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including

25 any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, for a reference polypeptide of amino acid sequence A, when compared to the derivative polypeptide of amino acid sequence B, the percent identity to an amino acid sequence is calculated as:

100 times the fraction X/Y,

30 where X is the number of amino acid sequence residues scored as identical matches between A and B, and where Y is the total number of amino acid residues in the polypeptide sequence of B.

Advantages

Compared to existing immunogenic conjugate technologies, the immunogenic conjugate

35 compositions of the present invention are simple to make, less expensive, and more adaptive to different antigens of interest and carrier proteins than existing conjugate technologies.

Petitioner Merck Ex-1013, 0012

The immunogenic conjugates of the present invention do not require that each combination of carrier protein and the antigen intended to evoke an immune response be conjugated by a tailored ligation process unique to their respective chemical properties. Polysaccharide (PS)-protein immunogenic conjugates have been prohibitively expensive to produce and sell in the developing world, 5 and conventional immunogenic conjugates are difficult to produce cheaply because of the highly specialized chemistry required for each antigen-protein conjugate. Thus, the present invention simplifies the method of making these conjugates and reduces the cost of their preparation compared to current immunogenic conjugate technology.

10 The immunogenic conjugates of the present invention also address a need for immunogenic conjugates that can safely induce immunity against previously intractable antigens. Immunogenic conjugates containing TLR (Toll-like receptor) ligands have been shown to evoke immune responses for otherwise intractable antigens, but they tend to be unsafe because TLR ligands are often proinflammatory, toxic in even small doses, reactogenic, and likely to cause adverse symptoms compared 15 to immunogenic conjugates of the invention.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

20

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a schematic of a conjugation reaction between a protein (Protein 1) and a peptidoglycan (Polysaccharide 1) catalyzed by a sortase in *Staphylococcus aureus*. In this model, sortase recognizes a C-terminus peptide signal ("LPXTGXX") on a protein and forms an intermediate 25 conjugate with a protein before recognizing an N-terminus peptide signal ("GGG") on a peptidoglycan. In the final step, sortase covalently links a protein to a peptidoglycan to form a final conjugate.

FIGURE 2 is a schematic of a conjugation reaction between a polysaccharide (polysaccharide 2) and a protein (protein 2) catalyzed by a sortase. In this model, sortase recognizes a C-terminus peptide 30 signal ("LPXTGXX") on a PS and forms an intermediate PS-sortase conjugate (conjugate 1). In the presence of a protein containing an N-terminus recognition sequence ("GGG"), sortase covalently links a protein to a PS to form a final PS-protein conjugate (conjugate 2).

DETAILED DESCRIPTION

35

The invention features novel immunogenic compositions and methods of making and administering such compositions to provide immunity against T-cell independent antigens or antigens

Petitioner Merck Ex-1013, 0013

which normally invoke weak immune responses, such as, e.g., polysaccharides (PS), polyalcohols, poly amino acids, and other organic polymers. The immunogenic compositions of the invention show the efficacy of a new method for antigenic conjugation. Specifically, a carrier protein and an antigenic polysaccharide can be conjugated in the presence of an enzyme from the sortase family of proteins. Thus, the carrier protein can effectively display and facilitate a robust immune response to the antigenic polysaccharide. The immune response to the antigenic polysaccharide can also be enhanced in the immunogenic conjugate relative to the isolated antigenic polysaccharide. Unlike other chemical conjugations, all immunogenic conjugates produced by the method described herein are homogenous in their structure and thus offer compositions with a high and reproducible specific activity. Thus, the new conjugation procedures can be applied to a wide range of antigens and carrier proteins to provide improved and highly active immunogenic conjugate compositions.

Polysaccharides are polymers of saccharides (sugars). PS derived from capsules are the primary antigenic components involved in protective immunity against encapsulated bacterial pathogens such as *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Salmonella typhi*, and *Haemophilus influenzae* Type B. Immunization of adolescents and adults with immunogenic conjugates based on microbial PS has been successful in reducing disease burden, but has proven less effective in providing protective immunity to infants and young children (i.e., children less than 24 months of age). Young children have not yet developed a mature adaptive immune repertoire and T cell-independent antigens such as capsular PS are poorly immunogenic and do not lead to long-term protective immune responses (i.e., an immunological memory response) in such young immunogenic conjugate recipients.

A T-cell independent antigen such as PS can be converted to a T-cell dependent antigen by chemical coupling of PS to protein; this process is called "conjugation" and involves the formation of covalent bonds between atoms in the PS structure and side chain atoms of amino acids present in the "carrier" protein. Such "immunogenic conjugates" more efficiently promote the induction of B-cell maturation and isotype switching leading to much higher levels of antibody with the correct anti-PS protective profile. Protective antibodies have high affinity for their PS antigens, and typically are of the Immunoglobulin G (IgG) subclass, a long-lived antibody with complement fixing and opsonic effector activity.

A non-limiting pathway for induction of an anti-PS IgG immune response is exemplified by a conjugate made between a PS and the carrier protein tetanus toxoid. In this model, only B-cells that display antibody receptors that recognize the PS bind the PS-carrier protein conjugate. Thus, the carrier protein is bound to the surface of the B-cell that displays the correct PS binding specificity. The carrier protein-PS complex is taken up by these B-cells into the intracellular vacuolar compartment where the carrier is processed by proteolytic degradation. Peptides derived from the carrier protein are transported

Petitioner Merck Ex-1013, 0014

and loaded into the presentation groove of the MHC-Class II receptor (MHC-II). This MHC-II-carrier peptide complex is displayed on the surface of the B-cell. Upon recognition of the MHC-II-peptide complex by the T-cell receptor (TCR), T-cells become activated and secrete cytokines that provide "help" for the induction of B-cell differentiation. B-cells expand in numbers and differentiate into "plasma cells" which now secrete antibody. Initially Immunoglobulin M (IgM) is produced by plasma cells but eventually the T-cell help causes the plasma cells to class switch and produce other isotype classes of antibody such as IgG. This process continues with plasma cells undergoing mutational changes leading to production of antibody receptors that have even higher affinity for the PS-carrier protein conjugates. As antigen is cleared, only the higher affinity plasma cells are activated by residual PS-carrier protein conjugate remaining in circulation. The process of T-cell dependent maturation of plasma cells continues, leading to the expansion of plasma cell populations which produce high affinity antibodies of the IgG class. The expansion can be easily monitored by measuring the levels of anti-PS IgG antibodies in the serum of an immunized subject, e.g., a human.

Eventually the maturation and switching process leads to the production of Memory B-cells which are long lived and specific for the PS. Memory B-cells have a unique property in that they can be immediately activated if exposed to PS. Activation causes memory B-cells to multiply and quickly produce anti-PS IgG. The activation of memory B cells that occurs during a second exposure of to PS antigen is called a "booster response" and is indicative of a long lived "secondary" memory immune response. Primary immunization may stimulate the production of IgM antibodies and some IgG antibodies. Upon secondary immunization, i.e., the "booster" shot, memory cells already programmed by the first immunization are stimulated to produce large quantities of IgG, the memory immune response.

A T-cell independent antigen generally does not stimulate lasting immunity, i.e., the production of IgG antibodies, but may stimulate the production of less potent and more temporary IgM antibodies. As such, PS antigens alone do not typically produce booster responses of IgG. However, PS do produce booster responses if primary immunization is performed with a PS-carrier protein conjugate because memory cells induced by the conjugate have already been programmed to produce IgG. Indeed, the booster response in immunized animals or humans is thought to mimic the protective response due to exposure to a microbe displaying the PS; this long term memory is critical for an immunogenic conjugate to work in protecting immunized subjects years after their immunization with immunogenic conjugates. Thus, PS-carrier protein conjugates are valued for (1) their ability to induce high levels of IgG against PS antigens, and (2) their ability to induce memory immune responses against PS antigens. PS antigens typically do not display these properties and thus are inferior antigens. The difficulty in synthesizing immunogenic conjugates and their cost of production has slowed the development of immunogenic conjugates for many bacterial diseases where an immune response to PS may be protective.

Petitioner Merck Ex-1013, 0015

Other T-cell independent antigens include homopolymers of amino acids, such as poly-gamma-D-glutamic acid (PGA), and polyalcohols. Indeed most biological polymers are T-cell independent antigens. Polymers can cross-link Immunoglobulin (Ig) receptors on B-cells that recognize them due to the repetitive nature of their chemical structures (and thus epitopes). Thus polymers can activate B-cells
5 for production of anti-polymer IgM in the same way that polysaccharides do. For example, an amino acid homopolymer, poly-gamma-D-glutamic acid (PGA) of *Bacillus anthracis*, is a capsular polymer that is poorly immunogenic and also a T-cell independent antigen. Immunogenic conjugates composed of PGA linked to protein carriers are highly immunogenic, able to induce anti-PGA IgG and immunological memory to PGA. Hence, most polymers respond like PS in terms of their immunogenicity because they
10 cannot be processed and displayed in the context of MHC-II and thus cannot recruit T-cell help. An exception is found in some naturally-occurring polymers that interact with another class of receptor termed Toll-like receptors (TLRs). Once activated, TLRs can induce production of cytokines by host cells and produce changes in the adaptive immune response. Some PS are covalently attached to TLR ligands or contaminated with such ligands. For example, lipopolysaccharides (LPS) are PS that are
15 highly immunogenic and induce IgG and memory responses; the lipid A moiety of LPS is a TLR ligand and may be responsible for the immunological properties.

Immunogenic conjugates are difficult to produce cheaply because of the specialized chemistry required. PS-carrier protein conjugation by covalent linkage procedures have numerous drawbacks,
20 including the need for organic solvents and other reagents that can adversely affect the structure and/or epitope presentation of carrier proteins; highly specialized chemical linkage reactions to selectively target a reactive site within the target protein; and time-consuming additional processing steps for carrying out the conjugation. Typically, coupling chemistry must be worked out for various PS that is unique for the chemistry of the PS and the carrier protein that has been selected. This coupling chemistry introduces
25 functional groups in the PS that then can be linked to carrier protein typically through the epsilon amino side chains of lysine residues. For conventional PS-protein immunogenic conjugates, the PS structure, nature of the carrier protein selected, and the type of linkage chemistry can all affect immunogenicity of the conjugate. As such, for example, in the case of pneumococcal disease where each of the 90+ known serotypes has a different PS structure (Bentley et al., *PLOS Genetics* 2(3):e31 262-269, 2006), one single
30 conjugation method may not be appropriate for all serotypes. Reproducibly synthesizing immunogenic conjugates with reproducible immunological properties involves careful control of the PS structure, the nature of the carrier selected, and the type of linkage chemistry, all of which dramatically increase the cost of manufacture of immunogenic conjugates. Thus, there is a need for a simplified, reproducible, cost-effective mechanism of producing PS-protein conjugates.

35

The sortase family of bacterial proteins includes well-conserved enzymes encoded by the genomes of numerous Gram positive bacterial organisms. Their enzymatic activity was first described in

Petitioner Merck Ex-1013, 0016

Staphylococcus aureus. As shown in Fig. 1, naturally occurring sortases modify surface proteins by recognizing and cleaving a carboxyl-terminal recognition signal. For most substrates of sortase enzymes, the recognition signal consists of the motif LPXTG (Leu-Pro-any amino acid (AA)-Thr-Gly), followed by a highly hydrophobic transmembrane sequence, and a cluster of basic residues such as Arg. Sortase
5 peptidase activity results in cleavage between the T and G residues of the sortase recognition sequence. Furthermore, a sortase active site, containing a sulfhydryl group within the Cys residue, is exposed to the carboxyl group of the Thr residue, allowing for formation of a thioester bond between C and T residues. A transamidation reaction between the sortase-protein conjugate and a polysaccharide polymer, peptidoglycan, displaying a polyglycine motif results in a covalent linkage between the carboxyl group of
10 the protein and the amino group of the Gly of the polysaccharide. The formation of this peptide bond leads to the covalent attachment of the protein to the bacterial cell wall (Marraffini et al., *Microbiol Mol Biol Rev*. 70: 192-221, 2006).

Sortases have been classified into four major families, designated sortase A (SrtA), sortase B
15 (SrtB), sortase C (SrtC), and sortase D (SrtD), respectively, based on sequence alignment and phylogenetic analysis of 61 sortases from Gram-positive bacterial genomes (Dramsi et al., *Res Microbiol*. 156: 289-97, 2005). Sortase A of *Staphylococcus aureus* recognizes a LPXTG like sequence motif located near the C-terminus of the target proteins, cleaves at the Thr-Gly peptide bond, and catalyzes the formation of a new peptide bond between a threonyl carboxyl and an amino group of the peptidoglycan
20 penta-glycine cross-bridges (Marraffini et al., *Microbiol Mol Biol Rev*. 70: 192-221, 2006; Ton-That et al., *Proc Natl Acad Sci USA*. 96: 12424-12429, 1999). The immunogenic composition of the invention encompasses embodiments relating to a SrtA, SrtB, SrtC, and/or SrtD from any bacterial species or strain, as well as evolved sortases that have been modified from their wild type amino acid sequences to include at least one mutation in the encoding sequence, examples of which are provided herein.
25

Immunogenic Conjugates

The immunogenic conjugates of the invention have the potent immunological properties of typical PS-carrier protein immunogenic conjugates but desirably differ from previously known immunogenic conjugates in that an antigen of interest, e.g., PS or capsular organic polymer, may be coupled to a
30 desired carrier protein without utilizing differing chemical linkages specialized to produce each combination. Rather, as depicted in Fig. 2, a novel sortase reaction is performed that is the reverse of a sortase reaction in nature where an antigenic polysaccharide is coupled to a sortase or a carrier protein. In the immunogenic conjugates of the invention, an antigen of interest, e.g., PS or capsular organic polymers, is covalently linked by a thioester bond to a sortase which is then optionally capable of coupling
35 the antigen with a carrier protein by a peptide bond. For example, a PS-carrier protein conjugate may be formed by first covalently linking a sortase to a soluble antigen, e.g., PS or capsular organic polymers: these immunogenic conjugates are referred to as a PS-sortase immunogenic conjugates (conjugate 1 in

Petitioner Merck Ex-1013, 0017

Fig. 2). The novel PS-sortase conjugate may be enzymatically stabilized by chemical cross-linking, for example, such that sortase is the carrier protein of the immunogenic conjugate. Alternatively, an un-stabilized PS-sortase immunogenic conjugate in the presence of a secondary carrier protein may catalyze the covalent linkage between the antigenic PS and the secondary carrier protein to produce a novel PS-
5 carrier protein conjugate: these immunogenic conjugates are referred to as PS-carrier protein immunogenic conjugates (conjugate 2 in Fig. 2).

In desirable embodiments, the immunogenic conjugates of the invention include a polysaccharide antigen conjugated to a sortase carrier protein capable of stimulating an immune response. In another
10 desirable embodiment, the immunogenic conjugates of the invention include a polysaccharide antigen conjugated to a carrier protein capable of stimulating an immune response.

Immunogenic conjugates of the invention may be prepared by attaching a sortase recognition sequence to a polysaccharide. In the presence of a sortase, the polysaccharide becomes a sortase
15 substrate and is covalently linked to a sortase by a sortase ligation sequence. In desirable embodiments, the thio-ester linkage between the PS and sortase conjugate may be stabilized to prevent reversal of the covalent bond formed. Methods of enzymatic stabilization by photo cross-linking, e.g., ultraviolet (UV) cross-linking, or chemical cross-linking, e.g., formaldehyde, glutaraldehyde, and formaldehyde/glutaraldehyde cross-linking, are well known in the art. Once the PS-sortase immunogenic
20 conjugate is formed, treatment with, for instance, a chemical cross-linker such as formaldehyde, cross-links the basic amino acid lysine residues of sortase, further stabilizing the enzymatic conjugate.

Further, a carrier protein of interest may be attached to a polyglycine motif to enable covalent linkage between itself and the PS. By mixing of the non-cross-linked PS-sortase conjugate with a carrier
25 protein attached to a polyglycine motif, a covalent bond between an antigenic PS and the carrier protein is formed by a sortase ligation sequence, which produces the PS-carrier protein conjugate. By attaching a synthetic peptide to a PS, an antigenic polysaccharide may be covalently linked to any sortase enzyme. Further, attachment of a polyglycine motif to a carrier protein enables the covalent linkage of an antigenic polysaccharide to any carrier protein, when a first PS-sortase conjugate is formed. Exemplary and
30 preferred carrier proteins and polysaccharide antigens of interest are described herein.

The methods of making immunogenic conjugates described herein are less complex than immunogenic conjugate technology because its chemistry depends only on the covalent-linking chemistry of a sortase recognition peptide of the antigenic PS with that of the Cys residue of a sortase active site; or
35 the polyglycine motif of the carrier protein (e.g., DNI, cholera toxin B subunit, diphtheria toxin, tetanus toxin Fragment C, or *Escherichia coli* beta-galactosidase). Furthermore, multiple antigenic polysaccharides can be coupled to a carrier protein; or, a mixture of carrier proteins can be conjugated to

Petitioner Merck Ex-1013, 0018

the antigenic PS in a single reaction or multiple sequential reactions. Thus, the method described herein enables multiplexing of the immunogenic conjugate, further reducing the cost of production.

Immunogenic conjugates of the invention may be made using a combination of a sortase
5 recognition peptide and polyglycine motif, such as, e.g., those described herein, to covalently link any carrier protein, such as, e.g., those described herein, in the presence of one or more antigens of interest, such as, e.g., those described herein. If one antigen of interest is used, the immunogenic conjugate of the invention is said to be monovalent. If more than one antigen of interest is used, the immunogenic conjugate of the invention is said to be multivalent.
10

The methods of making immunogenic compositions described herein may be used with any antigenic polysaccharide capable of being covalently linked by a free carboxyl group, e.g., any capsular polymer or any polymer, attached to a sortase recognition peptide, and any carrier protein capable of being covalently-linked by a free amino group, e.g., carrier proteins attached to a polyglycine motif.
15 Tetanus toxoid is one possible carrier protein. This toxin is detoxified by treatment with formaldehyde, a reagent that reacts with amino groups of proteins. Other desirable carrier proteins include the cholera toxin B subunit (available from SBL Vaccin AB), diphtheria toxin, tetanus toxin Fragment C (available from Sigma Aldrich), DNI, or beta-galactosidase from *Escherichia coli* (available from Sigma Aldrich). Further, immunogenic conjugates of the invention may include whole cell encapsulated pathogens as the antigen,
20 eliminating the need for purification and characterization of antigenic PS. Whole cell encapsulated pathogens may be killed by a chemical, e.g., formaldehyde, treatment or by heat-inactivation and subsequently conjugated to sortase as described herein.

The immunogenic conjugates of the invention may be used to immunize against, for example,
25 *Pneumococcus* infection, *Streptococcus* (groups A and B) infection, *Haemophilus influenzae* type B ("HiB") infection, meningococcal (e.g., *Neisseria meningitides*) infection, and may be used as O antigen immunogenic conjugates from Gram negative bacteria (e.g., *Pseudomonas aeruginosa*, *Francisella tularensis* (Thirumalapura et al., *J. Med. Microbiol.* 54:693-695, 2005; Vinogradov and Perry, *Carbohydr. Res.* 339:1643-1648, 2004; Vinogradov et al., *Carbohydr. Res.* 214:289-297, 1991), *Shigella* species,
30 *Salmonella* species, *Acinetobacter* species, *Burkholderia* species, and *Escherichia coli*).

Peptide Linkers

Peptide linkers used in the immunogenic conjugates of the invention desirably are polypeptide sequences that are substrates for a sortase enzyme. In a further embodiment, an immunogenic
35 conjugate composition is provided including an antigenic polysaccharide covalently linked to a carrier protein by a peptide bond of a sortase ligation sequence. Desirably, the immunogenic conjugate of the invention has the formula: PS-L-CP, where PS is an antigenic polysaccharide; L is a peptide linker; and

Petitioner Merck Ex-1013, 0019

CP is a carrier protein. For example, the covalent linkage between an antigenic polysaccharide and a carrier protein may consist of a peptide bond at the carboxyl-terminus of a synthetic peptide attached to a side chain of an antigenic polysaccharide. Exemplary PS side chain groups capable of attachment include, but are not limited to, vicinal hydroxyls, non-vicinal hydroxyls, carboxyl groups, amino groups and
5 reducing sugar aldehydes. Examples of a synthetic peptide utilized in the present invention include a sortase recognition sequence with a carboxyl-terminus, such that prior to linkage with the carrier protein, includes a sortase A, B, C or D recognition motif.

For example, such a synthetic peptide may, in certain embodiments, be engineered to include the
10 sortase recognition sequence LPXTG, where X is any amino acid, and includes but is not limited to residues with amino, carboxyl, thiol, halogen, and azide groups, such that these groups may chemically react with an activated polysaccharide. The sortase recognition sequence may be modified to include reactive residues located at the N-terminal of, internal to, or C-terminal of the synthetic peptide, such that the residues allow for covalent modification of a polysaccharide. The sortase recognition sequence of the
15 invention may further be covalently linked to a chemically activated antigenic polysaccharide. Antigenic polysaccharides of the invention may be chemically activated with periodate, cyanogen borohydride, or carbodiimide reagents.

In other desirable embodiments, a covalently linked polysaccharide to a synthetic peptide is
20 mixed with a sortase enzyme capable of recognizing the peptide sequence. In another desirable embodiment, a sortase enzyme is covalently linked to a polysaccharide by a sortase ligation sequence of LPXT, where X is any amino acid. A peptide linker of the invention may additionally include a polyglycine motif with a peptide sequence of GGGGG. Such a polyglycine motif is covalently bound to a free primary amino group (NH_2-R) of a desired carrier protein and is recognized by a sortase enzyme. A carrier
25 protein of the invention may encompass a carrier protein further chemically modified to covalently link the carrier protein with at least one peptide and/or at least one protein that displays the polyglycine motif. In other desirable embodiments, a covalently linked carrier protein to a polyglycine motif is mixed with a polysaccharide covalently linked to a sortase enzyme that is capable of recognizing the polyglycine motif. In some embodiments, an antigenic polysaccharide and a carrier protein include the sortase ligation
30 sequence LPXT at the covalent linkage.

In some embodiments, a sortase ligation sequence includes a sortase recognition sequence. In some embodiments, a sortase ligation sequence is located C-terminal of an antigenic PS. In some embodiments, a sortase ligation sequence includes a polyglycine motif of 2, 3, 4 or 5 Gly residues. In
35 some embodiments, a sortase ligation sequence is located N-terminal of a carrier protein. In other embodiments, a sortase ligation sequence is located C-terminal of an antigenic polysaccharide, and N-terminal of a carrier protein. In some embodiments, an immunogenic conjugation includes formation of

Petitioner Merck Ex-1013, 0020

an amide bond between a C-terminal carboxyl group of a cleaved sortase recognition sequence and an N-terminal amino group of a polyglycine sequence.

In some embodiments, a sortase recognition sequence is a sortase A recognition sequence having a consensus sequence $X_1PX_2X_3G$, where X_1 is Leu, Ile, Val or Met, P is Pro, X_2 is any amino acid, X_3 is Ser, Thr or Ala, and G is Gly. In further embodiments, X_2 is Asp, Glu, Ala, Gln, Lys or Met. In some embodiments, a sortase recognition sequence is a sortase A recognition sequence having the consensus sequence LPX_1TG, where X_1 is any amino acid. Exemplary sortase A recognition sequences include but are not limited to the following sequences: LPKTG, LPATG, LPNTG, LPETG, LPNAG, LPNTA, LGATG, IPNTG, or IPETG.

In some embodiments, a sortase recognition sequence is a sortase B recognition sequence having a consensus sequence NPX_1TX_2, where N is Asn, P is Pro, X_1 is Lys or Gln, T is Thr, and X_2 is Asn, Asp, or Gly. In further embodiments, a sortase B recognition sequence is NPQTN. Exemplary sortase B recognition sequences include but are not limited to the following sequences: NPKTG, NSKTA, NPQTG, NAKTN, or NPQSS

In some embodiments, a sortase recognition sequence is a sortase C recognition sequence and may utilize as its substrate LPX_1TX_2, where L is Leu, P is Pro, and T is Thr, as a recognition motif, with each X_1 and X_2 independently representing any amino acid residue.

In some embodiments, a sortase recognition sequence is a sortase D recognition sequence. In some embodiments, consensus sequences for sortase D are LPX_1TA or LAX_1TG, where L is Leu, P is Pro, X_1 is any amino acid, T is Thr, A is Ala, and G is Gly.

Additional embodiments of the immunogenic conjugate may include a combination of any of the above sortase recognition sequences. For example, the invention may include an antigenic polysaccharide covalently linked to at least one sortase recognition sequence. Furthermore, sortase recognition sequences linked to an antigenic polysaccharide may be the same sequence or include any of the differing sequences, such as recognition sequences provided for SrtA, SrtB, SrtC, and/or SrtD. In additional embodiments, an antigenic polypeptide of the invention is covalently linked to at least one carrier protein.

Carrier Proteins

Carrier proteins used in the immunogenic conjugates of the invention desirably are proteins that, either alone or in combination with an antigen, invoke an immune response in a subject. Desirably, the carrier protein contains at least one epitope recognized by a T-cell. Desirably, the epitope is capable of

Petitioner Merck Ex-1013, 0021

inducing a T-cell response in a subject, and induce B-cells to produce antibodies against the entire antigen of interest. Epitopes as used in describing this invention, include any determinant on an antigen that is responsible for its specific interaction with an antibody molecule or fragment thereof. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or
5 sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. To have immunogenic properties, a protein or polypeptide generally is capable of stimulating T-cells. However, a carrier protein that lacks an epitope recognized by a T-cell may also be immunogenic.

10 By selecting a carrier protein that is known to elicit a strong immunogenic response, a diverse population of subjects can be treated by an immunogenic conjugate described herein. The carrier protein desirably is sufficiently foreign to elicit a strong immune response to the immunogenic conjugate. Typically, the carrier protein used is a molecule that is capable of imparting immunogenicity to the antigen of interest. In a desirable embodiment, a carrier protein is one that is inherently highly immunogenic.
15 Thus, a carrier protein that has a high degree of immunogenicity and is able to maximize antibody production to the antigens complexed with it is desirable.

Various carrier proteins of the invention include, e.g., toxins and toxoids (chemical or genetic), which may or may not be mutant, such as anthrax toxin, PA and DNI (PharmAthene, Inc.), diphtheria
20 toxoid (Massachusetts State Biological Labs; Serum Institute of India, Ltd.) or CRM 197, tetanus toxin, tetanus toxoid (Massachusetts State Biological Labs; Serum Institute of India, Ltd.), tetanus toxin fragment Z, exotoxin A or mutants of exotoxin A of *Pseudomonas aeruginosa*, bacterial flagellin, pneumolysin, an outer membrane protein of *Neisseria meningitidis* (strain available from the ATCC (American Type Culture Collection, Manassas, Va.)), *Pseudomonas aeruginosa* Hcp1 protein,
25 *Escherichia coli* heat labile enterotoxin, shiga-like toxin, human LTB protein, a protein extract from whole bacterial cells, and any other protein that can be cross-linked by a peptide linker. Desirably, the carrier protein is the cholera toxin B subunit (available from SBL Vaccin AB), diphtheria toxin (Connaught, Inc.), tetanus toxin Fragment C (available from Sigma Aldrich), DNI, or beta-galactosidase from *Escherichia coli* (available from Sigma Aldrich). Other desirable carrier proteins include bovine serum albumin (BSA),
30 P40, and chicken riboflavin. (Unless otherwise indicated, the exemplary carrier proteins are commercially available from Sigma Aldrich.) Other exemplary carrier proteins are MAPs (multi-antigenic peptides), which are branched peptides. By using a MAP, cross-linking density is maximized because of multiple branched amino acid residues. An exemplary amino acid that can be used to form a MAP is, but is not limited to, lysine.
35

Both BSA and keyhole limpet hemocyanin (KLH) have commonly been used as carriers in the development of immunogenic conjugates when experimenting with animals. Carrier proteins which have

been used in the preparation of therapeutic immunogenic conjugates include, but are not limited to, a number of toxins of pathogenic bacteria and their toxoids. Examples include diphtheria and tetanus toxins and their medically acceptable corresponding toxoids. Other candidates are proteins antigenically similar to bacterial toxins referred to as cross-reacting materials (CRMs). Carrier proteins of the invention
5 may also include any protein not derived from humans and not present in any human food substance.

In another embodiment, DNI is used as the carrier protein because it is nontoxic leaving no need to detoxify the protein before use. Furthermore, the use of DNI is desirable because DNI may also induce a protective immune response to *B. anthracis*, in addition to the protective immune response to the
10 antigen of interest.

Sortases

Other exemplary carrier proteins of the invention include members of the sortase family of enzymes because sortases may also induce a protective immune response to Gram-positive bacterium in
15 addition to the protective immune response to the antigen of interest. A sortase carrier protein may be derived from a sortase A, sortase B, sortase C, or sortase D. Carrier proteins of the invention may also include a catalytically active fragment, derivative, or variant of a sortase A, sortase B, sortase C, or sortase D. For example, the carrier protein of the invention may include a soluble fragment of sortase A including the C-terminal catalytic domain. In another exemplary embodiment, the carrier protein of the
20 invention may include a central catalytic domain of a sortase B.

Examples of suitable sortases are described in Dramsi et al., *Res. Microbiol.* 156:289-297, 2005; Comfort et al., *Infect. Immun.,* 72:2710-2722, 2004; Chen et al., *Proc. Natl. Acad. Sci. USA.* 108:11399-11404, 2011; and Pallen et al., *Trends in Microbiology*. 9: 97-101, 2001, the entire contents of each of
25 which are incorporated herein by reference. The sortase carrier protein may also be isolated from but not limited to one of the following bacterial strains: *Bacillus anthracis*, *Bacillus cereus*, *Bacillus halodurans*, *Clostridium acetobutylicum* (SortaseD), *Clostridium perfringens*, *Clostridium tetani* (SortaseD), *Enterococcus faecalis*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Listeria innocua*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus agalactiae*,
30 *Streptococcus gordonii*, *Streptococcus mutans*, *Streptococcus phenumoniae*, *Streptococcus pyogenes*, and *Streptococcus suis*.

The sequences of many sortases and of the naturally occurring nucleic acids that encode them are found in publicly available databases such as those of the National Center for Biotechnology
35 Information (NCBI) available at Entrez (http://www.ncbi.nlm.nih.gov/Entrez), e.g., GenBank. The sequences of sortase proteins having the accession numbers provided herein are hereby incorporated by reference.

Petitioner Merck Ex-1013, 0023

The carrier protein of the invention may include a sortase A derived from class A sortases, e.g., *S. aureus* sortase A. The prototypical class A sortase, *S. aureus* sortase A, has been purified and characterized (Ton-That et al., *Proc. Natl. Acad. Sci. USA*. 96:12424-12429, 1999), and the gene that
5 encodes it has been cloned and sequenced (Mazmanian, et al., *Science*. 285:760-763, 1999.) The gene has been assigned accession number AF162687, and the protein sequence has accession number AAD48437.1. Additional exemplary sequences of class A sortases from a variety of other bacterial species are available under the following GenBank accession numbers: *S. pyogenes* (Spyog) SrtA, AAK34025; *S. gordonii* (Sgord) SrtA, AAG41778; *L. lactis* (Llact) hypO, AAK0521 1 ; *S. aureus* (Saure)
10 SrtA, AAD48437; and *A. naeslundii* (Anaes) fimbria-associated protein (fimassoc), AAC13546; *Staphylococcus aureus* subsp. *aureus* MSSA476, CAG44229.

In additional embodiments, the carrier protein of the invention features a sortase B derived from class B sortases identified from the *Streptococcus*, *Bacillus*, *Staphylococcus*, *Clostridia*, and *Listeria*
15 genera, among others. Exemplary sequences of several class B sortases are available at GenBank accession numbers as follows: *S. pyogenes*, NP_268518; *B. anthracis*, NP_846988; *C. perfringens*, NP_561429; *E. faecalis*, AAQ16264; *Staphylococcus aureus* subsp. *aureus* MRSA252, CAG401 10; *L. monocytogenes*, CAD00259.

20 The carrier protein of the invention may include a sortase C derived from class C sortases found among species in the *Streptococcus*, *Enterococci*, *Bacillus*, and *Clostridia* genera. Exemplary sequences of several class C sortases are available under the following accession numbers: *S. pyogenes*, AAL1 1468; *C. diphtheriae*, NP_940532.1; *Streptococcus suis*, BAB83966.

25 In some embodiments, the invention features a sortase D carrier protein derived from class D sortases found among species in the *Streptomyces*, *Corynebacterium*, *Clostridium*, and *Bacillus* genera. Sequences of several class D sortases are available under the following accession numbers: *Streptomyces coelicolor*, NP_628037; *B. subtilis*, CAB12748, *C. tetani*, NP_781831.

30 The amino acid sequences of sortases and the nucleotide sequences that encode them are known to those of skill in the art and are disclosed in a number of references cited herein, the entire contents of all of which are incorporated herein by reference. Those of skill in the art will appreciate that any sortase and any sortase recognition motif can be used in some embodiments of this invention, including, but not limited to, the sortases and sortase recognition motifs described in Ploegh et al.,
35 International PCT Patent Application, PCT/US2010/000274, filed Feb. 1, 2010, published as WO/2010/087994 on Aug. 5, 2010 (e.g., paragraphs [0089]-[0101]); and Ploegh et al., International Patent Application PCT/US2011/033303, filed Apr. 20, 2011, published as WO/2011/133704 on Oct. 27,

2011 (e.g., paragraphs [0085-0094]); the entire contents of each of which are incorporated herein by reference.

The carrier protein of the invention may also include evolved sortases. An evolved sortase exhibits enhanced reaction kinetics, for example, in that it catalyzes a transpeptidation reaction at a greater speed or turnover rate than the respective wild type sortase. An evolved sortase may exhibit a modified substrate preference, for example, in that it utilizes a different substrate (e.g., a polypeptide comprising an altered sortase recognition sequence) or binds a given substrate with higher or lower affinity, or with higher or lower specificity than the respective wild type sortase. For instance, the evolved sortase recognizes a sortase recognition sequence that the respective wild type sortase does not recognize or bind. See, e.g., Liu et al., United States Patent Application, 13/922,812, filed Jun. 20, 2013, published as US2014/0057317A1 on Feb. 27, 2014, the entire contents of each of which are incorporated herein by reference, for exemplary sortases, recognition motifs, reagents, and methods for directed evolution of bond-forming sortases.

For example, some embodiments of the invention provide a sortase comprising a polypeptide sequence of *S. aureus* Sortase A (SEQ ID NO: 1) that is homologous to the polypeptide sequence of a wild type Sortase A (NP_647265.1), or a fragment thereof, as provided below:

SEQ ID NO: 1:
MKKWTNRLMTIAGVVLILVAAYLFAKPHIDNYLHDKDKDEKIEQYDKNVKEQASKDKKQQAKPQIPKDKSK
VAGYIEIPDADIKEPVYPGPATPEQLNRGVSFAEENESLDDQNISIAGHTFIDRPNYQFTNLKAAKKGSMVY
FKVGNETRKYKMTSIRDVKPTDVEVLDEQKGKDKQLTLITCDDYNEKTGVWEKRKIFVATEVK.

In some embodiments, the polypeptide sequence of the provided sortase may include one or more amino acid mutations as compared to the wild type sequence of the respective sortase (e.g., the sequence of SEQ ID NO: 1). For example, the evolved sortase sequence provided may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or more mutations. In some embodiments, the polypeptide sequence of the provided sortase is at least 90% identity (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%) to a wild type sortase sequence (e.g., the sequence of SEQ ID NO: 1).

For example, in a desirable embodiment of the invention an evolved *S. aureus* sortase A is provided. An evolved sortase A, or a fragment thereof, may include a mutation relative to the sequence of SEQ ID NO: 1 described herein, for example, P86L, P94S, P94R, N98S, A104T, E106G, A118T, F122S, F122Y, D124G, N127S, K134R, F154R, D160N, D165A, K173E, G174S, K177E I182V K190E, or K196T, or any combination of any one of these mutations. In some embodiments, an evolved sortase is provided herein that includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or all 19 of these

Petitioner Merck Ex-1013, 0025

mutations. The aforementioned amino acid substitution may provide an evolved sortase that efficiently uses substrates not bound by the respective parent wild type sortase. For example, in some embodiments, an evolved sortase is provided that is derived from a wild type *S. aureus* sortase A as the parent sortase A, which utilizes substrates including a C-terminal sortase recognition motif of the
5 sequence LPXTG and substrates including an N-terminal polyglycine motif in a transpeptidation reaction. In some embodiments, the evolved sortases utilize a substrate different from those used by the parent sortase, e.g., substrates including a C-terminal LPXS, LAXT, LAXTG, MPXT, MPXTG, LAXS, LAXSG, NPXT, NPXTG, NAXT, NAXTG, NAXS, NAXSG, LPXP, LPXPG, or LPXTA motif.

10 *Antigens of Interest*

The composition of the immunogenic conjugate of the invention and methods of making and administering such immunogenic conjugates can be used for any antigen of interest, e.g., a polysaccharide, polyalcohol, or poly amino acid. Desirably, the antigen of interest carries no primary groups that can be destroyed by the chemical reactions employed by the method of making immunogenic
15 conjugates, e.g., the denaturing of an antigen caused by the destruction of antigen disulfide bonds by borohydride reduction. In yet other desirable embodiments of the invention, the antigen of interest is an organic polymer consisting of monomers having at least three atoms, where each of the atoms is independently selected from carbon, oxygen, hydrogen, phosphate, nitrogen, and sulfate. Exemplary antigens of interest include organic polymers such as polysaccharides (e.g., polysaccharides having at
20 least 18 residues), phosphopolysaccharides, polysaccharides with amino sugars with N-acetyl substitutions, polysaccharides containing sulfonylated sugars, other sulfate-modified sugars, or phosphate-modified sugars, polyalcohols, poly amino acids, teichoic acids, O side chains of lipopolysaccharides.

25 Exemplary antigens of interest also include capsular organic polymers including those synthesized by microbes, e.g., bacteria, fungi, parasites, and viruses, and then purified from such a biological source using standard methods. Exemplary antigens of interest include microbial capsular organic polymers including those purified from bacterial organisms such as *Bacillus* species (including *B. anthracis*) (Wang and Lucas, *Infect. Immun.* 72(9):5460-5463, 2004), *Streptococcus pneumoniae*
30 (Bentley et al., *PLoS Genet.* 2(3):e31, Epub 2006; Kolkman et al., *J. Biochemistry* 123:937-945, 1998; and Kong et al., J. *Med. Microbiol.* 54:351-356, 2005), *Shigella* (Zhao et al., *Carbohydr. Res.* 342(9):1275-1279, Epub 2007), *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Salmonella*, (including *Salmonella typhi*), *Streptococcus pyogenes*, *Escherichia coli* (Zhao et al., *Carbohydr. Res.* 342(9):1275-1279, Epub 2007), *Francisella tularensis*, and *Pseudomonas aeruginosa*,
35 and fungal organisms such as *Cryptococcus* and *Candida*, as well as many other microorganisms (see, e.g., Ovodov, *Biochemistry* (Mosc.) 71(9):937-954, 2006; Lee et al., *Adv. Exp. Med. Biol.* 491:453-471,

Petitioner Merck Ex-1013, 0026

2001; and Lee, *Mol. Immunol.* 24(10):1005-1019, 1987). Exemplary antigens of interest also include polymers that do not occur in nature and thus are non-biological in origin.

In other particularly desirable embodiments, the *Francisella tularensis* polysaccharide is the O antigen. Desirably, the microbial capsular polymer is poly-gamma-D-glutamic acid (PGA) from *Bacillus*
5 *anthracis*. In desirable embodiments of the invention, the *Streptococcus pneumoniae* polysaccharide is one of capsular types described in Kong et al. (*J. Med. Microbiol.* 54:35-356, 2005). For example, *Streptococcus pneumoniae* polysaccharide capsular type desirably is 1 (e.g., 1-g or 1-q), 2 (e.g., 2-g, 2-q, or 2-41 A), 3 (e.g., 3-g, 3-q, 3-c, or 3-nz), 4, 5 (e.g., 5-q, 5-c, 5-qap, or 5-g), 6A (e.g., 6A-g, 6A-cl, 6A-c2, 6A-n, 6A-qap, 6A-6B-g, 6A-6B-q, or 6A-6B-s), 6B (e.g., 6B-c, 6A-6B-g, 6A-6B- q, or 6A-6B-s), 7F (e.g.,
10 7F-7A), 7A (e.g., 7A-cn or 7F-7A), 7B (e.g., 7B-40), 7C (e.g., 7C-19C-24B), 8 (e.g., 8-g or 8-s), 9A (e.g., 9A-9V), 9L, 9N, 9V (e.g., 9A-9V), 9V and 14, 1OF (e.g., 10F-q, lOF-ca, or lOF-lOC), 1OA (e.g., 10A-17A or 10A-23F), 1OB (e.g., lOB-lOC), HF, l lA (e.g., l lA-nz or 11A-11D-18F), HB (e.g., 1 lB-11C), HC (e.g., 1 lB-11C or HC-cn), HD (e.g., 11A-11D-18F), 12F (e.g., 12F-q or 12F-12A-12B), 12A (e.g., 12A-cn, 12A-46, or 12F-12A-12B), 12B (e.g., 12F-12A-12B), 13 (e.g., 13-20), 14 (e.g., 14-g, 14-q, 14-v, or 14-c), 15F (e.g.,
15 15F-cnl or 15F-cn2), 15A (e.g., 15A-cal, 15A-ca2, or l5A-chw), 15B (e.g., 15B-C, 15B-15C, 15B-15C-22F-22A), 15C (e.g., 15C- ca, 15C-ql, 15C-q2, 15C-q3, 15C-S, 15B-15C, or 15B-15C-22F-22A), 16F (e.g., 16F-q or 16F-nz), 16A, 17F (e.g., 17F-n and 17F-35B-35C-42), 17A (e.g., 17A-ca or 10A-17A), 18F (e.g., 18F-ca, 18F-W, or 1 lA-l lD- 18F), 18A (e.g., 18A-nz or 18A-q), 18B (e.g., 18B-18C), 18C (e.g., 18B-18C), 19F (e.g., 19F-gl, 19F-g2, 19F-g3, 19F-q, 19F-n, or 19F-C), 19A (e.g., 19A-g, 19A-, or 19A-ca), 19B, 19C
20 (e.g., 19C-cnl, 19C-cn2, or 7C- 19C-24B), 20 (e.g., 13-20), 21 (e.g., 21-ca or 21-cn), 22F (e.g., 15B-15C-22F-22A), 23F (e.g., 23F-C, 10A-23F, or 23F-23A), 23B (e.g., 23B-C or 23B-q), 24F (e.g., 24F-cnl, 24F-cn2, or 24F-cn3), 24A, 24B (e.g., 7C-19C-24B), 25F (e.g., 25F-38), 25A, 27, 28F (e.g., 28F-28A or 28F-cn), 28A (e.g., 28F-28A), 29 (e.g., 29-ca or 29-q), 31, 32F (e.g., 32F- 32A), 32A (e.g., 32A-cn or 32F-32A), 33F (e.g., 33F-g, 33F-q, 33F-chw, 33F-33B, or 33F-33A-35A), 33A (e.g., 33F-33A-35A), 33B (e.g.,
25 33B-q, 33B-s, or 33F-33B), 33D, 34 (e.g., 34-ca or 34s), 35F (e.g., 35F-47F), 35A (e.g., 33F-33A-35A), 35B (e.g., 17F- 35B-35C-42), 36, 37 (e.g., 37-g or 37-ca), 38 (e.g., 25F-38), 39 (e.g., 39-cnl or 39-cn2), 40 (e.g., 7B-40), 41F (e.g., 41F-cn or 41F-s), 41A (e.g., 2-41A), 42 (e.g., 17B-35B-35C- 42), 43, 44, 45, 46 (e.g., 46-s or 12A-46), 47F (e.g., 35F-47F), 47 A, 48 (e.g., 48-cnl or 48-cn2), or GenBank Accession Number AF532714 or AF532715.

30 Alternatively, exemplary antigens of interest include killed whole cell encapsulated pathogens such as *Bacillus* species (including *B. anthracis*) (Wang and Lucas, *Infect. Immun.* 72(9):5460-5463, 2004), *Streptococcus pneumoniae* (Bentley et al., *PLoS Genet.* 2(3):e31, Epub 2006; Kolkman et al., *J. Biochemistry* 123:937-945, 1998; and Kong et al., J. *Med. Microbiol.* 54:351-356, 2005), *Shigella* (Zhao et al., *Carbohydr. Res.* 342(9):1275-1279, Epub 2007), *Haemophilus influenzae*, *Neisseria meningitidis*,
35 *Staphylococcus aureus*, *Salmonella*, (including *Salmonella typhi*), *Streptococcus pyogenes*, *Escherichia coli* (Zhao et al., *Carbohydr. Res.* 342(9):1275-1279, Epub 2007), *Francisella tularensis*, and

Petitioner Merck Ex-1013, 0027

Pseudomonas aeruginosa, and fungal organisms such as *Cryptococcus* and *Candida*, as well as many other microorganisms (see, e.g., Ovodov, *Biochemistry* (Mosc.) 71(9):937-954, 2006; Lee et al., *Adv. Exp. Med. Biol.* 491:453-471, 2001; and Lee, *Mol. Immunol.* 24(10):1005-1019, 1987). Whole cell pathogens may contain, but are not limited to, one or more of the aforementioned antigens of interest, e.g., microbial
5 capsular organic polymers, O-antigen, and PGA.

Immunogenic Conjugate Compositions: antigenic PS-carrier protein

The immunogenic conjugates of the invention may be used in combination, for example, in pediatric immunizations. In addition, the immunogenic conjugates of the invention may be used to immunize against, for example, *Pneumococcus* infection, *Haemophilus influenzae* type B ("HiB")
10 infection, *Streptococcus* (groups A and B) infection, meningococcal (e.g., *Neisseria meningitides*) infection, and may be used as O antigen immunogenic conjugates from Gram negative bacteria (e.g., *Pseudomonas aeruginosa*, *Francisella tularensis*, *Shigella* species, *Salmonella* species, *Acinetobacter* species, *Burkholderia* species, and *Escherichia coli*).

15 The immunogenic conjugate formulation desirably includes at least one carrier protein, at least one antigen of interest, and a pharmaceutically acceptable carrier or excipient (e.g., aluminum phosphate, sodium chloride, or sterile water). An immunogenic conjugate composition may also include an adjuvant system for enhancing the immunogenicity of the formulation, such as oil in a water system and other systems known in the art or other pharmaceutically acceptable excipients. A carrier protein-antigenic PS
20 complex that is insoluble under physiological conditions is desirable to slowly release the antigen after administration to a subject. Such a complex desirably is delivered in a suspension containing pharmaceutically acceptable excipients. However, the carrier protein-antigenic PS complex may also be soluble under physiological conditions.

25 Typically the immunogenic conjugate is in a volume of about 0.5 mL for subcutaneous injection, 0.1 mL for intradermal injection, or 0.002-0.02 mL for percutaneous administration. A 0.5 ml dose of the immunogenic conjugate may contain approximately 2-500 µg of the antigen covalently linked with approximately 2-500 µg of the carrier protein. In a desirable embodiment, in a 0.5 ml dose, approximately 10 µg of the antigen are conjugated with approximately 10 µg of the carrier protein. The molar ratio of
30 antigen to carrier protein desirably is 1:1 (e.g., 1 part antigen to 1 part carrier protein).

Because the peptides or conjugates may be degraded in the stomach, the immunogenic conjugate is desirably administered parenterally (for instance, by subcutaneous, intramuscular, intravenous, or intradermal injection). While delivery by a means that physically penetrates the dermal
35 layer is desirable (e.g., a needle, airgun, or abrasion), the immunogenic conjugates of the invention can also be administered by transdermal absorption.

Petitioner Merck Ex-1013, 0028

In particular, the immunogenic conjugates of the invention may be administered to a subject, e.g., by intramuscular injection, intradermal injection, or transcutaneous immunization with appropriate immune adjuvants. Immunogenic conjugates of the invention may be administered, one or more times, often including a second administration designed to boost production of antibodies in a subject to prevent infection by an infectious agent. The frequency and quantity of immunogenic conjugate dosage depends on the specific activity of the immunogenic conjugate and can be readily determined by routine experimentation. While the age at which the first dosage is administered generally is two-months, an immunogenic conjugate may be administered to infants as young as six weeks of age. For children who are beyond the age of a routine infant vaccination schedule, the immunogenic conjugates of the invention may be administered according to the following exemplary schedule.

Age of first dosage	**Dosage schedule**
6 weeks-5 years of age	Total of four 0.5 ml doses; the first three at least eight weeks apart and the fourth at least six months after the third dose.
6-17 years of age	One 0.5 ml dose.
50 years of age and older	One 0.5 ml dose.

A booster dose is desirably given as early as four years following the last dose in subjects who are at a high risk for infection; or 10 years after the last dose to previously immunized adults and children above fifteen years of age.

The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier immediately prior to use. Immunogenic conjugates of the invention can be formulated in pharmacologically acceptable vehicles, e.g., alum hydroxide gel, adjuvant preparation, or saline, and then administered, e.g., by intramuscular injection, intradermal injection, or transcutaneous immunization with appropriate immune adjuvants.

Petitioner Merck Ex-1013, 0029

Immunogenic Conjugate Compositions: whole cell pathogen-carrier protein

The immunogenic conjugates of the invention may be used in combination, for example, in pediatric immunizations. In addition, the immunogenic conjugates of the invention may be used to immunize against, for example, *Pneumococcus* infection, *Haemophilus influenzae* type B ("HiB") infection, *Streptococcus* (groups A and B) infection, meningococcal (e.g., *Neisseria meningitides*) infection, and may be used as O antigen whole cell pathogen immunogenic conjugates from Gram negative bacteria (e.g., *Pseudomonas aeruginosa*, *Francisella tularensis*, *Shigella* species, *Salmonella* species, *Acinetobacter* species, *Burkholderia* species, and *Escherichia coli*).

For whole cell pathogen immunogenic conjugates, the formulation includes at least one inactivated whole cell pathogen including the antigenic polysaccharide of interest, at least one carrier protein, and a pharmaceutically acceptable buffer (e.g., bicarbonate buffer) to neutralize gastric acid. In this formulation, the immunogenic conjugate preparation is mixed with a buffer, e.g., 5.6 g of sodium hydrogen carbonate granules dissolved in 150 mL of sterile water.

Typically the immunogenic conjugate contains about 1 mg of whole cell pathogen immunogenic conjugate in a single dose for oral administration. A 1 mg dose of the immunogenic conjugate may contain at least 1×10^9 whole cell pathogens or a range of 1×10^9 to 1×10^{11} whole cell pathogens covalently linked with approximately 2-500 µg of the carrier protein.

In particular, the immunogenic conjugates of the invention may be administered to a subject enterally (for instance, by oral administration) by ingestion of an immunogenic conjugate in the form of a e.g., liquid, powder, capsule, or tablet. Immunogenic conjugates of the invention may be administered, one or more times, often including a second administration designed to boost production of antibodies in a subject to prevent infection by an infectious agent. The frequency and quantity of immunogenic conjugate dosage depends on the specific activity of the immunogenic conjugate and can be readily determined by routine experimentation. The age at which the first dosage is administered generally is two-years. The immunogenic conjugates of the invention may be administered according to the following exemplary schedule.

Age of first dosage	**Dosage schedule**
2 – 6 years of age	Three oral doses at least one week apart.
6 years of age and older	Two oral doses at least one week apart.

A booster dose is desirably given as early as six months following the last dose in subjects who are at a high risk for infection; or five years after the last dose to previously immunized adults and children above two years of age.

Petitioner Merck Ex-1013, 0030

The invention also includes kits that include an immunogenic conjugate described herein. The kits of the invention can also include instructions for using the kits in the immunization methods described herein.

5 The efficacy of the immunization schedule may be determined by using standard methods for measuring the antibody titer in the subject. In general, mean antibody titers (desirably IgG titers) of approximately 1 µg/ml are considered indicative of long-term protection.

The antigenic polysaccharide-carrier protein complexes for use in the immunogenic conjugate 10 compositions described herein are desirably between 10 nm and 100 µm in diameter. Viruses can be 100 nm in diameter and are immunogenic. Whole bacteria are 1-10 µm in diameter and are also immunogenic. A small clump of bacteria can be about 100 µm in diameter. In particular embodiments, an antigenic polysaccharide-carrier protein complex in an immunogenic conjugate composition desirably is between 100 nm and 10 µm in diameter. This complex may be soluble or insoluble.

15

The invention is described herein below by reference to specific examples, embodiments and figures, the purpose of which is to illustrate the invention rather than to limit its scope. The following examples are not to be construed as limiting.

20

EXAMPLES

Example 1: Sortase-mediated Immunogenic Conjugate 1 Preparations

An immunogenic conjugate can be prepared from an antigenic polysaccharide (PS) displaying a sortase recognition peptide and a sortase. For this reaction, polysaccharides are first 25 chemically activated with periodate, cyanogen borohydride, or carbodiimide reagents to allow for attachment of a sortase recognition peptide to PS using conventional chemistry. The sortase recognition peptide is a synthetic peptide that displays the LPXTGXX sequence but which then has unique N-terminal, internal, or C-terminal residues that allow for the covalent modification of activated polysaccharides. The chemical groups of these residues include amino, carboxyl, thiol, halogen, and 30 azide groups, as well as others. These chemical groups are selected to react with activated PS. Once modified, the PS covalently linked to the sortase recognition peptide is mixed with a sortase, for instance SrtA, to covalently attach sortase to the PS by a thioester bond. This reaction produces a PS-sortase conjugate (Fig. 2 conjugate 1). To prevent reversal of the sortase-PS thioester bond, the PS-sortase conjugate may be enzymatically stabilized by treatment with a chemical cross-linking agent such as 35 formalin or glutaraldehyde, further entrapping the antigenic polysaccharide to form a protein cross-linked cage or mesh. To cross-link the antigenic PS-sortase immunogenic conjugate, a 10% neutral buffered formalin is prepared by diluting a 37% aqueous formaldehyde solution with phosphate buffered saline

Petitioner Merck Ex-1013, 0031

(PBS) to about 3.7-4.0% formaldehyde. The PS-sortase immunogenic conjugate is treated with excess 10% formalin for about 10 minutes at room temperature to cross-link the antigen to the sortase. Subsequently, the fixation is quenched by addition of excess 1.25 M glycine in PBS.

5 *Example 2: Sortase-mediated Immunogenic Conjugate 2 Preparations*

An immunogenic conjugate can be prepared from an antigenic polysaccharide (PS) displaying a sortase recognition peptide and a carrier protein. For this reaction, polysaccharides are first chemically activated with periodate, cyanogen borohydride, or carbodiimide reagents to allow for attachment of a sortase recognition peptide to PS using conventional chemistry. The sortase recognition peptide is a 10 synthetic peptide that displays the LPXTGXX sequence but which then has unique N-terminal, internal, or C-terminal residues that allow for the covalent modification of activated polysaccharides. The chemical groups of these residues include amino, carboxyl, thiol, halogen, and azide groups, as well as others. These chemical groups are selected to react with activated PS. Once modified, the PS covalently linked to the sortase recognition peptide is mixed with a sortase, for instance SrtA, to covalently attach sortase 15 to the PS by a thioester bond. This reaction produces a PS-sortase conjugate (Fig. 2 conjugate 1).

A second immunogenic conjugate can be prepared from conjugate 1. For this reaction, a carrier protein is engineered to display polyglycine motif, where at least two glycine repeats are coupled to a carrier protein amino acid residue by a peptide bond. The carrier protein may be modified with glycine 20 repeats by sandwiching the glycine repeats between the carrier protein and a small ubiquitin-like modifier (SUMO) peptide. SUMOylation occurs when the C-terminal carboxyl group of a double-glycine motif in SUMO and the epsilon-amino group of a lysine residue in a carrier protein form an isopeptide bond. Upon addition of the SUMO protease that recognizes the SUMO peptide, the SUMO protease cleaves immediately following the glycine repeat precursor at the C-terminus of the SUMO peptide. This reaction 25 produces a polyglycine-modified carrier protein. The polyglycine decorated carrier protein is subsequently mixed with the PS-sortase conjugate (Fig. 1 conjugate 1) and sortase transfers itself off the recognition peptide modified PS and attaches the polyglycine modified carrier protein to the PS. Thus, this reaction produces a second PS-carrier protein conjugate (Fig. 2 conjugate 2) that is covalently linked by a peptide bond.

30

Example 3: Whole cell bacterial conjugate and oral administration

The sortase-mediated immunogenic conjugation method can be performed *in situ* on the surface of the polysaccharide-encapsulated organism, such as *Streptococcus pneumonia*. For instance, about 10^9-10^{11} cells of 23 *S. pneumoniae* serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 35 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F) are heat-inactivated by incubation of the cells at 55º - 60º C for 30 minutes. Alternatively, the cells may be chemically-inactivated by treatment with 55 µL of 10% neutral buffered formalin per 1 mL of bacterial cell suspension. The cells are incubated in formalin for at

Petitioner Merck Ex-1013, 0032

37º C for one hour. Subsequently, the inactivated cells are washed in PBS and or additional gentle biologically compatible buffers and reagents. The killed cells are then modified to attach the sortase recognition peptide to the surface polysaccharides of the cells. By this approach, the polysaccharide purification and characterization steps are eliminated, and the whole cells conjugated to sortase or the carrier protein(s) of interest are incorporated into the immunogenic conjugate essentially as described in Example 1 or Example 2. The 23 valent pneumococcal whole cell immunogenic conjugate can be absorbed mucosally and thus allows for oral administration of the preparation.

Example 4: Generation and Characterization of Sortase-mediated Immunogenic Conjugates

The sortase-mediated immunogenic conjugation method can be applied to capsular antigens of various structures and ionic charges. For example, generation of an immunogenic conjugate against *Streptococcus pneumonia* can be produced by first purchasing 23 types of *Streptococcus pneumonia* PS from the American Type Culture Collection (ATCC), which are manufactured by Merck, Inc. These PS vary widely in their molecular structure and include PS that are strongly anionic, partially cationic, neutral in charge, phosphorylated, linear, have branching structures, and modified in various other ways. A subset of these PS correspond to the thirteen capsular types in the Wyeth product *Prevnar13®* (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) and can be assayed for their ability to induce IL-6 production by mouse macrophages. Since PS can also be contaminated with a TLR agonist, phenol extraction and ethanol precipitation can be performed to "clean up" (remove residual unknown TLR agonists) commercially prepared *S. pneumoniae* PS.

Removal of the contaminants is confirmed by testing the treated PSs for induction of IL-6 by peritoneal macrophages by standard methods. PSs that are devoid of IL-6 induction activity are used for production of immunogenic conjugates. Following examination of the 23 pneumococcal PS, each of the 23 capsular types is used to make an immunogenic conjugate using a sortase, essentially by the method described in Example 1. A one to one ratio of PS to a dominant negative mutant (DNI) carrier protein is used (approximately 1:1 by dry weight) for these initial immunogenic conjugate preparations. Each preparation is characterized by SDS-PAGE for evidence of covalent-linkage between pneumococcal PS and a carrier protein such as DNI. For some capsular types (e.g., 6B and 23F), other carrier proteins are used to make immunogenic conjugates.

For example, ten different immunogenic conjugates using five different matrix proteins and two different antigens are made as follows. The selection of the five matrix proteins is based on their current use in FDA-approved vaccines or other properties that allow them to serve as tracers for measuring the stability of immunogenic conjugate preparations. The following matrix proteins are used (1) cholera toxin B subunit (available from SBL Vaccin AB), (2) diphtheria toxin, (3) tetanus toxin Fragment C, "Frag C" (available from Sigma Aldrich), (4) DNI, and (5) beta-galactosidase from *Escherichia coli* (available from

Petitioner Merck Ex-1013, 0033

Sigma Aldrich). As capsular antigens poly-D-glutamic acid from *Bacillus anthracis* and *Streptococcus pneumoniae* capsule type 14 (Suarez et al., *Appl. Environ. Micobiol.* 67:969-971, 2001) are used. Each capsule antigen is combined with each of the five selected matrix proteins to produce 10 distinct immunogenic conjugates. All immunogenic conjugate preparations that show evidence of covalent
5 protein linkage (e.g., in SDS-PAGE) are tested for their immunogenicity.

Immunogenic conjugates can be tested for their ability to induce, in mice, isotype antibody switching to IgG as is observed in conventional conjugate vaccines. All antigens can be absorbed to alum and then typically groups of 5 mice per immunogenic conjugate preparation are used. Mice are pre-
10 bled to obtain baseline immune responses to the test antigens. Mice are then immunized three times (at day 0, 7, 14) by a standard IP injection protocol, and blood is collected at days 10, 20, 30, and 60 days post primary immunization. Mouse sera are analyzed by standard ELISA assay for IgG against the PS and carrier proteins used. In these experiments, control groups of mice immunized with only PS are included to assess the ability of various immunogenic conjugate preparations to induce anti-PS IgG
15 compared with the non-conjugated PS which should be poorly- or non-immunogenic. Promising immunogenic conjugates (i.e., immunogenic conjugates that induce high levels of IgG against PSs) undergo more careful immunological analysis, which seeks to establish the kinetics and dose response aspects of the immune response to the immunogenic conjugate in mice.

20 Alternatively, promising immunogenic conjugates and their corresponding controls can be sent to commercial vendors for production of rabbit anti-sera. Similar immunoassays are performed to assess the immunogenicity, class of antibody induced, and kinetics of immune response in rabbits. In these experiments the control is the commercial product *Prevnar13®* which is an alum absorbed mixture of 13 different conventional conjugate PS vaccines coupled to CRM197, the nontoxic mutant protein related to
25 diphtheria toxin.

The functionality of the antibody responses induced with immunogenic conjugates can be assessed. For example, functionality can be assessed by measuring the ability of the anti-PS antibody to opsonize encapsulated *S. pneumococcus* and lead to bacterial killing after phagocytosis by
30 macrophages. Protection of animals from lethal challenge with *S. pneumococcus* is another way to demonstrate the efficacy of the immunogenic conjugate in immunized animals.

Example 5: Combination of Immunogenic Conjugates with Adjuvants

The PS-carrier protein immunogenic conjugate can be modified to further stimulate the immune
35 response, and ultimately improve the efficacy of the immunization, by addition of an adjuvant. The immunogenic conjugate can be absorbed by an alum adjuvant such as aluminum hydroxide gel. Additionally, the immunogenic can be combined with an emulsion adjuvant such as squalene based oil in

Petitioner Merck Ex-1013, 0034

water nano emulsion. Adjuvants such as these can be used to create a delivery system for the immunogenic conjugate and function to create depots that trap the conjugated antigen-carrier protein at the site of injection to allow for its slow release. This allows for extended stimulation of the immune system by enabling the immunogenic conjugate to persist at the site of injection, increasing the
5 recruitment and activation of immune cells.

Petitioner Merck Ex-1013, 0035

CLAIMS

1. An immunogenic composition comprising a polysaccharide-sortase conjugate, wherein said polysaccharide is an antigen and said sortase is a carrier protein, and wherein said sortase is covalently linked to said polysaccharide antigen by a sortase recognition sequence.

2. An immunogenic composition comprising a polysaccharide-protein conjugate, said conjugate comprising a polysaccharide antigen and a carrier protein, wherein said polysaccharide antigen is covalently linked to said carrier protein by a sortase recognition sequence and a polyglycine motif present at the N-terminus of said carrier protein.

3. The immunogenic composition of claim 1 or 2, wherein said sortase is selected from the group consisting of sortase A, sortase B, sortase C, and sortase D.

4. The immunogenic composition of claim 3, wherein said sortase is sortase A, or a fragment thereof.

5. The immunogenic composition of claim 4, wherein said sortase A comprises the amino acid sequence of SEQ ID NO: 1.

6. The immunogenic composition of claim 5, wherein said sortase comprises an amino acid sequence that has at least 90% identity to the amino acid sequence of SEQ ID NO: 1.

7. The immunogenic composition of claim 6, wherein said sortase comprises an amino acid sequence that has at least 95% identity to the amino acid sequence of SEQ ID NO: 1.

8. The immunogenic composition of claim 7, wherein said sortase comprises an amino acid sequence that has at least 99% identity to the amino acid sequence of SEQ ID NO: 1.

9. The immunogenic composition of claim 3, wherein said sortase is sortase B, or a fragment thereof.

10. The immunogenic composition of claim 3, wherein said sortase is sortase C, or a fragment thereof.

11. The immunogenic composition of claim 3, wherein said sortase is sortase D, or a fragment thereof.

Petitioner Merck Ex-1013, 0036

12. The immunogenic composition of any one of claims 1 to 11, wherein said sortase comprises at least one mutation

13. The immunogenic composition of any one of claims 1 to 12, wherein said sortase comprises a substitution.

14. The immunogenic composition of claim 1 or claim 2, wherein said sortase recognition sequence has the formula $X_1PX_2X_3$ X_4, where X_1-X_4 are any amino acid.

15. The immunogenic composition of claim 14, wherein said sortase recognition sequence has the formula $X_1PX_2X_3G$ for a sortase A substrate, where X_1 is Leu, Ile, Val or Met, X_2 is any amino acid, and X_3 is Ser, Thr or Ala.

16. The immunogenic composition of claim 15, wherein the sortase recognition sequence is LPX_1TG for a sortase A substrate, where X_1 is any amino acid.

17. The immunogenic composition of claim 14, wherein the sortase recognition sequence is NPX_1TX_2 for a sortase B substrate, where X_1 is Lys or Gln and X_2 is Asn, Asp, or Gly.

18. The immunogenic composition of claim 14, wherein the sortase recognition sequence is LPX_1TX_2 for a sortase C substrate, where X_1 and X_2 are any amino acid.

19. The immunogenic composition of claim 14, wherein the sortase recognition sequence is LPX_1TA for a sortase D substrate, where X_1 is any amino acid.

20. The immunogenic composition of claim 14, wherein the sortase recognition sequence is LAX_1TG for a sortase D substrate, where X_1 is any amino acid.

21. The immunogenic composition of claim 2, wherein the polyglycine motif comprises an amino acid sequence selected from the group consisting of GG, GGG, GGGG, and GGGGG.

22. The immunogenic composition of claim 21, wherein the polyglycine motif comprises the amino acid sequence GGGGG.

23. The immunogenic composition of any one of claims 1 to 22, wherein said carrier protein molecules are selected from the group consisting of diphtheria toxin, diphtheria toxoid, tetanus toxin, tetanus toxoid, *Pseudomonas aeruginosa* exotoxin A, cholera toxin B subunit, tetanus toxin fragment C, bacterial flagellin, pneumolysin, an outer membrane protein of *Neisseria menningitidis*, *Pseudomonas aeruginosa* Hcp1 protein, *Escherichia coli* heat labile enterotoxin, shiga-like toxin, human LTB protein, pneumolysin, listeriolysin O, a protein extract from whole bacterial cells, the dominant negative mutant

Petitioner Merck Ex-1013, 0037

(DNI) of the protective antigen of *Bacillus anthracis*, and *Escherichia coli* beta-galactosidase.

24. The immunogenic composition of claim 23, wherein said whole bacterial cells are *Pseudomonas aeruginosa* or *Streptococcal* cells.

25. The immunogenic composition of claim 23, wherein said bacterial flagellin is the *Vibrio cholerae* flagellin protein.

26. The immunogenic composition of claim 23, wherein said shiga-like toxin is the *Shigella* SltB2 protein.

27. The immunogenic composition of claim 23, wherein said carrier protein molecules are pneumolysin.

28. The immunogenic composition of claim 23, wherein said carrier protein molecules are listeriolysin O.

29. The immunogenic composition of claim 23, wherein said carrier protein molecules are diphtheria toxin.

30. The immunogenic composition of claim 23, wherein said carrier protein molecules are diphtheria toxoid.

31. The immunogenic composition of claim 23, wherein said carrier protein molecules are tetanus toxin.

32. The immunogenic composition of claim 23, wherein said carrier protein molecules are tetanus toxoid.

33. The immunogenic composition of any one of claims 1 to 32, wherein said antigen of interest is a polysaccharide, a polyalcohol, or a poly amino acid.

34. The immunogenic composition of claim 33, wherein said polysaccharide comprises at least 18 residues.

35. The immunogenic composition of claim 33, wherein said polysaccharide is a *Streptococcus pneumoniae* polysaccharide, *Francisella tularensis* polysaccharide, *Bacillus anthracis* polysaccharide,

Petitioner Merck Ex-1013, 0038

Haemophilus influenzae polysaccharide, *Salmonella typhi* polysaccharide, *Salmonella* species polysaccharide, *Shigella* polysaccharide, or *Neisseria meningitidis* polysaccharide.

36. The immunogenic composition of claim 35, wherein said *Streptococcus pneumoniae* polysaccharide is capsular type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, or 48.

37. The immunogenic composition of claim 35, wherein said *Francisella tularensis* polysaccharide is O antigen.

38. The immunogenic composition of any one of claims 33 to 37, wherein said antigen of interest is a microbial capsular polymer.

39. The immunogenic composition of claim 38, wherein said microbial capsular polymer is poly-gamma-D-glutamic acid from *Bacillus anthracis*.

40. The immunogenic composition of any one of claims 33 to 39, wherein said antigen of interest is an organic polymer consisting of monomers having at least three atoms, wherein each of said atoms is independently selected from the group consisting of carbon, oxygen, hydrogen, phosphate, nitrogen, and sulfate.

41. The immunogenic composition of claim 40, wherein said organic polymer is obtained from a microbe.

42. The immunogenic composition of claim 40, wherein said organic polymer does not occur in nature.

43. The immunogenic composition of any one of claims 1 to 42, wherein said immunogenic composition further comprises a second antigen of interest.

44. The immunogenic composition of any one of claims 1 to 43, wherein said immunogenic composition further comprises a third antigen of interest.

Petitioner Merck Ex-1013, 0039

45. The immunogenic composition of any one of claims 1 to 44, wherein said antigen of interest comprises whole cell pathogens.

46. The immunogenic composition of claim 45, wherein said whole cell pathogens comprise heat inactivated whole cell pathogens.

47. The immunogenic composition of claim 45, wherein said whole cell pathogens comprise chemically inactivated whole cell pathogens.

48. The immunogenic composition of any one of claims 45 to 47, wherein said whole cell pathogens comprise *Pseudomonas aeruginosa* or *Streptococcal* cells.

49. The immunogenic composition of claim 48, wherein said *Streptococcal* cells comprise *Streptococcus pneumonia*.

50. The immunogenic composition of claim 49, wherein said whole cell pathogens are *Streptococcus pneumonia* type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, or 48.

51. The immunogenic composition of claim 50, wherein said antigen of interest comprises at least one whole cell pathogen selected from the group consisting of *Streptococcus pneumonia* type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

52. The immunogenic composition of claim 51, wherein said antigen of interest comprises two or more whole cell pathogens selected from the group consisting of *Streptococcus pneumonia* type 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10F, 11A, 11B, 11C, 11D, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20, 21, 22F, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33D, 33F, 34, 35A, 35B, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

53. The immunogenic composition of any one of claims 1 to 52, wherein said complex, when administered to a mammal, elicits a T-cell dependent immune response in said mammal.

Petitioner Merck Ex-1013, 0040

54. The immunogenic composition of any one of claims 1 to 53, wherein the molar ratio of said antigen to said carrier protein molecules is 1 to 1.

55. A pharmaceutical composition in unit dosage form comprising (i) the immunogenic composition of any one of claim 1 to 54 and (ii) a pharmaceutically acceptable excipient.

56. A method of making an immunogenic composition of claim 1 or 2 comprising a polysaccharide-protein conjugate, said method comprising mixing a polysaccharide antigen comprising a sortase recognition sequence and a sortase, wherein said mixing results in formation of a thioester bond between said polysaccharide antigen and said sortase, yielding said polysaccharide-protein conjugate.

57. The method of making an immunogenic composition of claim 56, said method further comprising mixing said polysaccharide-protein conjugate and a carrier protein comprising a polyglycine motif at its N-terminus, wherein said mixing results in formation of a peptide bond between a terminal carboxyl group of a sortase recognition sequence attached to said polysaccharide antigen and a terminal amino group of said polyglycine motif of said carrier protein.

58. The method of making an immunogenic composition of claim 56 or 57, wherein said polysaccharide antigen is a whole cell pathogen.

59. A method of generating an immune response in a subject comprising administering the pharmaceutical composition of claim 55 to a subject, wherein said immunogenic composition elicits a T-cell dependent immune response in said subject.

60. The method of claim 59, wherein said subject is an infant, a child, or an adolescent.

Petitioner Merck Ex-1013, 0041

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1 Remove

Given Name	Middle Name	Family Name	City	State	Country i
John	J.	MEKALANOS	Brighton	MA	US

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE
Attorney Docket Number (if applicable)	00742-257001

Correspondence Address

Direct all correspondence to (select one):

◉ The address corresponding to Customer Number	○ Firm or Individual Name
Customer Number	21559

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

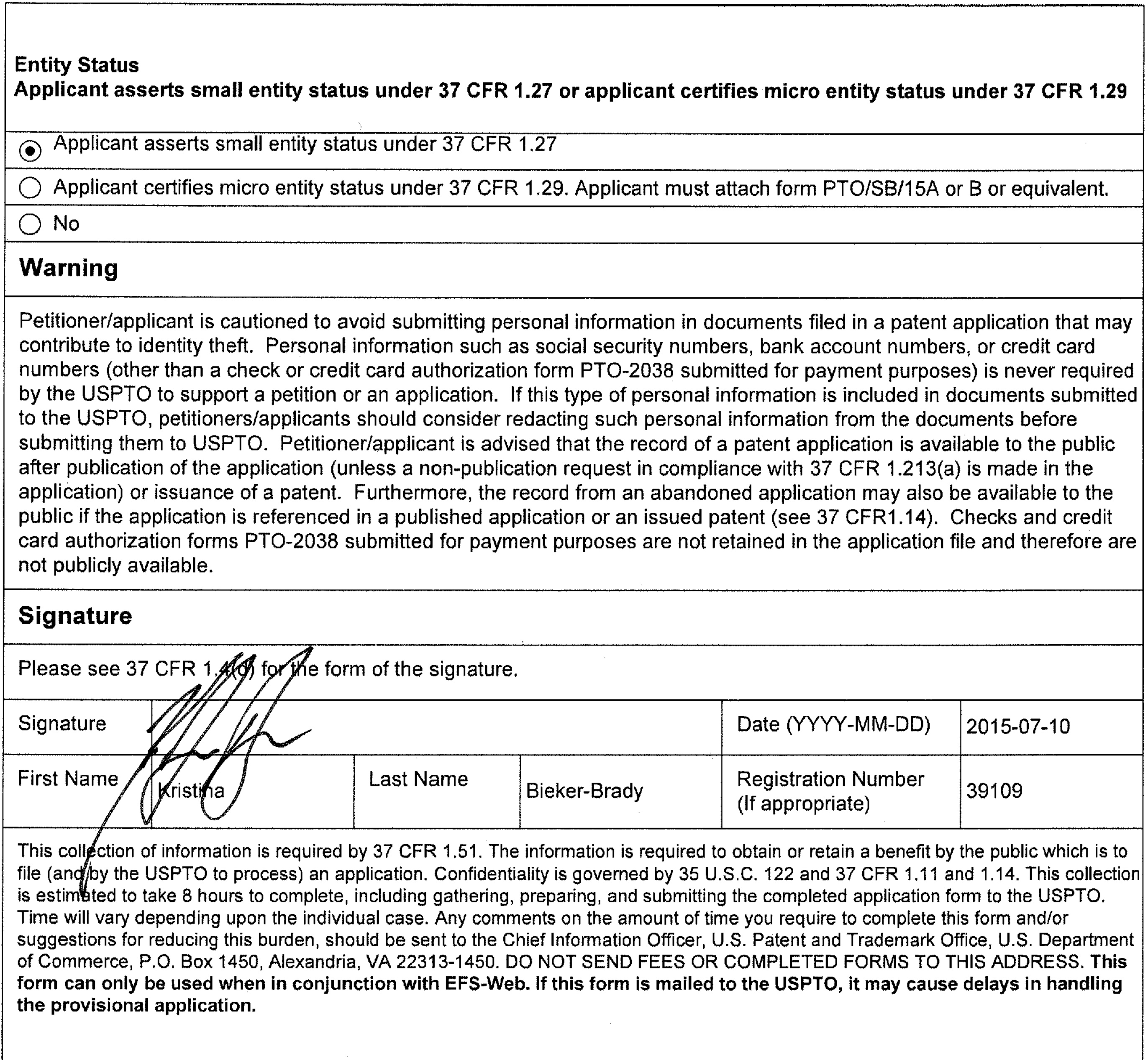
◉ No.

○ Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:

○ Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number



Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

- (●) Applicant asserts small entity status under 37 CFR 1.27
- ( ) Applicant certifies micro entity status under 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
- ( ) No

Warning

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Kristina/			Date (YYYY-MM-DD)	2015-07-10
First Name	Kristina	Last Name	Bieker-Brady	Registration Number (If appropriate)	39109

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **This form can only be used when in conjunction with EFS-Web. If this form is mailed to the USPTO, it may cause delays in handling the provisional application.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or paten. Accordingly, pursuant to the requirements of the Act, please be advised that : (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, t o a n other federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	00742-257001
	Application Number	
Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	John	J.	MEKALANOS	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Brighton	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	733-ch Cambridge Street		
Address 2			
City	Brighton	State/Province	MA
Postal Code	02135	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below. For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	21559
Email Address	Add Email Remove Email

Application Information:

Title of the Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE		
Attorney Docket Number	00742-257001	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	2	Suggested Figure for Publication (if any)	

Filing By Reference :

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	00742-257001
	Application Number	

Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	21559		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

Foreign Priority Information:

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	00742-257001
	Application Number	
Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE	

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

PTO/AIA/14 (07-14)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	00742-257001
	Application Number	

Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

- (●) Assignee
- ( ) Legal Representative under 35 U.S.C. 117
- ( ) Joint Inventor
- ( ) Person to whom the inventor is obligated to assign.
- ( ) Person who shows sufficient proprietary interest

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	President and Fellows of Harvard College

Mailing Address Information For Applicant:

Address 1	17 Quincy Street		
Address 2			
City	Cambridge	**State/Province**	MA
Country US		Postal Code	02138
Phone Number		Fax Number	

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	00742-257001
	Application Number	

Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE

Email Address	

Additional Applicant Data may be generated within this form by selecting the Add button.

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

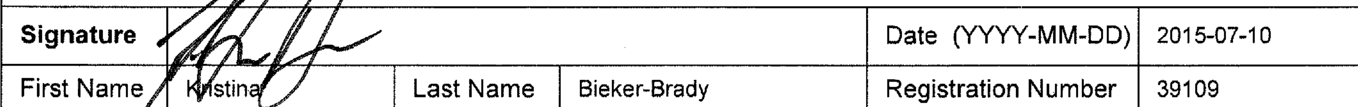
Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

Signature:

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications.



Signature				Date (YYYY-MM-DD)	2015-07-10
First Name	Kristina	Last Name	Bieker-Brady	Registration Number	39109

Additional Signature may be generated within this form by selecting the Add button.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	00742-257001
	Application Number	

Title of Invention	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Petitioner Merck Ex-1013, 0050

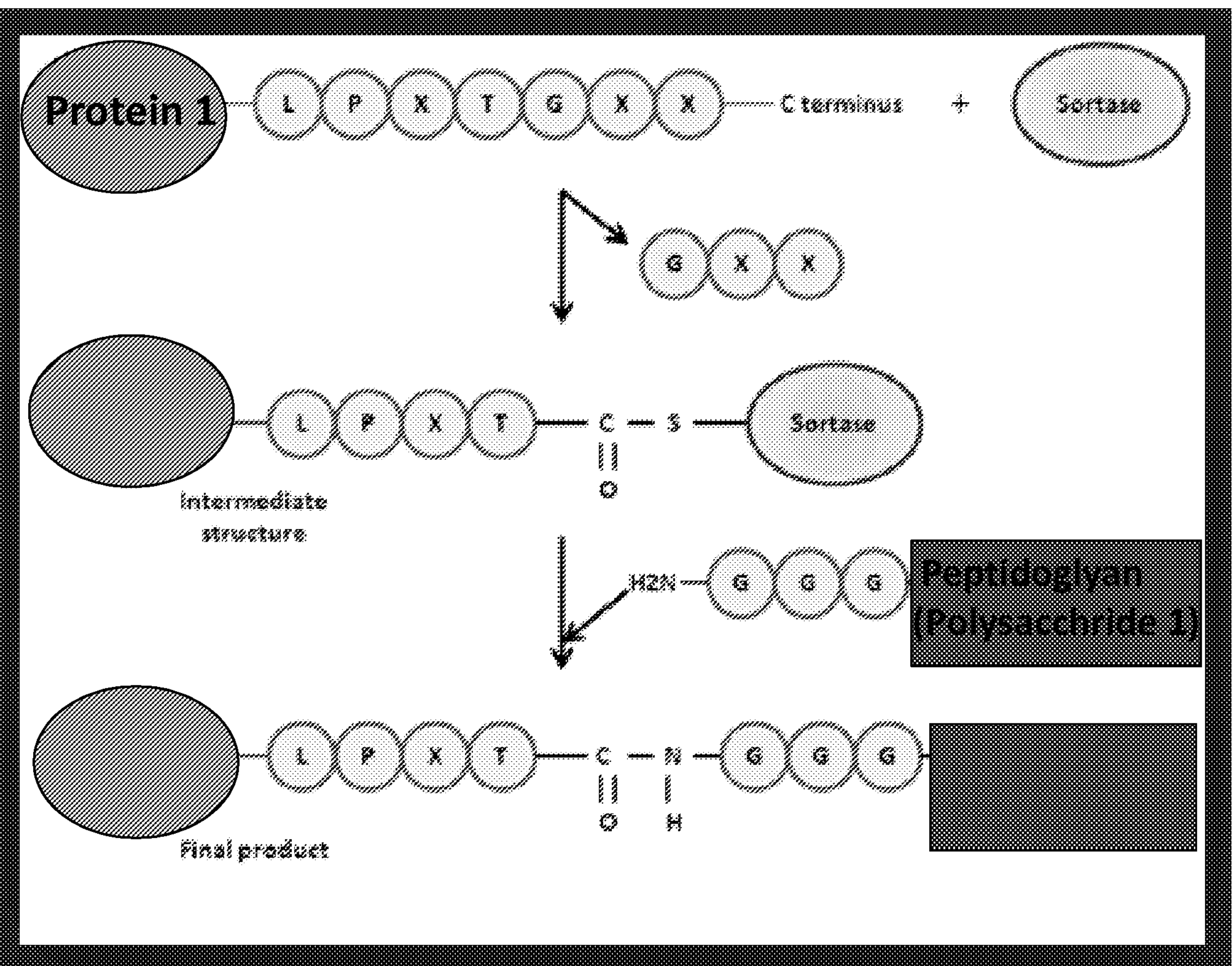
Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

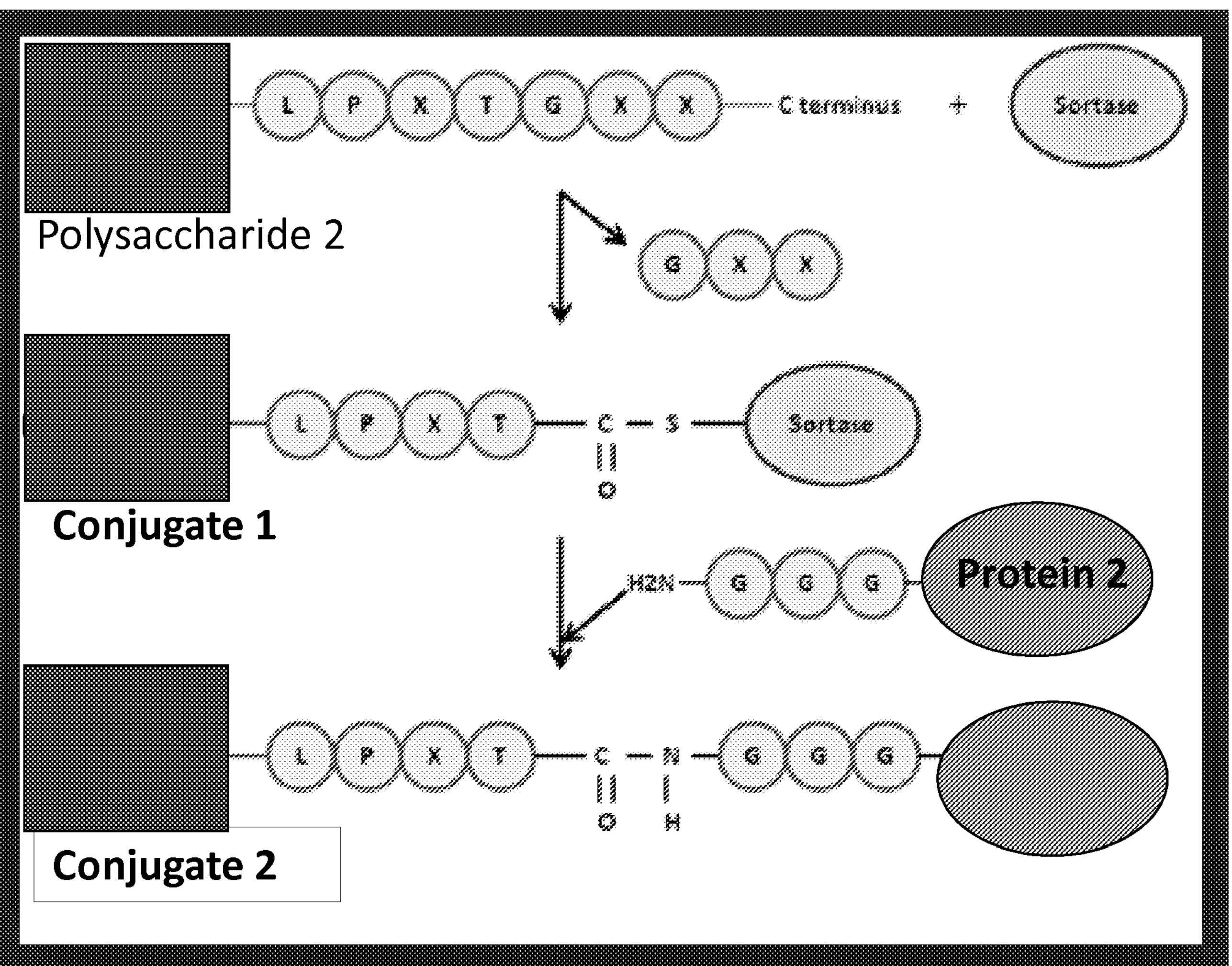
1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent C o o p eration Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Figure 1



Petitioner Merck Ex-1013, 0052

Figure 2



Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE
First Named Inventor/Applicant Name:	John J. MEKALANOS
Filer:	Kristina Bieker-Brady/Nathan Verry
Attorney Docket Number:	00742-257001

Filed as Small Entity

Filing Fees for **Provisional**

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Provisional Application Filing Fee	2005	1	130	130
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
	Total in USD ($)			**130**

Electronic Acknowledgement Receipt

EFS ID:	22885362
Application Number:	62191028
International Application Number:	
Confirmation Number:	3822
Title of Invention:	SORTASE-MEDIATED COUPLING OF IMMUNOGENIC POLYSACCHARIDE-PROTEIN CONJUGATES AND THEIR USE
First Named Inventor/Applicant Name:	John J. MEKALANOS
Customer Number:	21559
Filer:	Kristina Bieker-Brady/Alicia Briggs
Filer Authorized By:	Kristina Bieker-Brady
Attorney Docket Number:	00742-257001
Receipt Date:	10-JUL-2015
Filing Date:	
Time Stamp:	15:54:15
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$130
RAM confirmation Number	2424
Deposit Account	032095
Authorized User	BIEKER-BRADY, KRISTINA

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Specification	Draft_application.pdf	204709 db42ca753b52195fa66a09c9b859cbe6221 9ab0d	no	38
Warnings:					
Information:					
2	Provisional Cover Sheet (SB16)	Provisional_Cover_Sheet.PDF	355862 60976d680ac6a788349ac8565f4cea4df3be 3238	no	3
Warnings:					
This is not a USPTO supplied Provisional Cover Sheet SB16 form.					
Information:					
3	Application Data Sheet	ADS.PDF	763534 7331f122d037a97de7f13d4a8eb63a09e6a 9780e	no	7
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					
4	Drawings-only black and white line drawings	Figures.PDF	194196 c72bc38ae62677ddfaf7da9496159e6eeadc 1a52	no	2
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	30062 1c29bbd6ee95f47d7d32870a08cf19a50a2 b2259	no	2
Warnings:					
Information:					
			Total Files Size (in bytes):	1548363	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.