CLAIMS

Claim 1. A polypeptide-polysaccharide conjugate comprising:

(a) a GAS polypeptide antigen or a non-GAS carrier polypeptide comprising at least one non-natural amino acid (nnAA), wherein the nnAA comprises a click chemistry reactive group; and

(b) a purified cell wall polysaccharide or a peptidoglycan-bound capsular polysaccharide with a molecular weight of at least about 10 kDa to at least about 40 kDa.

Claim 2. The polypeptide-polysaccharide conjugate of claim 1, wherein the purified cell wall polysaccharide lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

Claim 3. The polypeptide-polysaccharide conjugate of claims 1-2, wherein the GAS polypeptide antigen is a full-length GAS polypeptide antigen or a fragment of a full-length GAS polypeptide antigen.

Claim 4. The polypeptide-polysaccharide conjugate of any one of claims 1-3, wherein the cell wall polysaccharide is a GAS polysaccharide, or a variant thereof.

Claim 5. The polypeptide-polysaccharide conjugate of claim 4, wherein between about 8 mol% and about 20 mol% of the polysaccharide repeat units of the GAS polysaccharide, or a variant thereof, are derivatized by a linker.

Claim 6. The polypeptide-polysaccharide conjugate of claims 4-5, wherein between about 10 mol% and about 20 mol% of the polysaccharide repeat units of the GAS polysaccharide, or a variant thereof, are derivatized by a linker.

Claim 7. The polypeptide-polysaccharide conjugate of claims 4-6, wherein between about 10 mol% and about 18 mol% of the polysaccharide repeat units of the GAS polysaccharide, or a variant thereof, are derivatized by a linker.

Claim 8. The polypeptide-polysaccharide conjugate of any one of claims 1-6, wherein the average molecular weight is between about 185 kDa and about 700.

Claim 9. The polypeptide-polysaccharide conjugate of any one of claims 1-7, wherein the average molecular weight is between about 200 kDa and about 700 kDa.

Claim 10. The polypeptide-polysaccharide conjugate of any one of claims 1-9, wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 300 kDa and about 600 kDa.

Claim 11. The polypeptide-polysaccharide conjugate of any one of claims 1-10, wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 400 kDa and about 500 kDa.

Claim 12. The polypeptide-polysaccharide conjugate of any one of claims 1-11, wherein the at least one nnAA is substituted for a lysine, a leucine, an isoleucine, or an arginine in the GAS polypeptide antigen or the non-GAS carrier polypeptide.

Claim 13. The polypeptide-polysaccharide conjugate of any one of claims 1-12, wherein the at least one nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

Claim 14. The polypeptide-polysaccharide conjugate of any one of claims 1-13, wherein the at least one nnAA is pAMF.

Claim 15. The polypeptide-polysaccharide conjugate of any one of claims 1-14, wherein the GAS polypeptide antigen is selected from C5a peptidase, streptolysin O (SLO), SpyAD, Sib35, and Sfb1.

Claim 16. The polypeptide-polysaccharide conjugate of any one of claims 1-15, wherein the GAS polypeptide antigen is a SpyAD polypeptide, or a fragment thereof.

Claim 17. The polypeptide-polysaccharide conjugate of claim 16, wherein the SpyAD polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

Claim 18. The polypeptide-polysaccharide conjugate of claim 16, wherein the SpyAD polypeptide is at least 95% identical to SEQ ID NO: 33.

Claim 19. The polypeptide-polysaccharide conjugate of any one of claims 16-18, wherein the SpyAD polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

Claim 20. The polypeptide-polysaccharide conjugate of any one of claims 16-19, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 34.

Claim 21. The polypeptide-polysaccharide conjugate of any one of claims 16-19, wherein the SpyAD polypeptide has the amino acid sequence of SEQ ID NO: 34.

Claim 22. The polypeptide-polysaccharide conjugate of any one of claims 16-19, wherein the SpyAD polypeptide comprises the amino acid sequence of SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

Claim 23. The polypeptide-polysaccharide conjugate of any one of claims 16-19, wherein the SpyAD polypeptide has the amino acid sequence of SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

Claim 24. The polypeptide-polysaccharide conjugate of any one of claim 1-2 or 4-14, wherein the non-GAS carrier polypeptide is selected from ADI, ferritin, Protein D, and eCRM197.

Claim 25. The polypeptide-polysaccharide conjugate of claim 24, wherein the non-GAS carrier polypeptide is eCRM197.

Claim 26. The polypeptide-polysaccharide conjugate of claim 25, wherein the eCRM197 has the sequence of SEQ ID NO: 25.

Claim 27. The polypeptide-polysaccharide conjugate of any one of claims 1-13, wherein the GAS polypeptide antigen or a non-GAS carrier polypeptide comprises or consists comprises or consists of the amino acid sequence of a polypeptide listed in Table 1.

Claim 28. The polypeptide-polysaccharide conjugate of any one of claims 1-13, wherein the GAS polypeptide antigen or a non-GAS carrier polypeptide comprises or consists comprises or consists of the amino acid sequence of a polypeptide listed in Table 1A.

Claim 29. An immunogenic composition comprising:

(a) a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

(b) a GAS streptolysin O (SLO) polypeptide antigen; and

(c) a polypeptide-polysaccharide conjugate comprising

a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide, or a fragment thereof, comprising at least one non-natural amino acid (nnAA), wherein the at least one nnAA comprises a click chemistry reactive group, and

a GAS polysaccharide, or a variant thereof, that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain;

wherein between about 8 mol% and about 20 mol% of the polysaccharide repeat units of the GAS polysaccharide, or a variant thereof, are derivatized by a linker; and

wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 185 kDa and about 700 kDa.

Claim 30. The immunogenic composition of claim 29, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

Claim 31. The immunogenic composition of claims 29 or 30, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 29 or SEQ ID NO: 30.

Claim 32. The immunogenic composition of any one of claims 29-31, wherein the C5a peptidase polypeptide antigen has the amino acid sequence of SEQ ID NO: 29 or SEQ ID NO: 30.

Claim 33. The immunogenic composition of any one of claims 29-32, wherein the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

Claim 34. The immunogenic composition of claims 29-33, wherein the SLO antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

Claim 35. The immunogenic composition of any one of claims 29-34, wherein the SLO polypeptide antigen has the amino acid sequence of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

Claim 36. The immunogenic composition of any one of claims 29-35, wherein the at least one nnAA is selected from 2-amino-3-(4-azidophenyl)propanoic acid (pAF), 2-amino-4-azidobutanoic acid, 2-azido-3-phenylpropionic acid, 2-amino-3-azidopropanoic acid, 2-amino-3-(4-(azidomethyl)phenyl)propanoic acid (pAMF), 2-amino-3-(5-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(4-(azidomethyl)pyridin-2-yl)propanoic acid, 2-amino-3-(6-(azidomethyl)pyridin-3-yl)propanoic acid, and 2-amino-5-azidopentanoic acid.

Claim 37. The immunogenic composition of any one of claims 29-36, wherein the at least one nnAA is pAMF.

Claim 38. The immunogenic composition of any one of claims 29-37, wherein the SpyAD conjugate polypeptide, or a fragment thereof, comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 33.

Claim 39. The immunogenic composition of any one of claims 29-38, wherein the SpyAD conjugate polypeptide comprises an amino acid sequence that is a fragment of SEQ ID NO: 33.

Claim 40. The immunogenic composition of any one of claims 29-38, wherein the SpyAD conjugate polypeptide has an amino acid sequence that is a fragment of SEQ ID NO: 33.

Claim 41. The immunogenic composition of any one of claims 29-40, wherein the SpyAD conjugate polypeptide comprises a pAMF substitution at positions K64, K287, K386, and K657 of SEQ ID NO: 33.

Claim 42. The immunogenic composition of any one of claims 29-41, wherein the SpyAD conjugate polypeptide comprises the amino acid sequence of SEQ ID NO: 34, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

Claim 43. The immunogenic composition of any one of claims 29-42, wherein the SpyAD conjugate polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 34, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

Claim 44. The immunogenic composition of any one of claims 29-43, wherein the SpyAD conjugate polypeptide has the amino acid sequence of SEQ ID NO: 34, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

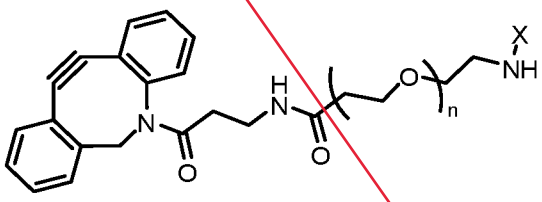
Claim 45. The immunogenic composition of any one of claims 29-44, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 30; the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 53; and the SpyAD conjugate polypeptide comprises an amino acid sequence that is at least 95% identical to SEQ ID NO: 34.

Claim 46. The immunogenic composition of any one of claims 29-45 wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 30; the SLO polypeptide antigen comprises the amino acid sequence of SEQ ID NO: 53; and the SpyAD conjugate polypeptide comprises the amino acid sequence of SEQ ID NO: 34.

Claim 47. The immunogenic composition of any one of claims 29-46, wherein the C5a peptidase polypeptide antigen has the amino acid sequence of SEQ ID NO: 30; the SLO polypeptide antigen has the amino acid sequence of SEQ ID NO: 53; and the SpyAD conjugate polypeptide has the amino acid sequence of SEQ ID NO: 34.

Claim 48. The immunogenic composition of any one of claims 29-47, wherein between about 15 mol% and about 20 mol% of the polysaccharide repeat units of the GAS polysaccharide, or a variant thereof, are derivatized by a linker.

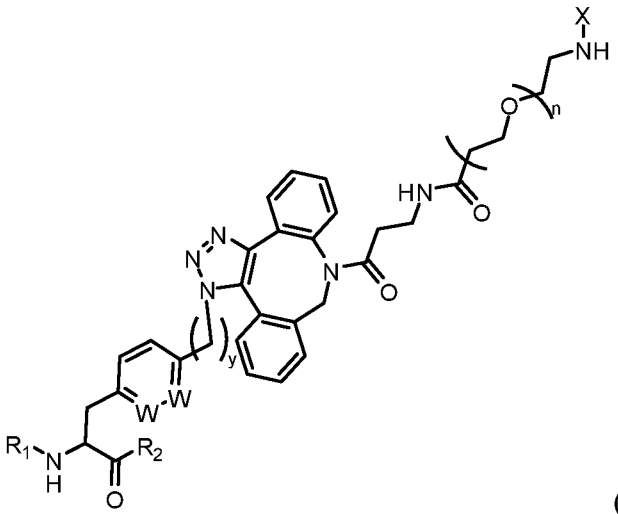
Claim 49. The immunogenic composition of any one of claims 29-48, wherein the linker, prior to reaction with the click chemistry reactive group of the nnAA, comprises a structure of Formula I:



(Formula I)

wherein, X is at least one polysaccharide repeat unit of a GAS polysaccharide, or a fragment thereof; and n is at least 1.

Claim 50. The immunogenic composition of any one of claims 29-49, wherein the SpyAD conjugate polypeptide, or fragment thereof, is linked to the GAS polysaccharide according to Formula II:



(Formula II)

wherein,

R_1 is H, formyl, or at least one amino acid of the SpyAD conjugate polypeptide;

R_2 is OH or at least one amino acid of the SpyAD conjugate polypeptide;

W is CH or N;

y is at least 1;

n is at least 1; and

X is at least one polysaccharide repeat unit of a GAS polysaccharide or variant thereof.

Claim 51. The immunogenic composition of any one of claims 29-50, wherein the GAS polysaccharide, or a variant thereof, has a molecular weight of at least about 10 kDa to at least about 40 kDa.

Claim 52. The immunogenic composition of any one of claims 29-51, wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 185 kDa and about 700.

Claim 53. The immunogenic composition of any one of claims 29-52, wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 200 kDa and about 700 kDa.

Claim 54. The immunogenic composition of any one of claims 29-53, wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 300 kDa and about 700 kDa.

Claim 55. The immunogenic composition of any one of claims 29-54, wherein the average molecular weight of the polypeptide-polysaccharide conjugate is between about 300 kDa and about 600 kDa.

Claim 56. The immunogenic composition of any one of claims 29-55 further comprising less than about 60% free GAS polysaccharide, or a variant thereof.

Claim 57. The immunogenic composition of any one of claims 29-56 further comprising less than about 50% free GAS polysaccharide, or a variant thereof.

Claim 58. The immunogenic composition of any one of claims 29-57 further comprising less than about 25% free GAS polysaccharide, or a variant thereof.

Claim 59. The immunogenic composition of any one of claims 29-58 further comprising less than about 15% free GAS polysaccharide, or a variant thereof.

Claim 60. The immunogenic composition of any one of claims 29-59 further comprising less than about 10% free GAS polysaccharide, or a variant thereof.

Claim 61. A method of inducing a protective immune response against a Group A Streptococcus (GAS) bacterium in a subject comprising administering the immunogenic composition of any one of claims 29-60 to the subject.

Claim 62. The method of claim 61, wherein the immunogenic composition induces an antibody response in the subject against the Group A Streptococcus (GAS) bacterium and does not induce an antibody response in the subject against human tissue.

Claim 63. The use of the immunogenic composition of any one of claims 29-60 in the manufacture of a medicament for inducing a protective immune response against a GAS bacterium in a subject.

Claim 64. Use of the immunogenic composition of any one of claims 29-60 for inducing a protective immune response against a GAS bacterium in a subject.

Claim 65. A process for purifying cell wall polysaccharides or peptidoglycan-bound capsular polysaccharides from a bacterial cell, the process comprising:

(a) hydrolyzing the bacterial cell in a solution comprising base and a reducing agent to form a lysate comprising polysaccharide; and

(b) incubating the lysate comprising polysaccharide with a muralytic enzyme to form a free polysaccharide solution.

Claim 66. The process according to claim 65, wherein the bacterial cell is a *Pseudomonas* bacterial cell, a *Streptococcus* bacterial cell, a *Staphylococcus* bacterial cell, a *Neisseria* bacterial cell, a *Haemophilus* bacterial cell, a *Listeria* bacterial cell,a *Enterococcus* bacterial cell, or a *Clostridium* bacterial cell.

Claim 67. The process according to claims 65 or 66, wherein the bacterial cell is selected from of *Pseudomonas aeruginosa*, *Streptococcus viridans*, *Streptococcus mutans*, and *Streptococcus pyogenes*.

Claim 68. The process according to claim 67, wherein the *Streptococcus pyogenes* bacterial cell is of a serotype selected from M1, M2, M3, M4, M5, M6, M9, M11, M12, M13, M18, M22, M25, M28, M62, M71, M72, M74, M75, M77, M80, M81, M83, M87, M89, or M92.

Claim 69. The process according to claim 68, wherein the *Streptococcus pyogenes* bacterial cell produces a polysaccharide or a variant thereof that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

Claim 70. The process according to claim 65, for purifying a peptidoglycan-bound capsular polysaccharide from a bacterial cell.

Claim 71. The process according to any one of claims 65-70, wherein the base of step (a) is NaOH, KOH, or LiOH.

Claim 72. The process according to any one of claims 65-71, wherein the base of step (a) is NaOH.

Claim 73. The process according to any one of claims 65-72, wherein the concentration of base is between about 2M to about 8M.

Claim 74. The process according to any one of claims 65-73, wherein the solution comprising base and a reducing agent is about pH 14.

Claim 75. The process according to any one of claims 65-72, wherein the reducing agent is sodium borohydride, sodium cyanoborohydride, sodium triacetoxyborohydride, dithiothreitol, or beta-mercaptoethanol.

Claim 76. The process according to any one of claims 65-73, wherein the reducing agent is sodium borohydride.

Claim 77. The process according to any one of claims 65-76, wherein the concentration of the reducing agent is between about 1 mM and 500 mM.

Claim 78. The process according to any one of claims 65-75, wherein step (a) further comprises incubating the solution between about 30 °C and about 100 °C.

Claim 79. The process according to any one of claims 65-78, wherein step (a) further comprises incubating the solution for between about 0.5 to about 20 hours.

Claim 80. The process according to any one of claims 65-79, wherein step (a) further comprises one or more pH adjustment steps.

Claim 81. The process according to claim 80, wherein the one or more pH adjustment steps are independently selected from:

(i) raising the lysate comprising polysaccharide pH, or

(ii) lowering the lysate comprising polysaccharide pH.

Claim 82. The process according to claim 81, comprising lowering the lysate comprising polysaccharide pH to between about 3 and 7.0.

Claim 83. The process according to claims 81 or 82, comprising lowering the lysate comprising polysaccharide pH to about 6.5.

Claim 84. The process according to claims 81 or 82, comprising lowering the lysate comprising polysaccharide pH to between about 3 and about 4.

Claim 85. The process according to claims 80 or 81, comprising

(i) lowering the lysate comprising polysaccharide pH to between about 5.5 and 7.0;

(ii) lowering the lysate comprising polysaccharide pH to about 3; and

(iii) raising the lysate comprising polysaccharide pH to between about 5.5 and 7.0.

Claim 86. The process according to any one of claims 80-85, wherein the lysate comprising polysaccharide is incubated at about room temperature (r.t.) after the one or more pH adjustment steps.

Claim 87. The process according to any one of claims 81-84, wherein the lysate comprising polysaccharide is incubated at between about 4 °C and about 30 °C after the one or more pH adjustment steps.

Claim 88. The process according to any one of claims 65-87, further comprising removing solids from the lysate comprising polysaccharide.

Claim 89. The process according to claim 88, wherein removing solids from the lysate comprising polysaccharide comprises filtration, centrifugation, or a combination thereof.

Claim 90. The process according to claim 89, wherein the filtration comprises depth filtration, tangential flow filtration (TFF), sterile filtration, or a combination of the foregoing.

Claim 91. The process according to claims 89 or 90, wherein the filtration comprises depth filtration followed by TFF.

Claim 92. The process according to claims 88 or 89, wherein solids are removed from the lysate comprising polysaccharide by centrifugation.

Claim 93. The process according to any one of claims 65-92, wherein the muralytic enzyme of step (b) is mutanolysin, lysozyme, or a bacteriophage hydrolase.

Claim 94. The process according to any one of claims 65-93, wherein step (b) further comprises incubating with a protease.

Claim 95. The process according to claim 94, wherein the protease is proteinase K, trypsin, chymotrypsin, endoproteinase Asp-N, endoproteinase Arg-C, endoproteinase Glu-C, endoproteinase Lys-C, pepsin, thermolysin, elastase, papain, substilisin, clostripain, carboxypeptidase A, carboxypeptidase B, carboxypeptidase P, carboxypeptidase Y, cathepsin C, acylamino-acid releasing enzyme, or pyroglutamate.

Claim 96. The process according to any one of claims 65-95, wherein step (b) further comprises warming the lysate comprising polysaccharide with the muralytic enzyme to between about 30 °C and about 65 °C.

Claim 97. The process according to any one of claims 94-96, wherein the lysate comprising polysaccharide with the protease is warmed to between about 45°C and 55 °C.

Claim 98. The process according to claims 96 or97, wherein the lysate is warmed between about 6 and about 20 hours.

Claim 99. The process according to any one of claims 65-98, wherein the free polysaccharide solution of step (b) is further purified to reduce the concentration of nucleic acids, enzymes, host cell proteins (HCPs), or a combination of the foregoing.

Claim 100. The process according to any one of claims 65-99, wherein the free polysaccharide solution of step (b) is further purified by precipitation.

Claim 101. The process according to any one of claims 65-100, wherein the free polysaccharide solution of step (b) is treated with cetyltrimethylammonium bromide (CTAB).

Claim 102. The process according to claim 101, wherein the concentration of CTAB in the free polysaccharide solution is about 0.1% to about 10%.

Claim 103. The process according to claims 101 or 102, the concentration of CTAB is between about 0.5% and about 3%.

Claim 104. The process according to any one of claims 101-103, wherein the free polysaccharide solution of step (b) is treated with potassium iodide (KI).

Claim 105. The process according to claim 104, wherein the concentration of KI in the free polysaccharide solution is between about 20 mM to about 400 mM.

Claim 106. The process according to any one of claims 65-105, wherein the free polysaccharide solution is further purified by filtration, centrifugation, chromatography, or a combination of the foregoing.

Claim 107. The process according to claim 106, wherein the filtration comprises depth filtration, tangential flow filtration (TFF), sterile filtration, or a combination of the foregoing.

Claim 108. The process according to claim 106, wherein the chromatography comprises hydrophobic interaction chromatography (HIC), anion-exchange chromatography (AEX), ceramic hydroxyapatite-type chromatography, or cation exchange chromatography (CEX).

CLAIMS

1. A polypeptide-polysaccharide conjugate comprising:

(a) a Group A Streptococcus (GAS) polypeptide antigen or a non-GAS carrier polypeptide comprising at least one non-natural amino acid (nnAA),

linked to

(b) a conjugate polysaccharide wherein the conjugate polysaccharide is a cell wall polysaccharide or a peptidoglycan-bound capsular polysaccharide.

2. The polypeptide-polysaccharide conjugate of claim 1, wherein the cell wall polysaccharide lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

3. The polypeptide-polysaccharide conjugate of any one of claims 1 and 2, wherein the cell wall polysaccharide is a GAS polysaccharide, or a variant thereof, or a Group A carbohydrate (GAC).

4. The polypeptide-polysaccharide conjugate of claim 3, wherein about 8 mol% to about 20 mol%, about 10 mol% to about 20 mol%, or about 10 mol% to about 18 mol% of the polysaccharide repeat units of the GAS polysaccharide or the variant thereof, are derivatized by a linker.

5. The polypeptide-polysaccharide conjugate of claim 4, wherein the purified cell wall polysaccharide or the peptidoglycan-bound capsular polysaccharide has a molecular weight of at least about 10 kDa to at least about 40 kDa.

6. The polypeptide-polysaccharide conjugate of any one of claims 1-5, wherein the at least one nnAA is substituted for a lysine, a leucine, an isoleucine, or an arginine in the GAS polypeptide antigen or the non-GAS carrier polypeptide.

7. The polypeptide-polysaccharide conjugate of claim 1, wherein the at least one nnAA is pAMF, and wherein the conjugate polysaccharide is linked to a triazole group formed by a click chemistry reaction with pAMF.

8. The polypeptide-polysaccharide conjugate of any one of claims 1-7, wherein the GAS polypeptide antigen is selected from the group consisting of C5a peptidase, streptolysin O (SLO), SpyAD, Sib35, and Sfb1.

9. The polypeptide-polysaccharide conjugate of claim 8, wherein the GAS polypeptide antigen is a SpyAD polypeptide, or a fragment thereof.

10. The polypeptide-polysaccharide conjugate of claim 9, wherein the SpyAD polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 33, a fragment thereof, or a sequence at least 95% identical thereto.

11. The polypeptide-polysaccharide conjugate of claim 10, wherein the SpyAD polypeptide comprises amino acids residues 1-667 of the amino sequence set forth in SEQ ID NO: 33, or a sequence at least 95% identical thereto.

12. The polypeptide-polysaccharide conjugate of any one of claims 9-11, wherein the SpyAD polypeptide comprises a nnAA substitution at one or more positions selected from the group consisting of K64, K287, K386, and K657 with reference to SEQ ID NO: 33.

13. The polypeptide-polysaccharide conjugate of any one of claims 9-12, wherein the SpyAD polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 78, or a sequence 95% identical thereto.

14. The polypeptide-polysaccharide conjugate of any one of claims 1-13, further comprising one or more antigens selected from the group consisting of C5a peptidase and Streptolysin O; wherein the one or more antigens comprise the amino acid sequence set forth in any one or more of the sequences set forth in SEQ ID NO: 2, SEQ ID NO: 29 SEQ ID NO: 30, and SEQ ID NO: 53.

15. An immunogenic composition comprising:

(a) a Group A Streptococcus (GAS) C5a peptidase polypeptide antigen;

(b) a GAS streptolysin O (SLO) polypeptide antigen; and

(c) a polypeptide-polysaccharide conjugate comprising a Streptococcus pyogenes Adhesion and Division (SpyAD) conjugate polypeptide, or a fragment thereof, comprising at least one non-natural amino acid (nnAA), wherein the at least one nnAA comprised a click chemistry reactive group, and is linked to a GAS polysaccharide, or a variant thereof, that lacks an immunodominant N-acetyl Glucosamine (GlcNAc) side chain.

16. The immunogenic composition of claim 15, wherein the C5a peptidase polypeptide antigen comprises the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 29, SEQ ID NO: 30, or an amino acid sequence at least 95% identical thereto.

17. The immunogenic composition of any one of claims 15 and 16, wherein the SLO polypeptide antigen comprises the amino acid sequence set forth in SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, or a sequence at 95% identical thereto.

18. The immunogenic composition of any one of claims 15-17, wherein the at least one nnAA was pAMF, and wherein the conjugate polysaccharide is linked to a triazole group formed by a click chemistry reaction with pAMF.

19. The immunogenic composition of any one of claims 15-18, wherein the SpyAD conjugate polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 33, a fragment thereof, or an amino acid sequence at least 95% identical thereto.

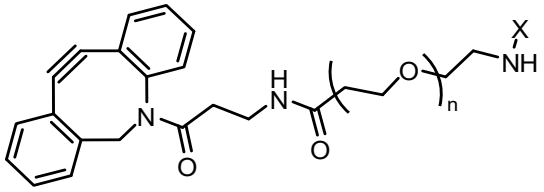
20. The immunogenic composition of any one of claims 18 and 19, wherein the SpyAD conjugate polypeptide comprises a nnAA substitution at one or more positions selected from K64, K287, K386, and K657 with reference to SEQ ID NO: 33.

21. The immunogenic composition of any one of claims 15-20, wherein the SpyAD conjugate polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 34, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, or a sequence at least 95% identical thereto.

22. The immunogenic composition of any one of claims 15-21, wherein the C5a peptidase polypeptide antigen comprises an amino acid sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 2, SEQ ID NO: 29, or SEQ ID NO: 30; the SLO polypeptide antigen comprises an amino acid sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 53; and the SpyAD conjugate polypeptide comprises an amino acid sequence that is at least 95% identical to the sequence set forth in SEQ ID NO: 78.

23. The immunogenic composition of any one of claims 15-22, wherein about 8 mol% and to about 20 mol% of the polysaccharide repeat units of the GAS polysaccharide, or a variant thereof, are derivatized by a linker.

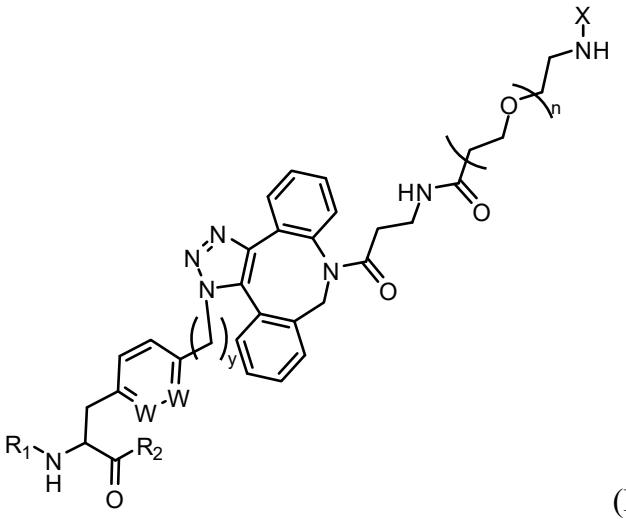
24. The immunogenic composition of claim 23, wherein the linker, prior to reaction with the click chemistry reactive group of the nnAA, comprises a structure of Formula I:



(Formula I)

wherein, X is at least one polysaccharide repeat unit of a GAS polysaccharide, or a fragment thereof; and n is at least 1.

25. The immunogenic composition of any one of claims 15-24, wherein the SpyAD conjugate polypeptide, or fragment thereof, is linked to the GAS polysaccharide according to Formula II:



(Formula II)

wherein,

R_1 is H, formyl, or at least one amino acid of the SpyAD conjugate polypeptide;

R_2 is OH or at least one amino acid of the SpyAD conjugate polypeptide;

W is CH or N;

y is at least 1;

n is at least 1; and

X is at least one polysaccharide repeat unit of a GAS polysaccharide or variant thereof.

26. The immunogenic composition of any one of claims 15-25, wherein the GAS polysaccharide, or a variant thereof, has a molecular weight of at least about 10 kDa to at least about 40 kDa.

27. The immunogenic composition of any one of claims 15-26 further comprising less than about 60% to less than about 10% free GAS polysaccharide, or a variant thereof.

28. Use of the immunogenic composition of any one of claims 15-27 in the manufacture of a medicament for the prophylactic treatment of a GAS bacterial infection.

29. A process for purifying cell wall polysaccharides or peptidoglycan-bound capsular polysaccharides from a bacterial cell, the process comprising:

(a) hydrolyzing the bacterial cell in a solution comprising base and a reducing agent to form a lysate comprising polysaccharide; and

(b) incubating the lysate comprising polysaccharide with a muralytic enzyme to form a free polysaccharide solution.