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.

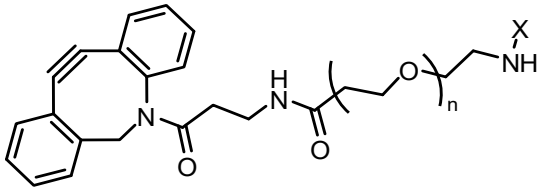
20. The immunogenic composition of any one of claims 18 and 19, wherein the SpyAD conjugate polypeptide comprises a nnAA substitution at one or more positions selected from K64, K287, K386, and K657 with reference to SEQ ID NO: 33.

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.

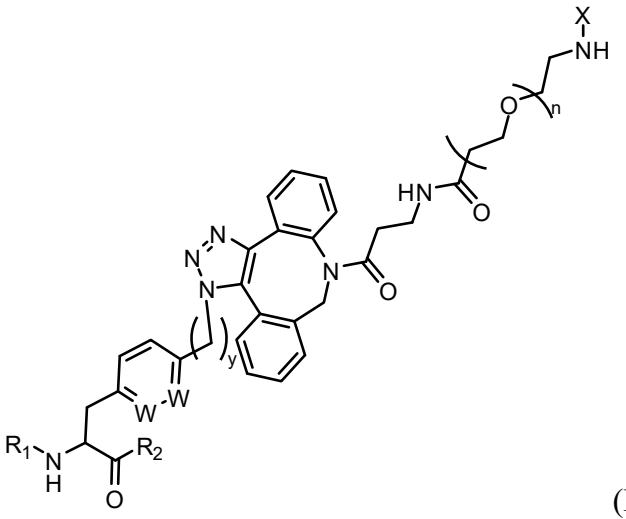
24. The immunogenic composition of claim 23, wherein the linker, prior to reaction with the click chemistry reactive group of the nnAA, comprises a structure of Formula I:



(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.