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(54) Title: ANTIBODY-MEDIATED DISRUPTION OF QUORUM SENSING IN BACTERIA

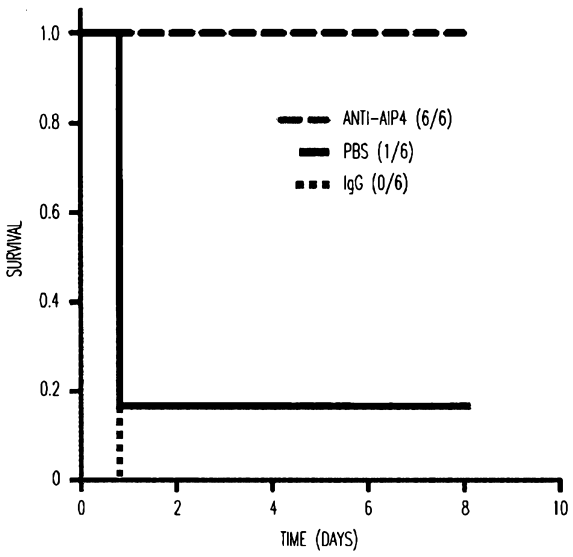

FIG. 8

(57) Abstract: The invention provides an immunogenic molecular entity, a supramolecular assembly, and an antibody that can be used to inhibit Gram-positive bacterial quorum sensing, prevent infection or development of a disease condition associated with a Gram-positive bacterial infection. The invention also provides methods of inhibiting Gram-positive bacterial quorum sensing, and methods of preventing infection or development of a disease condition associated with a Gram-positive bacterial infection.

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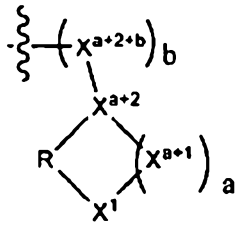
ANTIBODY-MEDIATED DISRUPTION OF QUORUM SENSING IN BACTERIA

Background of the Invention

Bacterial infections are becoming increasingly deadly as many strains that cause diseases are developing resistance to the array of antibiotics used to control them. *Staphylococcus aureus*, for example, is a common cause of hospital-acquired infections resulting in various diseases or conditions raging from skin infections and food poisoning to life-threatening nosocomial infections. Increasing resistance of *S. aureus* isolates to glycopeptide antibiotics, most prominently vancomycin, is a major concern in today's intensive care units. Therefore, an alternative strategy to combat bacterial infections is urgently needed.

Summary of the Invention

According to a first aspect of the invention, there is provided an immunogenic molecular entity comprising at least one hapten covalently linked to a macromolecular carrier, wherein the hapten comprises a cyclic peptide having a structure represented by Formula I:



wherein the cyclic peptide comprises an amino acid sequence selected from the group consisting of YST(X^{a+2})DFIM (SEQ ID: 92), YST(X^{a+2}) YFIM (SEQ ID: 93), IN(X^{a+2})DFLL (SEQ ID: 94), and GVNA(X^{a+2})SSLF (SEQ ID: 95);

X in the cyclic peptide is any amino acid residue;

X^1 is an amino acid residue that is covalently bonded to R by a carbonyl group;

X^{a+2} is an internal amino acid, a respective carbon atom of which is covalently bonded to R;

R is selected from the group consisting of -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, and -$(CH_2)_nNH$-,

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wherein

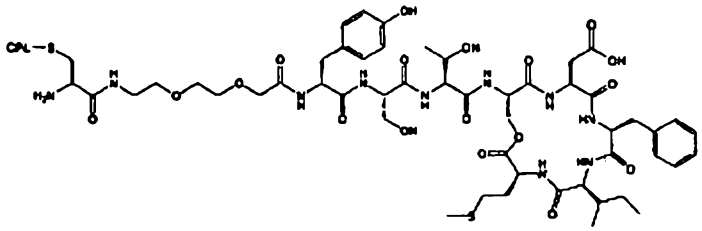
n is an integer from 1 to 4;

a is an integer from 1 to 9;

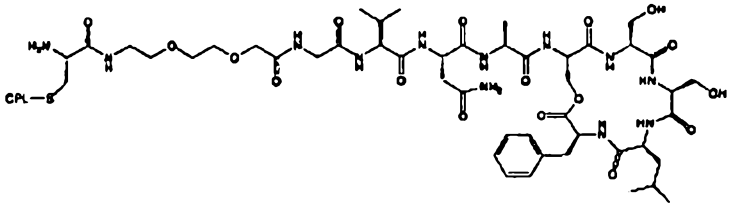
b is an integer from 1 to 8; and

a bond transected by a wavy line indicates a point of attachment of an N- terminal amino acid residue of the cyclic peptide to the macromolecular carrier.

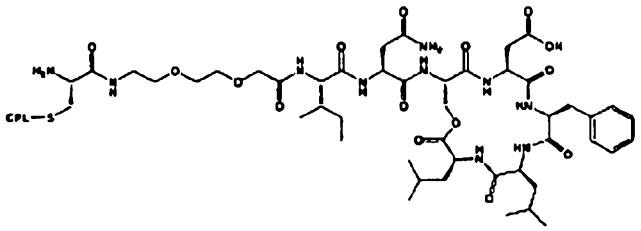
According to a second aspect of the invention, there is provided an immunogenic molecular entity having the structure:



SEQ ID NO: 3 (YSTSDFIM, not including protecting groups),

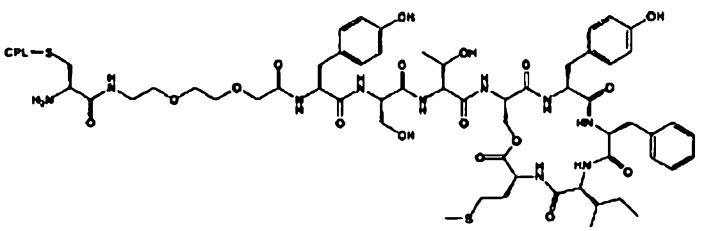


SEQ ID NO: 4 (GVNASSSLF, not including protecting groups),



SEQ ID NO: 2 (INSDFLL, not including protecting groups),

or



SEQ ID NO: 1 (YSTSYFIM, not including protecting groups), wherein CPL is a macromolecular carrier with optional linker covalently bonded to a cysteine thiol group.

According to a third aspect of the invention, there is provided an antibody that binds specifically with the immunogenic molecular entity thereof in accordance with the first or second aspect of the present invention.

According to a fourth aspect of the invention, there is provided a pharmaceutical composition comprising at least one antibody in accordance with the third aspect of the present invention, and a pharmaceutically-acceptable carrier.

According to a fifth aspect of the invention, there is provided a vaccine composition comprising at least one immunogenic molecular entity in accordance with the first or second aspect of the present invention and a pharmaceutically-acceptable carrier.

According to a sixth aspect of the invention, there is provided a method of eliciting an immune response in a mammal comprising administering to the mammal a composition comprising the immunogenic molecular entity in accordance with the first or second aspect of the present invention, in an amount effective to elicit an immune response in the mammal;

wherein the mammal is susceptible to infection by *Staphylococcus aureus*, or

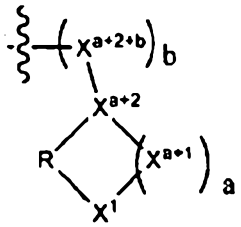
wherein the mammal is susceptible to a disease condition associated with *Staphylococcus aureus*.

According to a seventh aspect of the invention, there is provided a method of inhibiting quorum sensing in a mammal comprising administering to the mammal a composition comprising at least one antibody in accordance with the third aspect of the present invention in an amount effective to inhibit quorum sensing in the mammal.

According to an eighth aspect of the invention, there is provided a method of inhibiting quorum sensing in a mammal comprising administering to the mammal the immunogenic molecular entity in accordance with the first or second aspect of the present invention, in an amount effective to elicit an immune response and inhibit the quorum sensing in the mammal.

According to a ninth aspect of the invention, there is provided a method for preventing or treating infection of a mammal by *Staphylococcus aureus* comprising administering to the mammal, the immunogenic molecular entity in accordance with the first or second aspect of the present invention, or at least one antibody in accordance with the third aspect of the present invention in an amount effective to prevent or treat infection of the mammal by *Staphylococcus aureus*.

According to a tenth aspect of the invention, there is provided an immunogenic molecular entity comprising at least one hapten covalently linked to a macromolecular carrier, wherein the hapten comprises a cyclic peptide having a structure represented by Formula I:



wherein the cyclic peptide comprises an amino acid sequence selected from the group consisting of YST(X^{a+2})DFIM (SEQ ID: 92), YST(X^{a+2}) YFIM (SEQ ID: 93), IN(X^{a+2})DFLL (SEQ ID: 94), and GVNA(X^{a+2})SSLF (SEQ ID: 95);

X in the cyclic peptide is any amino acid residue;

X^1 is an amino acid residue that is covalently bonded to R by a carbonyl group;

X^{a+2} is an internal amino acid, a respective carbon atom of which is covalently bonded to R;

R is selected from the group consisting of -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, and -$(CH_2)_nNH$-,

wherein

n is an integer from 1 to 4;

a is an integer from 1 to 9;

b is an integer from 1 to 8; and

a bond transected by a wavy line indicates a point of attachment of an N- terminal amino acid residue of the cyclic peptide or analog thereof to the macromolecular carrier, for use as a vaccine.

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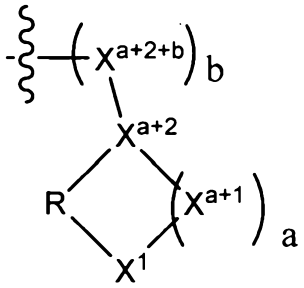
The invention relates to the discovery of an immunopharmacotherapeutic approach for the attenuation of quorum sensing. In particular, the invention involves the discovery of a monoclonal antibody elicited against a rationally-designed hapten that can inhibit quorum sensing, suppress bacterial pathogenicity in an abscess formation mouse model *in vivo*, and provide protection against a lethal bacterial challenge.

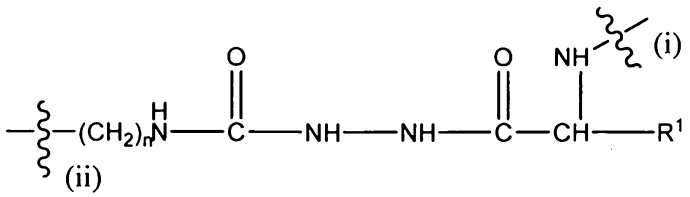
In one embodiment, the invention provides an immunogenic molecular entity comprising at least one hapten, the hapten being covalently linked to an macromolecular carrier, optionally via a linker moiety, the hapten comprising a cyclic peptide or an analog thereof, the cyclic peptide or analog thereof comprising a macrocyclic ring, wherein the cyclic peptide or analog thereof comprises about four to about nineteen amino acid residues, the cyclic peptide or analog thereof having a structure represented by Formula I:



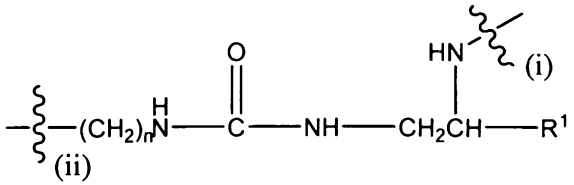
wherein each X is independently any amino acid residue; X^1 is an amino acid residue that is covalently bonded to R by a respective carbonyl group; X^{a+2} is an internal amino acid, a respective carbon atom of which is covalently bonded to R; R is a macrocyclizing moiety that covalently connects X^1 and X^{a+2} thereby forming the macrocyclic ring, wherein R comprises an ester, thioester, amide, carbamide, semicarbazide, or other amide-surrogate group, or any combination thereof; a is 1 to about 9; b is 1 to about 8; and a bond transected by a wavy line indicates a point of attachment of an N-terminal amino acid residue of the cyclic peptide or analog thereof to the macromolecular carrier, optionally via the linker moiety.

In some embodiments, the immunogenic molecular entity has the structure shown above, wherein a is 2-8, and R includes an *alkyloxy* or *alkaryloxy*, *alkylthio*, or *alkylamino* group covalently bonding X^{a+2} to the X^1 carbonyl group, thereby providing an ester, thioester, or amide bond, respectively, to form a lactone, thiolactone, or lactam macrocyclic ring, respectively. In some embodiments, the immunogenic molecular entity has the structure shown above, wherein R includes $-CH_2O-$, $-CH_2CH_2O-$, $-CH_2CH(CH_3)O-$, $-CH_2$-phenyl-O-, $-CH_2S-$, $-CH_2CH_2S-$, or $-(CH_2)_nNH-$, wherein n is 1 to about 4. In some embodiments, the immunogenic molecular entity has the structure shown above, wherein a is 2-8, and R includes at least one amide, urea, or semicarbazide group, or at least one amide-surrogate bond.

In some embodiments, R is represented by Formula (IIa) or Formula (IIb):



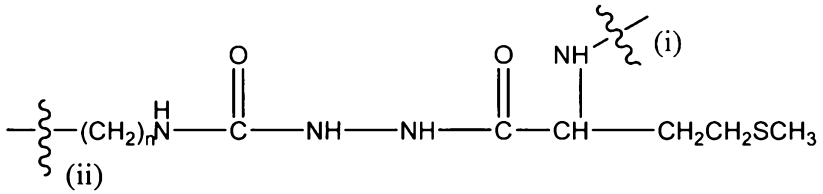
Formula (IIa),



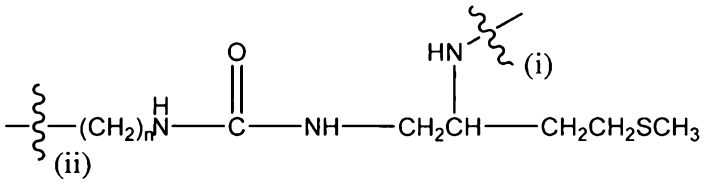
Formula (IIb),

wherein n is 1 to about 4, R^1 is the sidechain of a naturally occurring amino acid or an analog thereof, a bond transected by a wavy line indicates a point of attachment, wherein the point of attachment designated (i) is bonded to the carbonyl group of X^1 and the point of attachment designated (ii) is bonded to the alpha-carbon of X^{a+2}.

In some embodiments, R has the formula (IIa):



In some embodiments, R has the formula (IIb):



In some embodiments, the immunogenic molecular entity has the structure shown above, wherein X^1 and X^2 are hydrophobic amino acid residues, and in some embodiments, X^1 and X^2 are independently selected from the group of amino acid residues consisting of alanine, valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, or tryptophan, or analogs thereof. In some embodiments each of X^1 and X^2 is independently methionine, leucine, phenylalanine, tyrosine, alanine, isoleucine, or tryptophan.

In some embodiments, the cyclic peptide or analog of the immunogenic molecular entity has the amino acid sequence YST(X^{a+2})DFIM (SEQ ID NO: 92), YST(X^{a+2})YFIM (SEQ ID NO: 93), IN(X^{a+2})DFLL (SEQ ID NO: 94), GVNA(X^{a+2})SSLF (SEQ ID NO: 95), GVNP(X^{a+2})GGWF (SEQ ID NO: 96), KAKT(X^{a+2})TVLY (SEQ ID NO: 97), KTKT(X^{a+2})TVLY (SEQ ID NO: 98),

GANP(X^{a+2})OLYY (SEQ ID NO: 99), GANP(X^{a+2})ALYY (SEQ ID NO: 100), GYST(X^{a+2})SYYF (SEQ ID NO: 101), GYRT(X^{a+2})NTYF (SEQ ID NO: 102), YNP(X^{a+2})VGYF (SEQ ID NO: 103), GGKV(X^{a+2})SAYF (SEQ ID NO: 104), SVKP(X^{a+2})TGFA (SEQ ID NO: 105), DSV(X^{a+2})ASYF (SEQ ID NO: 106), KYNP(X^{a+2})SNYL (SEQ ID NO: 107), KYNP(X^{a+2})ASYL (SEQ ID NO: 108), KYNP(X^{a+2})ANYL (SEQ ID NO: 109), RIPT(X^{a+2})TGFF (SEQ ID NO: 110), DI(X^{a+2})NAYF (SEQ ID NO: 111), DM(X^{a+2})NGYF (SEQ ID NO: 112), KYNP(X^{a+2})LGFL (SEQ ID NO: 113), KYYP(X^{a+2})FGYF (SEQ ID NO: 114), GARP(X^{a+2})GGFF (SEQ ID NO: 115), GAKP(X^{a+2})GGFF (SEQ ID NO: 116), YSP(X^{a+2})TNFF (SEQ ID NO: 117), YSP(X^{a+2})TNF (SEQ ID NO: 118), or QN(X^{a+2})PNIFGQWM (SEQ ID NO: 119), wherein the last amino acid residue of each sequence is X^1, and (X^{a+2}) is the internal amino acid to which the carbonyl group of X^1 is covalently bonded via R.

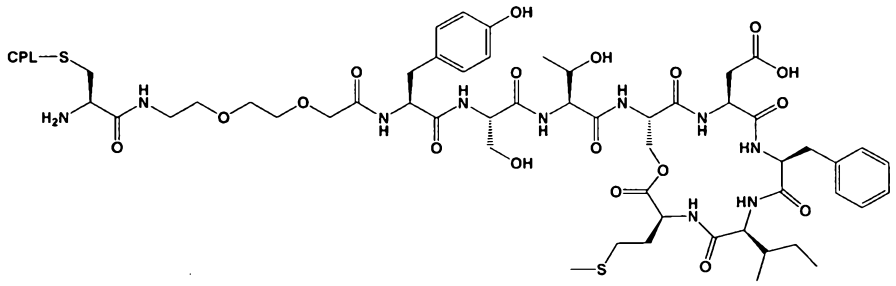
In some embodiments, the macromolecular carrier includes a protein, a polymer or a nanoparticle. In some embodiments, the polymer is a dendrimer. In some embodiments, the dendrimer is a MAP dendrimer. In some embodiments, the macromolecular carrier comprises a protein. In some embodiments, the protein is selected from the group consisting of keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), rabbit serum albumin (RSA), human serum albumin (HSA), *Concholepas concholepas* hemocyanin (CCH), cholera toxin B subunit, *E. coli* labile toxin B subunit, Diphtheria toxoid, tetanus toxoid, tetanus toxin C-fragment, recombinant *Pseudomonas aeruginosa* exoprotein A, CRM197 (cross-reactive material), cationized bovine serum albumin (cBSA), Thyroglobulin (Tg), avidin, bovine thyroglobulin (BTG), bovine G globulin, bovine immunoglobulin G (BIgG), conalbumin (CONA), colloidal gold, edestin, *Paralithodes camtschatica* heamocyanin (HC), helix promatia haemocyanin (HPH), soybean kunitz trypsin inhibitor (KTI), *Limulus polyphemus* heamocyanin (LPH), ovalbumin (OA), Pam3Cys-Th (lipopeptide/Th cell epitope), polylysine, porcine thyroglobulin (PTG), purified protein derivative (PPD), soybean trypsin inhibitor (STI), or sunflower globulin (SFG).

In some embodiments, the cyclic peptide analog is covalently linked to the macromolecular carrier via an amino group of an N-terminal amino acid

residue of the cyclic peptide analog or a thiol group of an N-terminal cysteine or homocysteine residue of the cyclic peptide analog.

In some embodiments, the molecular entity of the invention further includes a linker moiety that covalently links the cyclic peptide analog to the macromolecular carrier. In some embodiments, the cyclic peptide analog is bonded to the linker moiety via an amino group of an N-terminal amino acid residue of the cyclic peptide analog, or via a thiol group of an N-terminal cysteine or homocysteine residue of the cyclic peptide analog, the linker moiety being covalently bonded to the macromolecular carrier. In some embodiments, the linker moiety includes a moiety produced by reaction of MBS, sulfo-MBS, SMCC, or sulpho-SMCC. In some embodiments, the linker moiety includes adipic acid dihydrazide (ADH), a spacer peptide, hydroxymethyl hemisuccinate, or a polyethyleneglycol derivative.

In some embodiments, the molecular entity has the structure:



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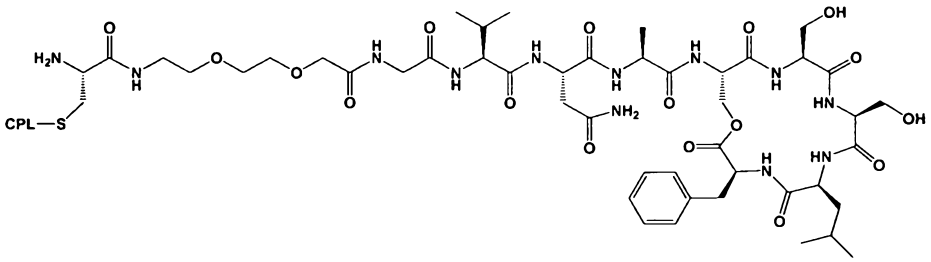

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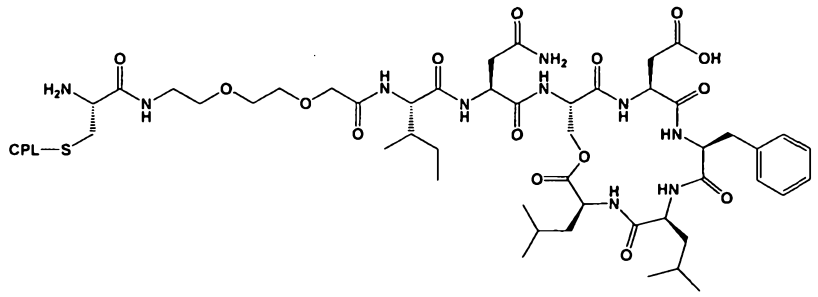
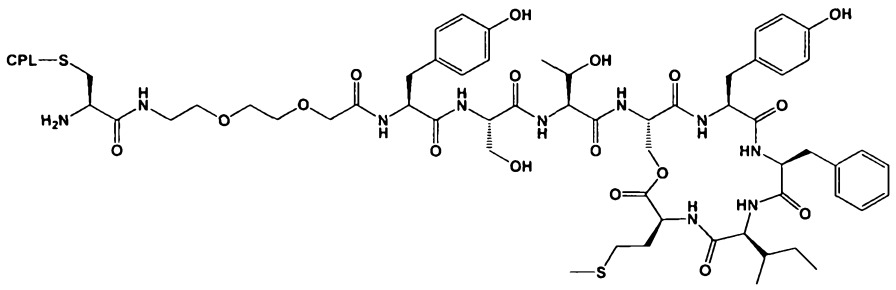


SEQ ID NO: 2 (INSDFLL, not including protecting groups),

or



SEQ ID NO: 1 (YSTSYFLM, not including protecting groups),

wherein CPL is a macromolecular carrier with optional linker covalently bonded to a cysteine thiol group.

In another aspect, the invention provides a supramolecular assembly that includes an immunogenic molecular entity of the invention. In some embodiments, the supramolecular assembly includes a liposome, a virosome, a bacteriophage, a viral particle, or a polymeric nanoparticle delivery system.

In another aspect, the invention provides an antibody that binds specifically with a cyclic peptide having the amino acid sequence YST(X^{a+2})DFIM (SEQ ID NO: 92), YST(X^{a+2})YFIM (SEQ ID NO: 93), IN(X^{a+2})DFLL (SEQ ID NO: 94), GVNA(X^{a+2})SSLF (SEQ ID NO: 95), GVNP(X^{a+2})GGWF (SEQ ID NO: 96), KAKT(X^{a+2})TVLY (SEQ ID NO: 97), KTKT(X^{a+2})TVLY (SEQ ID NO: 98), GANP(X^{a+2})OLYY (SEQ ID NO: 99), GANP(X^{a+2})ALYY (SEQ ID NO: 100), GYST(X^{a+2})SYYF (SEQ ID NO: 101), GYRT(X^{a+2})NTYF (SEQ ID NO: 102), YNP(X^{a+2})VGYF (SEQ ID NO: 103), GGKV(X^{a+2})SAYF (SEQ ID NO: 104), SVKP(X^{a+2})TGFA (SEQ ID NO: 105), DSV(X^{a+2})ASYF (SEQ ID NO: 106), KYNP(X^{a+2})SNYL (SEQ ID NO: 107), KYNP(X^{a+2})ASYL (SEQ ID NO: 108), KYNP(X^{a+2})ANYL (SEQ ID NO: 109), RIPT(X^{a+2})TGFF (SEQ ID NO: 110), DI(X^{a+2})NAYF (SEQ ID NO: 111),

DM(X^{a+2})NGYF (SEQ ID NO: 112), KYNP(X^{a+2})LGFL (SEQ ID NO: 113), KYYP(X^{a+2})FGYF (SEQ ID NO: 114), GARP(X^{a+2})GGFF (SEQ ID NO: 115), GAKP(X^{a+2})GGFF (SEQ ID NO: 116), YSP(X^{a+2})TNFF (SEQ ID NO: 117), YSP(X^{a+2})TNF (SEQ ID NO: 118), or QN(X^{a+2})PNIFGQWM (SEQ ID NO: 119); wherein the last amino acid residue of each sequence is X^1, and (X^{a+2}) is the internal amino acid to which the carbonyl group of X^1 is covalently bonded via R, wherein R is the sidechain moiety of X^{a+2} covalently bonded to the carbonyl group of X^1; and wherein R comprises -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, -CH_2S-, -CH_2CH_2S-, or -$(CH_2)_nNH$-, wherein n is 1 to about 4.

In another aspect, the invention provides an antibody that binds specifically with a cyclic peptide signaling molecule of a Gram-positive bacterium.

In some embodiments, the antibody binds specifically with a cyclic peptide signaling molecule having the sequence YST**C**DFIM (SEQ ID NO: 120); GVNA**C**SSLF (SEQ ID NO: 121); IN**C**DFLL (SEQ ID NO: 122); YST**C**YFIM (SEQ ID NO: 123); GVNP**C**GGWF (SEQ ID NO: 124); KAKT**C**TVLY (SEQ ID NO: 125); KTKT**C**TVLY (SEQ ID NO: 126); GANP**C**OLYY (SEQ ID NO: 127); GANP**C**ALYY (SEQ ID NO: 128); GYST**C**SYYF (SEQ ID NO: 129); GYRT**C**NTYF (SEQ ID NO: 130); YNPC**V**GYF (SEQ ID NO: 131); GGKV**C**SAYF (SEQ ID NO: 132); SVKP**C**TGFA (SEQ ID NO: 133); DSV**C**ASYF (SEQ ID NO: 134); KYNP**C**SNYL (SEQ ID NO: 135); KYNP**C**ASYL (SEQ ID NO: 136); KYNP**C**ANYL (SEQ ID NO: 137); RIPT**S**TGFF (SEQ ID NO: 138); DI**C**NAYF (SEQ ID NO: 139); DM**C**NGYF (SEQ ID NO: 140); KYNP**C**LGFL (SEQ ID NO: 141); KYYP**C**FGYF (SEQ ID NO: 142); VGARP**C**GGFF (SEQ ID NO: 143); GAKP**C**GGFF (SEQ ID NO: 144); YSP**C**TNFF (SEQ ID NO: 145); or QN**S**PNIFGQWM (SEQ ID NO: 146); wherein the alpha-carbonyl group of the underlined residue forms a thiolactone or lactone bond with the sulfhydryl or hydroxyl group of the bolded internal cysteine or serine residue, respectively.

In some embodiments, the antibody is a neutralizing antibody, e.g. a cross-neutralizing antibody. In some embodiments, it is a single chain variable fragment (scFv), a Fab or F(ab')$_2$ fragment. In some embodiments, the antibody

comprises the amino acid sequence of any one of SEQ ID NOs: 35-53. In some embodiments, the antibody is a monoclonal antibody. In some embodiment, the antibody comprises the amino acid sequence of any one of SEQ ID NOs: 19-26 and 147-154. In some embodiments, the antibody comprises an amino acid sequence of any one of SEQ ID NOs: 19-26 in covalent interaction with an amino acid sequence of any one of SEQ ID NOs: 147-154. In some embodiments, the antibody is a murine, bovine, or human antibody. In some embodiments, the antibody is a humanized or chimeric antibody. In some embodiments, the antibody is AP4-24H11.

In another aspect, the invention provides a composition that includes at least one antibody of the invention and a pharmaceutically-acceptable carrier. In some embodiments, the composition includes two to four antibodies that bind specifically with two to four cyclic peptide signaling molecules having the sequences YST**C**DFIM (SEQ ID NO: 120), GVNA**C**SSLF (SEQ ID NO: 121), IN**C**DFLL (SEQ ID NO: 122), and YST**C**YFIM (SEQ ID NO: 123); wherein the alpha-carbonyl group of the underlined residue forms a thiolactone bond with the sulfhydryl group of the bolded internal cysteine residue.

In another aspect, the invention provides a composition includes at least one immunogenic molecular entity of the invention and a pharmaceutically-acceptable carrier. In some embodiments, the immunogenic molecular entity includes a cyclic peptide having the sequence YST(X^{a+2})DFIM (SEQ ID NO: 92), YST(X^{a+2})YFIM (SEQ ID NO: 93), IN(X^{a+2})DFLL (SEQ ID NO: 94), GVNA(X^{a+2})SSLF (SEQ ID NO: 95), GVNP(X^{a+2})GGWF (SEQ ID NO: 96), KAKT(X^{a+2})TVLY (SEQ ID NO: 97), KTKT(X^{a+2})TVLY (SEQ ID NO: 98), GANP(X^{a+2})OLYY (SEQ ID NO: 99), GANP(X^{a+2})ALYY (SEQ ID NO: 100), GYST(X^{a+2})SYYF (SEQ ID NO: 101), GYRT(X^{a+2})NTYF (SEQ ID NO: 102), YNP(X^{a+2})VGYF (SEQ ID NO: 103), GGKV(X^{a+2})SAYF (SEQ ID NO: 104), SVKP(X^{a+2})TGFA (SEQ ID NO: 105), DSV(X^{a+2})ASYF (SEQ ID NO: 106), KYNP(X^{a+2})SNYL (SEQ ID NO: 107), KYNP(X^{a+2})ASYL (SEQ ID NO: 108), KYNP(X^{a+2})ANYL (SEQ ID NO: 109), RIPT(X^{a+2})TGFF (SEQ ID NO: 110), DI(X^{a+2})NAYF (SEQ ID NO: 111), DM(X^{a+2})NGYF (SEQ ID NO: 112), KYNP(X^{a+2})LGFL (SEQ ID NO: 113), KYYP(X^{a+2})FGYF (SEQ ID NO: 114), GARP(X^{a+2})GGFF (SEQ ID NO: 115), GAKP(X^{a+2})GGFF (SEQ ID NO: 116), YSP(X^{a+2})TNFF (SEQ ID NO: 117), YSP(X^{a+2})TNF (SEQ ID NO: 118), or

QN(X^{a+2})PNIFGQWM (SEQ ID NO: 119); wherein the last amino acid residue of each sequence is X^1, and (X^{a+2}) is the internal amino acid to which the carbonyl group of X^1 is covalently bonded via R; and wherein R comprises -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, -CH_2S-, -CH_2CH_2S-, or -$(CH_2)_nNH$-, wherein n is 1 to about 4.

In some embodiments, the composition includes two to four immunogenic molecular entities, the cyclic peptides of which have the sequence YST(X^{a+2})DFIM (SEQ ID NO: 92), YST(X^{a+2})YFIM (SEQ ID NO: 93), IN(X^{a+2})DFLL (SEQ ID NO: 94), GVNA(X^{a+2})SSLF (SEQ ID NO: 95), GVNP(X^{a+2})GGWF (SEQ ID NO: 96), KAKT(X^{a+2})TVLY (SEQ ID NO: 97), KTKT(X^{a+2})TVLY (SEQ ID NO: 98), GANP(X^{a+2})OLYY (SEQ ID NO: 99), GANP(X^{a+2})ALYY (SEQ ID NO: 100), GYST(X^{a+2})SYYF (SEQ ID NO: 101), GYRT(X^{a+2})NTYF (SEQ ID NO: 102), YNP(X^{a+2})VGYF (SEQ ID NO: 103), GGKV(X^{a+2})SAYF (SEQ ID NO: 104), SVKP(X^{a+2})TGFA (SEQ ID NO: 105), DSV(X^{a+2})ASYF (SEQ ID NO: 106), KYNP(X^{a+2})SNYL (SEQ ID NO: 107), KYNP(X^{a+2})ASYL (SEQ ID NO: 108), KYNP(X^{a+2})ANYL (SEQ ID NO: 109), RIPT(X^{a+2})TGFF (SEQ ID NO: 110), DI(X^{a+2})NAYF (SEQ ID NO: 111), DM(X^{a+2})NGYF (SEQ ID NO: 112), KYNP(X^{a+2})LGFL (SEQ ID NO: 113), KYYP(X^{a+2})FGYF (SEQ ID NO: 114), GARP(X^{a+2})GGFF (SEQ ID NO: 115), GAKP(X^{a+2})GGFF (SEQ ID NO: 116), YSP(X^{a+2})TNFF (SEQ ID NO: 117), YSP(X^{a+2})TNF (SEQ ID NO: 118), or QN(X^{a+2})PNIFGQWM (SEQ ID NO: 119); wherein the last amino acid residue of each sequence is X^1, and (X^{a+2}) is the internal amino acid to which the carbonyl group of X^1 is covalently bonded via R; and wherein R comprises -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, -CH_2S-, -CH_2CH_2S-, or -$(CH_2)_nNH$-, wherein n is 1 to about 4.

In some embodiments, the composition includes four immunogenic molecular entities, the cyclic peptides of which have the sequences YST**C**DFI<u>M</u> (SEQ ID NO: 120), GVNA**C**SSL<u>F</u> (SEQ ID NO: 121), IN**C**DFL<u>L</u> (SEQ ID NO: 122), and YST**C**YFI<u>M</u> (SEQ ID NO: 123); wherein the alpha-carbonyl group of the underlined residue forms a thiolactone bond with the sulfhydryl group of the bolded internal cysteine residue.

In some embodiments, the composition includes at least one additional immunogen. In some embodiments, the at least one additional immunogen elicits an immune response against hepatitis B, *Haemophilus influenzae* type b

bacteria, diphtheria, measles, mumps, pertussis, polio, rubella, tetanus, tuberculosis, varicella, or any combination thereof.

In another aspect, the invention provides an article of manufacture comprising the immunogenic molecular entity, supramolecular assembly, antibody or composition of the invention, and instructions for its use.

In another aspect, the invention provides a method of eliciting an immune response in a mammal that involves administering to the mammal a composition that includes the immunogenic molecular entity or the supramolecular assembly of the invention in an amount effective to elicit an immune response in the mammal. In some embodiments, the mammal is a goat, rabbit, sheep, pig, mouse, rat, guinea pig, hamster, cow, horse, monkey or human. In some embodiments, the composition is administered to the mammal by intravenous, intraperitoneal, subcutaneous, intradermal, or intramuscular injection. In some embodiments, the method further involves obtaining a biological sample from the mammal, wherein the biological sample comprises an antibody that binds specifically with a cyclic peptide signaling molecule and/or with the cyclic peptide of the immunogenic molecular entity. In some embodiments, the method further involves isolating an antibody-producing cell from the mammal, and fusing the antibody-producing cell with a myeloma cell to generate a hybridoma that produces an antibody that binds specifically with a cyclic peptide signaling molecule and/or with the cyclic peptide of the immunogenic molecular entity.

In some embodiments, the mammal is susceptible to infection by a Gram positive bacterium or is susceptible to a disease condition associated with a Gram positive bacterium. In some embodiments, theGram positive bacterium is a *Staphylococcus,* such as *S. aureus* or *S. epidermidis*. In some embodiments, the mammal is a human.

In some embodiments, the method further includes administering to the mammal at least one additional dose of the composition that include the immunogenic entity at selected time periods.

In another aspect, the invention provides a method of inhibiting quorum sensing in a mammal that involves administering to the mammal a composition that includes the antibody of the invention in an amount effective to inhibit the quorum sensing in the mammal.

In another aspect, the invention provides a method of inhibiting quorum sensing in a mammal that involves administering to the mammal an immunogenic molecular entity or the supramolecular assembly of the invention in an amount effective to elicit an immune response and inhibit the quorum sensing in the mammal. In some embodiments of the invention, the mammal is a human.

In another aspect, the invention provides a method for preventing or treating infection of a mammal by a Gram positive bacterium that involves administering to the mammal, an immunogenic molecular entity, supramolecular assembly, or the antibody of the invention in an amount effective to prevent or treat infection of the mammal by a Gram positive bacterium. In some embodiments, the mammal is a human. In some embodiments, the immunogenic molecular entity, supramolecular assembly or antibody is administered to the mammal by intravenous, intraperitoneal, subcutaneous, intradermal, or intramuscular injection.

In another aspect, the invention provides a method of identifying an antibody that binds specifically with a cyclic peptide signaling molecule that involves contacting an immunogenic molecular entity that includes a cyclic peptide analog of the signaling molecule covalently linked to a macromolecular carrier with a recombinant combinatorial immunoglobulin library, and identifying the recombinant immunoglobulin that binds specifically with the an immunogenic molecular entity as an antibody that binds specifically with the cyclic peptide signaling molecule.

In another aspect, the invention provides a method of preventing biofilm formation that involves coating a surface including a surface of a catheter with an antibody of the invention.

In another aspect, the invention provides an isolated nucleic acid having a sequence that encodes the antibody discussed herein. In some embodiments, the nucleic acid has the sequence of any one of SEQ ID NO: 54-91, 27-34 and 155-181. The term "nucleic acid," as used herein, refers to a polymer of deoxynucleic ribose nucleic acids (DNA), as well as ribose nucleic acids (RNA). The term includes linear molecules, as well as covalently closed circular molecules. It includes single stranded molecules, as well as double stranded molecules.

The term "isolated," as used herein with reference to a nucleic acid molecule, means that the nucleic acid molecule is free of unrelated nucleic acid sequences, or those involved in the expression of such other genes, that flank it's 5' and 3' ends in the naturally-occurring genome of the organism from which the nucleic acid of the invention is derived. Accordingly, an "isolated nucleic acid" of the invention has a structure that is different from that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. Thus, the term "isolated nucleic acid molecule" includes, for example, (1) a DNA molecule that has the sequence of part of a naturally occurring genomic DNA molecule, but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (2) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally-occurring vector or genomic DNA; (3) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (4) a recombinant nucleotide sequence that is part of a hybrid gene, i.e. a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of (1) DNA molecules, (2) transfected cells, and (3) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

In another aspect, the invention provides an expression vector that has a nucleic acid encoding the antibody discussed herein.

In some embodiments, the nucleic acid encoding the antibody is operably-linked to an expression control sequence. In some embodiments, the expression control sequence is a promoter. In some embodiments, the promoter is a phage, viral, bacterial or mammalian promoter.

The term "expression vector," as used herein, means a nucleic acid molecule capable of transporting and/or allowing for the expression of another nucleic acid to which it has been linked. The product of that expression is referred to as a messenger ribose nucleic acid (mRNA) transcript. Thus, expression vectors contain appropriate expression control sequences that may direct expression of a nucleic acid that is operably linked to the expression control sequence to produce a transcript. Thus, the phrase "expression control

sequence" means a nucleic acid sequence sufficient to direct transcription of another nucleic acid sequence that is operably linked to the expression control sequence to produce an RNA transcript when appropriate molecules such as transcriptional activator proteins are bound the expression control sequence. And the term "operably linked" means that a nucleic acid and an expression control sequence is positioned in such a way that the expression control sequence directs expression of the nucleic acid when the appropriate molecules such as transcriptional activator proteins are bound to the expression control sequence.

In another aspect, the invention provides a cell that has a nucleic acid encoding the antibody discussed above or an expression vector discussed above. The cell can be a bacterial or mammalian cell.

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

DESCRIPTION OF THE FIGURES

FIG. 1 illustrates the structures of the autoinducing peptides (AIPs) used by *S. aureus*. The oligopeptides are cyclized post-translationally to form a thioester linkage between the thiol moiety of the conserved $^{(*)}$Cys and the carboxyl group of the C-terminal residue (SEQ ID NOs: 120-123).

FIG. 2A-K are the ESI-MS spectra and HPLC chromatograms of the AIPs synthesized: AIP-1 (pure thiolactone) (**A & B**); AIP-2 (pure thiolactone) (**C & D**); AIP-3 (pure thiolactone) (**E & F**); AIP-4 (pure thiolactone) (**G & H**); AIP-IV (pure lactone) (**I & J**). HPLC was performed on a C18 column monitored at 214 nm by UV absorption using a gradient of 20 % B for 3 minutes and then increasing to 50 % B in 30 minutes. B is acetylnitrile run against HPLC grade water. FIG. 2K: MALDI-TOF analysis of AP4-BSA conjugate.

FIG. 3A-B are data illustrating the secretion of exoprotein in RN4850. **(A)** Analysis of exoprotein secretion in RN4850. After growth for 20-24 hours at 37 °C in the presence of the selected mAbs (200 μg/mL) as indicated, cells were centrifuged at 13,000 rpm for 2 minutes. The supernatants were analyzed by 10 % SDS-PAGE. The gels were stained using GelCode® Blue Stain Reagent (Pierce, Rockford IL). Solid arrows denote potential difference in exoprotein levels caused by AP4-24H11. **(B)** Hemolytic activity of the supernatants of *S. aureus* growing medium. Supernatants (150 μL) prepared above were dropped

onto the sheep blood agar plate. The plate was incubated at 37 °C for 24 hours and kept at room temperature for another 24 hours.

FIG. 4A-E are results illustrating the inhibition of quorum sensing signaling in *S. aureus* by AP4-24H11. **(A)** Western blot analyses of α-hemolysin and Protein A expression in *S. aureus* (RN4850 and Wood 46). *S. aureus* culture supernatants were prepared as described in the Examples. **(B)** Relative OD_{600} (%) of RN4850, NRS168 and Wood 46 after 20-24 hour incubation in the presence/absence of AP4-24H11. **(C)** Analysis of static biofilm formation in RN4850. **(D)** Real-Time PCR analysis. The amounts of the selected mRNAs were measured in RN4850 grown in the presence or absence of AP4-24H11. Relative quantification was performed using g*yrA* as a calibrator. At least two independent experiments were carried out for each experiment in duplicate. Actual numbers of fold-change; *rnaIII* (− 77±48), *eta* (− 8 .1 ±1), *hla* (− 5.2±3.1), *spa* (+ 5.7±3.6), *sarA* (− 2.1±0.6) and *saeR* (+ 1.4±0.4). **(E)** Suppression of AP4-24H11-mediated QS inhibition in *S. aureus* by AIP-4. AP4-24H11 (≈ 1.3 μM) was incubated with the native AIP-4 (2.5 μM) in CYPG medium for 20 minutes at room temperature. Overnight cultured *S. aureus* cells were diluted into the above medium ($OD_{600} \approx 0.03$) and grown for 20 to 24 hours at 37 °C under the static condition. The supernatants were prepared and analyzed. See the Examples for a detailed discussion of the experimental procedures.

FIG. 5A-B are data illustrating the inhibition of *S. aureus*-induced PARP cleavage by AP4-24H11. PARP cleavage in Jurkat cells after treating with supernatants from *S. aureus* RN4850 **(A)** and Wood 46 **(B)**. Human Jurkat leukemic T cells were maintained in RPMI 1640 supplemented with 10 % heat-inactivated fatal bovine serum, 10 mM $_{(L)}$-glutamine, and 50 mg/mL of streptomycin and penicillin (GIBCO, Invitrogen Corp.). *S. aureus* supernatants were prepared as described in the Examples, and the supernatants of RN4850 were further concentrated to 1/3 of original volume using Amicon Ultra-4 (5,000 NMWL) centrifugal filter devices (MILLIPORE, Billerica MA). Confluent cells were distributed to 24-well plate in fresh medium (0.5 mL) and incubated for 6 hours before adding the *S. aureus* supernatants. After 4 hours incubation with the indicated amount of *S. aureus* supernatants, cell extracts were prepared and analyzed by Western blotting using anti-PARP antibody.

FIG. 6A-B are results showing the inhibition of *S. aureus*-induced abscess formation by AP4-24H11 in mice models. **(A)** *S. aureus* (1×10^7) + PBS (upper panel); *S. aureus* (1×10^7) + AP4-24H11 (0.6 mg) (lower panel). **(B)** *S. aureus* (1×10^8) + Control mAb (0.6 mg) (upper panel); *S. aureus* (1×10^8) + AP4-24H11 (0.6 mg) (lower panel).

FIG. 7A-D are results illustrating the inhibition of *S. aureus*-induced abscess formation by AP4-24H11 in mice models. SKH1 euthymic hairless mice (6-8 weeks old) received 200 μL intradermal flank injections containing *S. aureus* (1×10^8 bacteria), 4 μL packed volume Cytodex beads, DPBS, mAb AP4-24H11 or control IgG (0.06 mg or 0.6 mg). Additional control animals received 200 μL intradermal injections containing Cytodex beads or beads plus antibody. After injections were made the mice were monitored at least three times each day over a period of 4-7 days. At the conclusion of the monitoring period the mice were euthanized and tissues harvested for bacteriologic and histologic analysis. **(A)** *S. aureus* + PBS; **(B)** *S. aureus* + AP4-24H11 (0.06 mg); **(C)** *S. aureus* + AP4-24H11 (0.6 mg); **(D)** Cytodex + AP4-24H11 (0.6 mg).

FIG. 8 illustrate survival data obtained from passive immunization of mice with AP4-24H11 against *S. aureus* infection. Survival in mice that were pretreated with mAb AP4-24H11 or control IgG followed two hours later by *S. aureus* injection (3×10^8 i.p.). The numbers in parenthesis show number of survivors/number per group. The log rank statistic, $p = 0.001$; $n = 6$ for each group.

FIG. 9 is result showing the suppression of α-hemolysin expression in the *agr* group I strains by anti-AP1 monoclonal antibodies.

FIG. 10A-B are the results of a biochemical evaluation of anti-AIP1 mAbs. **A.** α-hemolysin expression in *agr* I *S. aureus* RN6390B in the presence of anti-AIP1 mAbs (0.2 mg/mL). 1: AP1-2C2; 2: AP1-9A9; 3: AP1-9F9; 4: AP1-15B4; †: control mAb; ‡: no antibody. **B.** Static biofilm formation of *S. aureus* RN6390B in the presence of the anti-AIP1mAbs.

FIG. 11 is the result of a western analysis of the culture supernatants of *S. aureus* RN4850 grown in the presence of the human anti-AIP4 scFv 4-20 antibody for α-hemolysin expression.

FIG. 12 is the result of an experiment demonstrating the protection of mice from lethal MRSA USA300 challenge by mAb AP1-15B4. Mice were

treated with AP1-15B4 (1mg) or control IgG (1mg) 2 hours after *S. aureus* injection (1-3x10^8 i.p.). The numbers in parenthesis show survivors per group, p = 0.02; n = 6 for each group.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to the discovery that an antibody specific for the *Staphylococcus aureus* AP-4 signaling peptide can block quorum sensing and prevent *Staphylococcal* infection in mice. Thus, the invention provides an immunogenic molecular entity that can be used to elicit the production of an immune response against a native cyclic signaling peptide produced by a Gram-positive bacterium that regulates the expression of virulence factors through quorum sensing. The immunogenic molecular entity comprising at least one hapten, the hapten being covalently linked to an macromolecular carrier, optionally via a linker moiety, wherein the linker moiety is covalently bonded to the hapten and to the macromolecular carrier, the hapten comprising a cyclic peptide or an analog thereof, the cyclic peptide or analog thereof comprising a macrocyclic ring, wherein the cyclic peptide or analog thereof comprises about four to about nineteen amino acid residues as defined in the statements of the invention.

The invention also provides an antibody that binds specifically with a cyclic peptide signaling molecule. The antibody is a neutralizing antibody that can be used to inhibit quorum sensing in a mammal. In addition, the invention provides a composition that includes the immunogenic molecular entity or the neutralizing antibody, and a pharmaceutically-acceptable carrier. Additional embodiments of the invention include a method for eliciting an immune response in a mammal against a cyclic peptide signaling molecule, and a method of inhibiting bacterial quorum sensing in a mammal.

An immunogenic molecular entity of the invention is composed of a cyclic peptide or analog thereof covalently bonded to a macromolecular carrier, optionally via a linker moiety. The immunogenic molecular entity can be further included in a supramolecular assembly, such as a viral particle. Thus, an immunogenic molecular entity of the invention can elicit an immune response from an animal that has been administered the molecular entity. The animal can

be, for example, any mammal, such as a goat, pig, rabbit, mouse, rat, horse, or human.

Definitions

As used herein, the term "immunogenic" refers to the suitability of a molecular entity to generate an immune response in a vertebrate animal, for example, in a mammal including a mouse, a rat, a primate, or a human. A molecular entity is immunogenic when it is of sufficient molecular size and possesses other necessary molecular properties to generate an immune response such that antibodies are produced by the animal challenged by the molecular entity. It is well known in the art that to be immunogenic, a molecular entity such as a protein must have a molecular weight of at least about 10 kDa.

By the term "molecular entity" is meant a molecule or assembly of molecules defined by a chemical structure or assembly of chemical structures respectively. For example, a molecular entity of the invention can be a carrier protein or other immunogenically competent polymer, such as a dendrimer, covalently coupled to a hapten, optionally by a linker moiety. A "supramolecular assembly" can be an assembly of different macromolecules including the immunogenic molecular entity, such as a viral infectious particle that comprises the immunogenic molecular entity. A supramolecular assembly can also be an virosome displaying the hapten portion of the immunogenic molecular entity on its external surface.

As used herein, a "hapten" is a molecular moiety or fragment that is by itself insufficient in molecular size or weight, for example, to stimulate an immune response in an animal. When coupled to a carrier, however, antibodies can be raised that bind specifically to the hapten.

As the term is used herein, a "cyclic peptide or analog thereof" refers to an organic structure formed at least in part of multiple amino acid residues or analogous units covalently linked in a linear oligomeric form, wherein the linear chain is further internally cyclized to create a macrocyclic ring. The linear oligomeric form comprises monomeric units, each monomeric unit made up of an amino acid residue, bonded in a linear manner, but with additional formation of a loop produced by covalent attachment of the carboxy-terminal amino acid residue of the linear chain to a sidechain of an internal amino acid residue. See, for example, **FIG. 1**.

The term "amino acid residue" is meant an amino acid or an analog thereof as it is covalently bonded in an oligomeric chain, for example, in a natural peptide as is well known in the art. An amino acid residue is also known as an "anhydro amino acid unit" due to the formation of an amide bond between the amino group or the carboxylic acid group of the amino acid residue and the carboxylic acid group or the amino group, respectively, of an adjacent amino acid residue in the oligomer. Both the amino group and the carboxylic acid group of an amino acid or an amino acid analog can be combined in amide or amide-analogous linkages with adjacent amino acid residues in an oligomer. However the cyclic peptide or analog thereof as referred to herein need not be composed only of the residues of naturally occurring amino acids.

While a cyclic peptide, as the term is used herein, can be formed of ribosomal amino acid residues, that is, the approximately 20 L-α-amino acids that can be coded in DNA without posttranslational modification, it can include enantiomeric D-amino acid forms of these natural amino acids, as well as unnatural amino acids such as amino acids bearing sidechains other than those of the approximately 20 ribosomal amino acids. A cyclic peptide can also include amino acids of types other than α-amino acids such as β- or γ-amino acids, or amino groups wherein the carboxylic acid and amino groups are separated by larger numbers of atoms. For example, the cyclic peptide or analog can include an amino acid wherein an alkyl amino group and a carboxylic acid group are separated by various lengths of polyethyleneglycol (PEG) chains or simple alkylene chains. All of these are considered "amino acid residues" within the meaning herein. Thus, a cyclic peptide or analog thereof of the present invention can be made from genetically encoded amino acids, naturally occurring non-genetically encoded amino acids, or synthetic amino acids. The amino acid notations used herein for the twenty genetically encoded L-amino acids and some examples of non-encoded amino acids are provided in Table 1:

Table 1

Amino Acid	One-Letter Symbol	Common Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn

Amino Acid	One-Letter Symbol	Common Abbreviation
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val
Â-Alanine		Bala
2,3-Diaminopropionic acid		Dpr
Á-Aminoisobutyric acid		Aib
N-Methylglycine (sarcosine)		MeGly
Ornithine		Orn
Citrulline		Cit
t-Butylalanine		t-BuA
t-Butylglycine		t-BuG
N-methylisoleucine		MeIle
Phenylglycine		Phg
Cyclohexylalanine		Cha
Norleucine		Nle
Naphthylalanine		Nal
Pyridylalanine		
3-Benzothienyl alanine		

Amino Acid	One-Letter Symbol	Common Abbreviation
4-Chlorophenylalanine		Phe(4-Cl)
2-Fluorophenylalanine		Phe(2-F)
3-Fluorophenylalanine		Phe(3-F)
4-Fluorophenylalanine		Phe(4-F)
Penicillamine		Pen
1,2,3,4-Tetrahydro-isoquinoline-3-carboxylic acid		Tic
Â-2-thienylalanine		Thi
Methionine sulfoxide		MSO
Homoarginine		Harg
N-acetyl lysine		AcLys
2,4-Diamino butyric acid		Dbu
Ñ-Aminophenylalanine		Phe(pNH_2)
N-methylvaline		MeVal
Homocysteine		Hcys
Homoserine		Hser
α-Amino hexanoic acid		Aha
α-Amino valeric acid		Ava
2,3-Diaminobutyric acid		Dab

Irrespective of the amino acid make up, the structure of the cyclic peptide or analog thereof includes a macrocyclic ring. As the term is used herein, a cyclic peptide or analog thereof contains a macrocyclic ring that includes the C-terminal amino acid residue covalently bonded to the sidechain of an amino acid residue that is situated within the chain, that is, an "internal" amino acid residue. Therefore the "immunogenic molecular entity" comprising a "cyclic peptide or analog thereof" can be conceptualized as a molecule having a "lasso" like loop form, wherein the loop of the lasso is free while the tail of the lasso is bonded to the macromolecular carrier. As described below, the tail of the lasso can be bonded to the macromolecular carrier by a linker moiety, as well as directly bonded.

A cyclic peptide or analog thereof, as used herein, can also include molecular segments that do not include amino acid residues. For example,

spacer segments, such as polyethyleneglycol (PEG) segments, can be included in the cyclic peptide or analog. The spacer segment, typically disposed in the tail of the lasso-like loop, can serve to hold the hapten off the surface of the macromolecular carrier to increase its accessibility to antibodies.

The loop is completed by a set of covalently bonded atoms, referred to herein as a "macrocyclizing moiety" and shown as "R" in Formula (I), intervening between a carbonyl group of the C-terminal amino acid, that is, the carbonyl group of the amino acid's carboxyl group, and a carbon atom of an internal amino acid.

The "macrocyclizing moiety" as the term is used herein refers to a group of covalently bonded atoms which can include carbon, nitrogen, oxygen, sulfur and hydrogen that forms a bridge between the carboxy-terminal carbonyl group of the C-terminal amino acid residue and an atom, such as the alpha-carbon, of an internal amino acid residue. The macrocyclizing moiety can include amide bonds; for example, the moiety may be a group that includes a carboxylic acid group, that can be covalently bonded by an amide bond to an amino group of a sidechain of an internal amino acid residue, and can also include an amino group that can be covalently bonded by an amide bond to the carbonyl of the carboxylic acid group of the C-terminal amino acid residue. The macrocyclizing moiety, designated "R" in Formula (I), can also include other group types, such as ester, thioester, ether, thioether, carbonyl, olefin or hydrocarbon groups. The macrocyclizing moiety can contain any amide-surrogate group, or several such groups, for example, as are described in "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins," volume 7, by Arno F. Spatola, (1983) Marcel Dekker, New York / Basel, which is incorporated herein by reference in its entirety. Amide surrogate groups can include ketones, amines, ethers, thioethers, sulfones, sulfoxides, sulfonamides, sulfonates, aryls, heteroaryls, alkyls, alkenyls, hydrazines, amidines, guanidines, ureas, thioureas, semicarbazides, boronates, phosphonates, and the like.

By a "macrocyclic ring" as the term is used herein is meant a ring formed entirely of covalently bonded atoms, wherein the ring size is greater than about 9 atoms. A macrocyclic ring can include up to 20 atoms, or 30 atoms, or more. The macrocyclic ring can include carbon-carbon bonds, as well as carbon-nitrogen, carbon-oxygen, carbon-sulfur, nitrogen-nitrogen, and other covalent

bonds including atoms with valences greater than one. In the inventive cyclic peptide or analog thereof, the macrocyclic ring includes some atoms of at least three, and up to about 10, amino acid residues, as well as the macrocyclizing moiety described above that completes the macrocyclic ring structure.

An " macromolecular carrier" as the term is used herein refers to a macromolecular entity that is of sufficient size, in conjunction with the hapten bonded to it, to trigger the mounting of an immune response by an organism challenged by the composition. Typically, a hapten is bonded to a protein, for example, keyhole limpet hemocyanin, in order to trigger the immune response and bring about the formation of antibodies to the attached hapten by the challenged organism. Thus, the macromolecular carrier can be a protein, particularly a protein known as a good carrier for presentation of haptens, that is, where most of the antibodies raised have the hapten and not the carrier protein as their antigenic structures. However, the macromolecular carrier of the invention can be entities other than proteins. For example, the macromolecular carrier can comprise a dendrimer, such as a Multiple Antigen Peptide (MAP) dendrimer such as was developed by J. Tam et al., (see, for example Posnett, D., McGrath, H., and Tam, J. P. "A novel method for producing anti-peptide antibodies" *J. Biol. Chem.* 263, 1719-1725 (1988), and Tam, J. P. "Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system" PNAS USA 85, 5409-5413 (1988), which are incorporated by reference herein in their entireties) for the presentation of haptens to immune systems. Such dendrimers, which can be formed by star polymerization of multifunctional monomers such as lysine, present multiple functional groups on the surface of a globular macromolecule to which haptens can be bonded.

Further, the macromolecular entity can be a part of a supramolecular assembly of macromolecules, such as a viral particle. For example, a phage display system can be used wherein the phage surface is adapted for covalent attachment of the cyclic peptide or an analog. Or, the macromolecular carrier can include a virosome, that is, a micellar structure formed of phospholipids, wherein membrane-spanning proteins are embedded and serve as the macromolecular carrier to which the hapten is attached.

A "linker moiety" as the term is used herein refers to a molecular segment that is incorporated between the cyclic peptide or analog thereof, and

the macromolecular carrier. The hapten can include the linker moiety, which is introduced as a bifunctional reagent that can serve to couple the N-terminus of the cyclic peptide or analog to the carrier by reaction with both. It is understood that in some cases, a cyclic peptide or analog thereof can be directly coupled to a macromolecular carrier, such as a protein. For example, the N-terminal amino group of a cyclic peptide can be directly linked to a protein, for example a carboxylic acid group of a protein amino acid bearing an acidic sidechain such as aspartate or glutamate, by use of a dehydrating reagent such as EDC (ethyl dimethylaminopropyl carbodiimide) to form a direct amide bond without any intervening linker moiety. However, a linker reagent, consisting of a bifunctional reagent, as is well known in the art, can carry out the same function. The atoms of this linker reagent, when incorporated into the inventive immunogenic molecular entity, form the "linker moiety" as the term is used herein.

Many types of linker reagents are known to skilled artisans. Examples include reagents that have one functional group adapted to react with thiol groups, for example N-alkylmaleimide derivatives, that can react with an N-terminal cysteine or homocysteine residue of an inventive cyclic peptide or analog thereof. The linker also has a second functional group that is adapted to react with a group present on the surface of the macromolecular carrier, for example a carboxylate group or an amino group of an amino acid sidechain in a protein. For example, an N-hydroxysuccinimide ester of an acyl group can react to form an amide bond with a protein surface lysine residue. The two functional groups of the linker reagent are covalently bonded, usually through intervening atoms, such that reaction at the two ends serves to covalently couple the reactive molecules to each other via the linker moiety. Examples of linker chemistry can be found in the catalog of Pierce, P.O. Box 117, Rockford, IL 61105, which may be viewed at the website http://piercenet.com/products/browse.cfm?fldID=0203, the information of which is incorporated herein by reference. Some examples of linker reagents that can react to form linker moieties include MBS, sulfo-MBS, SMCC, or sulpho-SMCC, as are well known in the art.

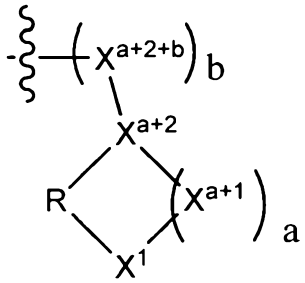
The term "quorum sensing" refers to the phenomenon wherein certain bacterial species detect their own population levels and, when a certain

population level is reached, initiate or amplify the expression of certain traits, such as secretion of virulence factors.

The term "immunogen" refers to the active ingredient of an active vaccine and can be a polypeptide, a hapten linked to a carrier as described herein or any macromolecular entity or assembly that is capable of eliciting an inmmune response in a mammal that has been exposed or come into contact with the immunogen.

An Immunogenic Molecular Entity of the Invention

The invention provides an immunogenic molecular entity comprising at least one hapten, the hapten being covalently linked to an macromolecular carrier, optionally via a linker moiety, the hapten comprising a cyclic peptide or an analog thereof, the cyclic peptide or analog thereof comprising a macrocyclic ring, wherein the cyclic peptide or analog thereof comprises about four to about nineteen amino acid residues, the cyclic peptide or analog thereof having a structure represented by Formula I:



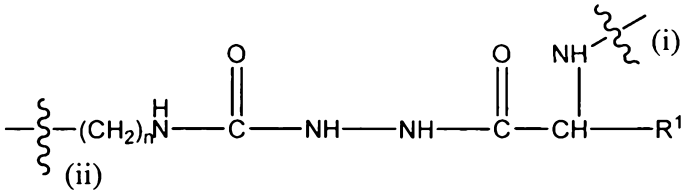
wherein each X is independently any amino acid residue; X^1 is an amino acid residue that is covalently bonded to R by a respective carbonyl group; X^{a+2} is an internal amino acid, a respective carbon atom of which is covalently bonded to R; R is a macrocyclizing moiety that covalently connects X^1 and X^{a+2} thereby forming the macrocyclic ring, wherein R comprises an ester, thioester, amide, carbamide, semicarbazide, or other amide-surrogate group, or any combination thereof; a is 1 to about 9; b is 1 to about 8; and a bond transected by a wavy line indicates a point of attachment of an N-terminal amino acid residue of the cyclic peptide or analog thereof to the macromolecular carrier, optionally via the linker moiety.

In one embodiment, the cyclic peptide or analog thereof includes structures of Formula (I) wherein a is 2-8, or alternatively a can be 2-4, and R

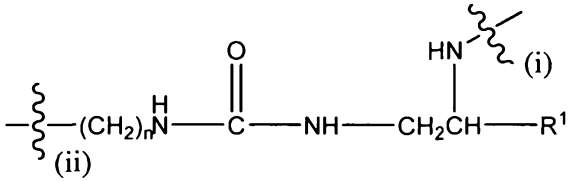
comprises an alkyloxy or alkaryloxy, alkylthio, or alkylamino group covalently bonding X^{a+2} to the X^1 carbonyl group, thereby providing an ester, thioester, or amide bond, respectively, to form a lactone, thiolactone, or lactam macrocyclic ring, respectively.

More specifically, the cyclic peptide or analog thereof includes structures of Formula (I), wherein R comprises $-CH_2O-$, $-CH_2CH_2O-$, $-CH_2CH(CH_3)O-$, $-CH_2$-phenyl-O-, $-CH_2S-$, $-CH_2CH_2S-$, or $-(CH_2)_nNH-$ wherein n is 1 to about 4. In these embodiments, the cyclic peptide or analog thereof can be viewed as including a macrocyclic ring wherein the carboxy-terminal carbonyl group is bonded to the sidechain of a serine, homoserine, threonine, or tyrosine residue respectively, forming a lactone ring; or to a sidechain of a cysteine or a homocysteine residue respectively, forming a thiolactone; or to a sidechain of a diaminopropropionate (n=1), diaminobutyrate (n=2), ornithine (n=3), or lysine (n=4) residue respectively, forming a lactam.

In another embodiment, the cyclic peptide or analog thereof includes structures of Formula (I), wherein a is 2-8, or alternatively a can be 2-4, and the macrocyclizing group R comprises at least one amide, urea, or semicarbazide group, or at least one amide-surrogate bond. For example, R can be represented by Formula (IIa) or Formula (IIb):



Formula (IIa),

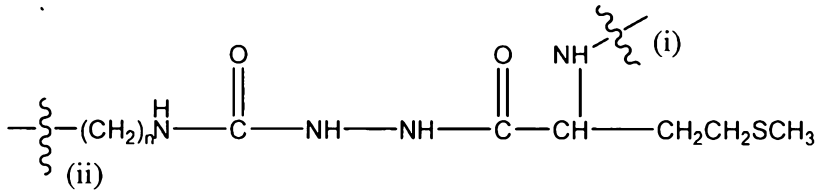


Formula (IIb),

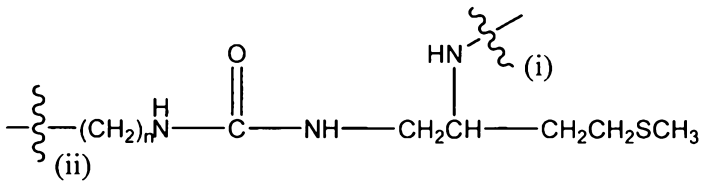
wherein n is 1 to about 4, R^1 is the sidechain of a naturally occurring amino acid or an analog thereof, a bond transected by a wavy line indicates a point of attachment, wherein the point of attachment designated (i) is bonded to the carbonyl group of X^1 and the point of attachment designated (ii) is bonded to the alpha-carbon of X^{a+2}. The sidechain of a naturally occurring amino acid can be

the sidechain of any of the ribosomal amino acids, or analogs thereof. Thus the sidechain represented by R^1 can be the sidechain of ribosomal amino acids like alanine, phenylalanine, histidine, methionine, asparagine, glutamine, tryptophan, etc. Alternatively the sidechain can be a structure analogous to these naturally occurring sidechains, for example, an ethyl group in place of an alanine methyl group, a phenethyl group in place of a phenylalanine benzyl group, and the like. An analog of an amino acid residue, or an amino acid sidechain, as the term is used herein, refers to a chemical structure that is not identical to the natural structure but differs only by addition of a short alkyl group, or addition of a □ubstituents that does not change the fundamental physical properties of the sidechain. For example, an analog of alanine would include a fluorinated derivative of alanine such as trifluoroalanine, as the size, ionicity and hydrophobicity of the residue would not be greatly altered by the substitution.

A non-limiting example of formula (IIa) is:



It is recognized that this R1 group corresponds to a methionine sidechain. Correspondingly, R can be a group of formula (IIb) bearing a methionine sidechain:



In another embodiment according to the invention, the cyclic peptide or analog thereof can include hydrophobic C-terminal amino acid residues. For example, in one embodiment, X^1 and X^2 of Formula (I) are hydrophobic amino acid residues. More specifically, X^1 and X^2 can be independently selected from the group of amino acid residues consisting of alanine, valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, or tryptophan, or analogs thereof. Yet more specifically, each of X^1 and X^2 can be independently methionine, leucine, phenylalanine, tyrosine, alanine, isoleucine, or tryptophan.

In further embodiments, the cyclic peptide or analog thereof can include sequences YST(X^{a+2})DFIM (SEQ ID NO: 92), YST(X^{a+2})YFIM (SEQ ID NO: 93), IN(X^{a+2})DFLL (SEQ ID NO: 94), GVNA(X^{a+2})SSLF (SEQ ID NO: 95), GVNP(X^{a+2})GGWF (SEQ ID NO: 96), KAKT(X^{a+2})TVLY (SEQ ID NO: 97), KTKT(X^{a+2})TVLY (SEQ ID NO: 98), GANP(X^{a+2})OLYY (SEQ ID NO: 99), GANP(X^{a+2})ALYY (SEQ ID NO: 100), GYST(X^{a+2})SYYF (SEQ ID NO: 101), GYRT(X^{a+2})NTYF (SEQ ID NO: 102), YNP(X^{a+2})VGYF (SEQ ID NO: 103), GGKV(X^{a+2})SAYF (SEQ ID NO: 104), SVKP(X^{a+2})TGFA (SEQ ID NO: 105), DSV(X^{a+2})ASYF (SEQ ID NO: 106), KYNP(X^{a+2})SNYL (SEQ ID NO: 107), KYNP(X^{a+2})ASYL (SEQ ID NO: 108), KYNP(X^{a+2})ANYL (SEQ ID NO: 109), RIPT(X^{a+2})TGFF (SEQ ID NO: 110), DI(X^{a+2})NAYF (SEQ ID NO: 111), DM(X^{a+2})NGYF (SEQ ID NO: 112), KYNP(X^{a+2})LGFL (SEQ ID NO: 113), KYYP(X^{a+2})FGYF (SEQ ID NO: 114), GARP(X^{a+2})GGFF (SEQ ID NO: 115), GAKP(X^{a+2})GGFF (SEQ ID NO: 116), YSP(X^{a+2})TNFF (SEQ ID NO: 117), YSP(X^{a+2})TNF (SEQ ID NO: 118), or QN(X^{a+2})PNIFGQWM (SEQ ID NO: 119), wherein the last amino acid residue of each sequence is X^1, and (X^{a+2}) is the internal amino acid to which the carbonyl group of X^1 is covalently bonded via R.

In an embodiment, the cyclic peptide or analog thereof can mimic any of the sequences determined for naturally occurring cyclic peptide signaling molecule, as shown in the following Table:

Bacterium	**Native cyclic signaling peptides**
S. aureus I	YSTCDFIM (SEQ ID NO: 120)
S. aureus II	GVNACSSLF (SEQ ID NO: 121)
S. aureus III	INCDFLL (SEQ ID NO: 122)
S. aureus IV	YSTCYFIM (SEQ ID NO: 123)
S. arlettae	GVNPCGGWF (SEQ ID NO: 124)
S. auricularis I	KAKTCTVLY (SEQ ID NO: 125)
S. auricularis II	KTKTCTVLY (SEQ ID NO: 126)
S. capitis I	GANPCOLYY (SEQ ID NO: 127)
S. capitis II	GANPCALYY (SEQ ID NO: 128)
S. caprae I	GYSTCSYYF (SEQ ID NO: 129)

S. caprae II	GYRTCNTYF (SEQ ID NO: 130)
S. carnosus	YNPCVGYF (SEQ ID NO: 131)
S. cohnii ssp. cohnii	GGKVCSAYF (SEQ ID NO: 132)
S. cohneii ssp. urealyticum	SVKPCTGFA (SEQ ID NO: 133)
S. epidermis I	DSVCASYF (SEQ ID NO: 134)
S. epidermis II	KYNPCSNYL (SEQ ID NO: 135)
S. epidermis III	KYNPCASYL (SEQ ID NO: 136)
S. epidermis IV	KYNPCANYL (SEQ ID NO: 137)
S. intermedius	RIPTSTGFF (SEQ ID NO: 138)
S. lugdunensis I	DICNAYF (SEQ ID NO: 139)
S. lugdunensis II	DMCNGYF (SEQ ID NO: 140)
S. simulans I	KYNPCLGFL (SEQ ID NO: 141)
S. simulans II	KYYPCFGYF (SEQ ID NO: 142)
S. gallinarum	VGARPCGGFF (SEQ ID NO: 143)
S. xylosus	GAKPCGGFF (SEQ ID NO: 144)
S. warneri (RN 833)	YSPCTNFF (SEQ ID NO: 145)
E. faecalis	QNSPNIFGQWM (SEQ ID NO: 146)

NOTE: the alpha-carbonyl group of the underlined residue forms a thiolactone bond with the sulfhydryl group of the bolded internal cysteine residue

The cyclic peptides and analogs thereof of the hapten can be synthesized in linear form using standard solid phase peptide synthesis techniques, wherein the sidechain of the internal amino acid residue to which the X^1 carbonyl group will be bonded either directly or through a more complex macrocyclizing moiety, such as the groups of Formulas (IIa) and (IIb), is appropriately blocked, such that selective deblocking of this amino acid residue sidechain can be achieved. The selectively deblocked sidechain can then be reacted either directly with the C-terminal carboxyl group, thereby bonding the sidechain to the C-terminal carbonyl wherein the sidechain is represented by the R group of Formula (I), or can be reacted with the more complex macrocyclizing moiety to form the macrocyclic ring therethrough. Synthetic examples are provided below.

The macromolecular carrier to which the hapten is covalently bonded or coupled is of sufficient size, molecular weight, and composition to stimulate an

immune response in an animal challenged with the hapten-carrier complex. The hapten, including the cyclic peptide or cyclic peptide analog, can be directly coupled to the macromolecular carrier. For example, a covalent bond can be formed between a functional group of the carrier such as a carboxylic acid and a functional group of the cyclic peptide or analog, such as between an N-terminal amino group, using an amide-forming reagent such as EDC (ethyl dimethylaminopropyl carbodiimide), optionally with N-hydroxysuccinimide. Alternatively, an N-terminal amino acid residue of the cyclic peptide can have carboxylic functionality, for example the N-terminal residue can be an aspartate or glutamate residue. In that case it can be directly coupled to an amino group on the carrier, using the same chemical synthesis approach. The amino group can be present, for example, in the sidechain of a lysine residue on the surface on a protein. Alternatively, an amino group to which the peptide carboxylate can be coupled could be on the surface of a synthetic dendrimer, such as a MAP structure. Other schemes for direct coupling of the cyclic peptide or analog thereof to a macromolecular carrier will be apparent to those of ordinary skill in the art.

The macromolecular carrier can comprise a polypeptide. For example, the macromolecular carrier can be a protein, and nonlimiting examples of such suitable carrier proteins include keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), rabbit serum albumin (RSA), human serum albumin (HAS), *Concholepas concholepas* hemocyanin (CCH), cholera toxin B subunit, *E. coli* labile toxin B subunit, Diphtheria toxoid, tetanus toxoid, tetanus toxin C-fragment, recombinant *Pseudomonas aeruginosa* exoprotein A, CRM197 (cross-reactive material), cationized bovine serum albumin (cBSA), Thyroglobulin (Tg), avidin, bovine thyroglobulin (BTG), bovine G globulin, bovine immunoglobulin G (BigG), conalbumin (CONA), colloidal gold, edestin, *Paralithodes camtschatica* heamocyanin (HC), helix promatia haemocyanin (HPH), soybean kunitz trypsin inhibitor (KTI), *Limulus polyphemus* heamocyanin (LPH), ovalbumin (OA), Pam3Cys-Th (lipopeptide/Th cell epitope), polylysine, porcine thyroglobulin (PTG), purified protein derivative (PPD), soybean trypsin inhibitor (STI), or sunflower globulin (SFG). Thus, in some embodiments, the immunogenic molecular entity comprises a hapten

covalently linked to a polypeptide such as, without limitation, the above exemplified polypeptides.

The macromolecular carrier can be a polymer, such as a linear polymer adapted for covalent attachment of haptens, or can be another type of synthetic carrier such as, for example, a dendrimer. A dendrimer produced by star polymerization of monomers with more than two reactive groups can be adapted to provide functional groups to which a synthetic cyclic peptide or analog thereof can be coupled using chemistry known to those of skill in the art. For example, a MAP dendrimer, which provides multiple amino groups on its surface, can be coupled to a sidechain carboxyl group of an N-terminal amino acid residue of an inventive cyclic peptide. See, for example, Sakarellos-Daitsiotis *et al.*, *Current Topics in Medicinal Chemistry* 6:1715-35 (2006); Saupe et al., *Expert Opin. Drug.Deliv.* 3:345-354 (2006); McDermott *et al.*, *Immunology and Cell Biology* 76: 256-62 (1998); and Shahiwala *et al.*, *Recent Patents on Drug Delivery & Formulation* 1:1-9 (2007).

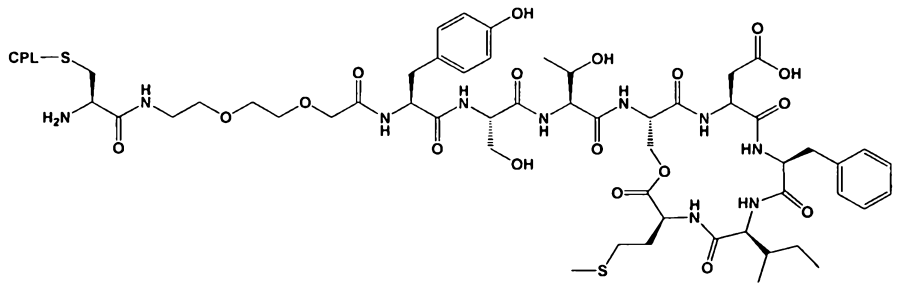
In another embodiment, the immunogenic molecular entity can include a linker moiety, disposed between the cyclic peptide or analog, and the macromolecular carrier. A linker moiety can be used to physically separate the domain(s) of the hapten for which antibodies are desired to be specific, i.e., the cyclic peptide or analog, from the surface of the macromolecular carrier. A linker moiety can be derived from a linker reagent, such as MBS (m-maleimidobenzoyl N-hydroxysuccinimide ester), sulfo-MBS (m-maleimidobenzoyl N-hydroxy-2-sulfosuccinimide ester), SMCC (succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate), sulfo-SMCC (2-sulfosuccinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate), as are well known in the art. Reaction of the linker reagent with the cyclic peptide and with the carrier yield the linker moiety coupled to both. For example, the linker reagents recited above are adapted to couple a thiol-containing N-terminal amino acid residue of the cyclic peptide and an amino group of the macromolecular carrier through addition of the thiol group to the maleimide group, and by acylation of the carrier amino group with the N-hydroxy ester group. Other linker reagents are adapted to react in different ways with different groups. Other types of structures can be included within linker moieties. For example a linker moiety can include adipic acid dihydrazide (ADH), a spacer peptide,

hydroxymethyl hemisuccinate, or a polyethyleneglycol derivative. It is within ordinary skill to select a linker reagent adapted to react with the particular cyclic peptide N-terminus and with the particular macromolecular carrier in the desired manner.

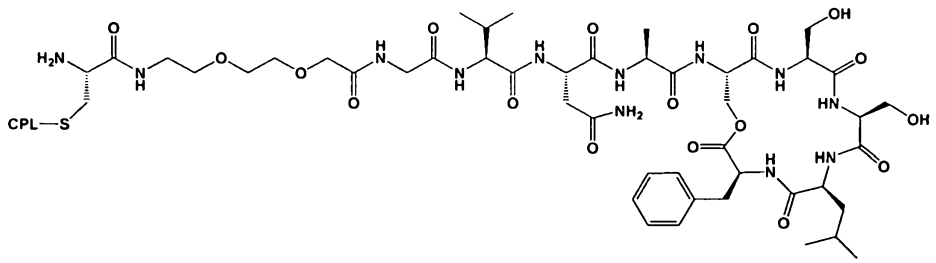
The macromolecular carrier and covalently bound hapten can be included within a supramolecular assembly. The supramolecular assembly can be a liposome or virosome, that is, a micellar structure including membrane-spanning proteins. See, for example, Westerfeld & Zurbriggen, *J. Peptide Sci.* 11:707-712 (2005) and Felnerova *et al.*, *Current Opinion in Biotechnology* 15:518-29 (2004). The supramolecular assembly can be a virus particle, such as in a phage display system, wherein a bacteriophage is adapted to express surface functional groups.

In other embodiments, the macromolecular carrier and covalently bound hapten need not be included within a supramolecular assembly to be immunogenic.

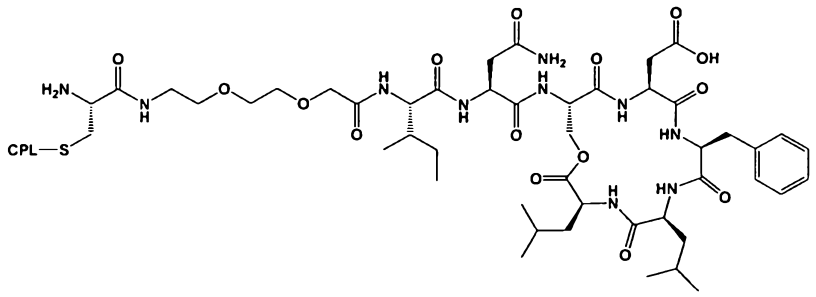
Specific examples of immunogenic molecular entities of the invention are shown below for exemplary purposes:



SEQ ID NO: 3 (YSTSDFIM, not including protecting groups),

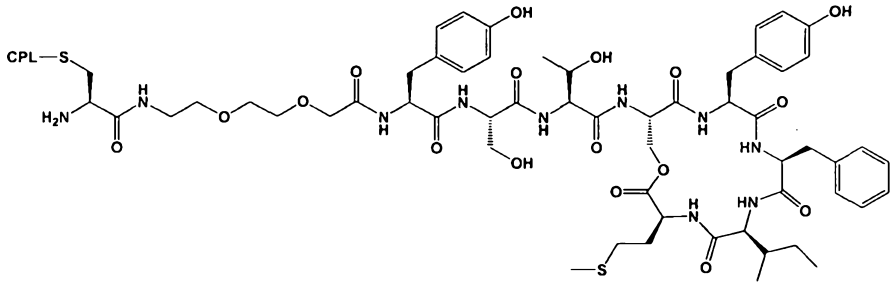


SEQ ID NO: 4 (GVNASSSLY, not including protecting groups),



SEQ ID NO: 2 (INSDFLL, not including protecting groups),

or



SEQ ID NO: 1 (YSTSYFLM, not including protecting groups),

wherein CPL is a macromolecular carrier with optional linker covalently bonded to a cysteine thiol group. It can be seen in these examples that the macrocyclic ring includes a lactone group that is formed between an internal serine amino acid residue and the carboxy terminus, which is a methionine, phenylalanine, or leucine residue. The macrocyclic ring of each of these examples includes five amino acid residues, four additional natural amino acid residues, a synthetic amino acid residue comprising a PEG group, and an N-terminal cysteine residue bonded via an optional linker group to a macromolecular carrier, for example a macromolecular polypeptide. These compositions exemplify structures that can be used to induce antibody formation in an animal, wherein at least some of the antibodies formed in response are specific for the cyclic peptide analog of the hapten.

The immunogenic molecular entity of the invention can be used to screen a recombinant combinatorial immunoglobulin library (for example, an antibody phage display library) for an antibody specific for a native cyclic signaling peptide. For example, an immunogenic molecular entity of the invention that has a hapten corresponding to the lactone, lactam, carbamide or semicarbazide analog of the *S. aureus* AIP IV cyclic signaling peptide, can be used to screen a

recombinant combinatorial immunoglobulin library for an antibody that will bind specifically with the AIP IV cyclic signaling peptide. Uses of an antibody that will bind specifically with a cyclic signaling peptide are discussed below.

A immunogenic molecular entity of the invention can also be used to elicit an immune response in a mammal directed against selected cyclic signaling peptide. For example, an immunogenic molecular entity of the invention that has a hapten corresponding to the lactone, lactam, carbamide or semicarbazide analog of the *S. aureus* AIP IV cyclic signaling peptide, can be used to elicit an immune response against the AIP-IV cyclic signaling peptide in a mammal.

The resulting mammal can be a source of antibody specific for the cyclic signaling peptide. For example, antibodies against AIP-IV can be isolated from the blood of the mammal. In addition, antibody-producing cells can be isolated and used to make antibody-producing hybridomas for the production of monoclonal antibodies as discussed below.

The immunogenic molecular entity of the invention can also be used as a vaccine in that the immune response generated in the mammal can protect the mammal from infection by a Gram positive bacteria that utilizes the selected cyclic signaling peptide in quorum sensing and expression of virulence genes or prevent the mammal from developing a disease or condition associated with infection. For example, an immunogenic molecular entity of the invention that has a hapten corresponding to the lactone, lactam, carbamide or semicarbazide analog of the *S. aureus* AIP IV cyclic signaling peptide can be used to elicit an immune response against the AIP-IV cyclic signaling peptide such that the mammal is protected from developing a disease condition or complications associated with *S. aureus* virulence.

Uses of an immunogenic molecular entity of the invention are further described below, for example, in the Methods and EXAMPLES sections.

An Antibody of the Invention

An antibody of the invention is one that binds specifically with a cyclic signaling peptide. As used herein, the term "cyclic signaling peptide" refers to a cyclic peptide produced by a Gram positive bacterium that utilizes quorum sensing to regulate the expression of virulence genes. The cyclic signaling peptide is a signaling molecule that binds to a membrane-bound histidine kinase sensor molecule, which then interacts with an intracellular response regulator.

Cyclic signaling peptides are produced by Gram-positive bacteria that employ quorum sensing including, without limitation, various *Staphylococci* species and *Enterococcus faecalis*. Non-limiting examples of cyclic signaling peptides and the producer bacteria are provided in the following table. The signaling peptide is composed of an N-terminal tail and a thiolactone- or lactone-containing ring that is formed by reaction of the alpha-carboxyl group of the "C-terminal" amino acid residue (underlined) with the sidechain sulfhydryl or hydroxyl group of an internal amino acid (bolded).

Bacterium	Native cyclic signaling peptides
S. aureus I	YSTCDFIM (SEQ ID NO: 120)
S. aureus II	GVNACSSLF (SEQ ID NO: 121)
S. aureus III	INCDFLL (SEQ ID NO: 122)
S. aureus IV	YSTCYFIM (SEQ ID NO: 123)
S. arlettae	GVNPCGGWF (SEQ ID NO: 124)
S. auricularis I	KAKTCTVLY (SEQ ID NO: 125)
S. auricularis II	KTKTCTVLY (SEQ ID NO: 126)
S. capitis I	GANPCOLYY (SEQ ID NO: 127)
S. capitis II	GANPCALYY (SEQ ID NO: 128)
S. caprae I	GYSTCSYYF (SEQ ID NO: 129)
S. caprae II	GYRTCNTYF (SEQ ID NO: 130)
S. carnosus	YNPCVGYF (SEQ ID NO: 131)
S. cohnii ssp. cohnii	GGKVCSAYF (SEQ ID NO: 132)
S. cohneii ssp. urealyticum	SVKPCTGFA (SEQ ID NO: 133)
S. epidermis I	DSVCASYF (SEQ ID NO: 134)
S. epidermis II	KYNPCSNYL (SEQ ID NO: 135)
S. epidermis III	KYNPCASYL (SEQ ID NO: 136)
S. epidermis IV	KYNPCANYL (SEQ ID NO: 137)
S. intermedius	RIPTSTGFF (SEQ ID NO: 138)
S. lugdunensis I	DICNAYF (SEQ ID NO: 139)
S. lugdunensis II	DMCNGYF (SEQ ID NO: 140)
S. simulans I	KYNPCLGFL (SEQ ID NO: 141)

S. simulans II	KYYPCFGYF (SEQ ID NO: 142)
S. gallinarum	VGARPCGGFF (SEQ ID NO: 143)
S. xylosus	GAKPCGGFF (SEQ ID NO: 144)
S. warneri (RN 833)	YSPCTNFF (SEQ ID NO: 145)
E. faecalis	QNSPNIFGQWM (SEQ ID NO: 146)

Thus, a cyclic signaling peptide can have a ring of three to eleven-amino acids and a tail of one to about nine amino acids. The ring structure is formed between the alpha-carbonyl group of the "C-terminal amino acid residue," that is the carboxy-terminal amino acid of a corresponding linear peptide, and an alkyloxy or alkylthio group on the sidechain of an internal serine or cysteine residue, in particular, the 4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th} or 9^{th} residue from the carboxy-terminal amino acid. For example, the *S. aureus* AIP4 signaling molecule is a cyclic thiolactone peptide analog composed of the amino acid sequence YSTCYFIM (SEQ ID NO: 123). The cyclic thiolactone ring structure results from a bond between the alpha-carboxyl group of methionine (M), the "C-terminal amino acid residue," and the sulfhydryl group of cysteine HAS, the fifth amino acid from the "C-terminal" methionine (M) residue.

Thus, a cyclic signaling peptide can have a five-amino acid ring, for example, a thiolactone or lactone ring, and a linear two- to five-amino acid tail.

An antibody can be an immunoglobulin molecule or an immunologically-active fragment thereof that binds specifically with a particular antigen. An antibody of the invention is one that binds specifically with a native cyclic signaling peptide, or a hapten that includes the lactone, lactam, carbamide or semicarbazide analog of the cyclic signaling peptide. As used herein, the term "bind specifically" or "specifically binds" in reference to an antibody of the invention means that the antibody of the invention will bind with the cyclic signaling peptide or corresponding hapten, but does not substantially bind to other unrelated molecules including the carrier protein alone or other unrelated molecules that may be present with the immunogenic molecular entity, supramolecular assembly, or a biological sample from a mammal. For example, an antibody that binds specifically with an immunogenic molecular entity of the invention in which the hapten is a lactone, lactam, carbamide or semicarbazide analog of the *S. aureus* AIP IV cyclic peptide signaling molecule is one that will

bind with the *S. aureus* AIP IV cyclic peptide, but will not bind substantially with the carrier alone or an unrelated molecule.

An antibody of the invention is also a neutralizing antibody. As used herein, the term "neutralizing antibody" refers to an antibody that will bind to a cyclic signaling peptide and prevent the binding of the cyclic signaling peptide with its membrane-associated receptor. The term "neutralizing antibody" also includes a cross-neutralizing antibody, an antibody that will bind to and prevent bidning of at least two cyclic signaling peptides with their receptors, for example, cyclic signaling peptides from different agr groups. Whether an antibody is a neutralizing antibody can be determined using the methods known to those of skilled in the art including those described herein, for example, in the EXAMPLES section. The term

An antibody of the invention can be a polyclonal or monoclonal antibody. Polyclonal antibodies can be obtained by immunizing a mammal with an immunogenic molecular entity of the invention, and then isolating antibodies from the blood of the mammal using standard techniques including, for example, enzyme linked immunosorbent assay (ELISA) to determine antibody titer and protein A chromatography to obtain the antibody-containing IgG fraction.

A monoclonal antibody is a population of molecules having a common antigen binding site that binds specifically with a particular antigenic epitope. A monoclonal antibody can be obtained by selecting an antibody-producing cell from a mammal that has been immunized with an immunogenic molecular entity of the invention and fusing the antibody-producing cell, e.g. a B cell, with a myeloma to generate an antibody-producing hybridoma. A monoclonal antibody of the invention can also be obtained by screening a recombinant combinatorial library such as an antibody phage display library using, for example, an immunogenic molecular entity of the invention. See, for example, Barbas, C.F., 3rd, D.R. Burton, J.K. Scott, and G.J. Silverman, *Phage Display – A Laboratory Manual*. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; and Kontermann, R., Dübel, S., Antibody Engineering, 2001, Berlin, Heidelberg: Springer-Verlag

An immunologically-active fragment of an antibody is the biologically active fragment of an immunoglobulin molecule, for example, the F(ab) or $F(ab')_2$ fragment generated by cleavage of the antibody with an enzyme such as

pepsin. An immunologically-active fragment can also be a single chain variable fragment (scFv) that results from the joining of the variable fragments of the heavy and light chains.

An antibody of the invention can also be a murine, chimeric, humanized or fully human antibody. A murine antibody is an antibody derived entirely from a murine source, for example, an antibody derived from a murine hybridoma generated from the fusion of a mouse myeloma cell and a mouse B-lymphocyte cell. A chimeric antibody is an antibody that has variable regions derived from a non-human source, e.g. murine or primate, and constant regions derived from a human source. A humanized antibody has antigen-binding regions, e.g. complementarity-determining regions, derived from a mouse source, and the remaining variable regions and constant regions derived from a human source. A fully human antibody is antibody from human cells or derived from transgenic mice carrying human antibody genes.

Methods to generate antibodies are well known in the art. For example, a polyclonal antibody of the invention can be prepared by immunizing a suitable mammal with an immunogenic molecular entity of the invention. The mammal can be, for example, a rabbit, goat, or mouse. At the appropriate time after immunization, antibody molecules can be isolated from the mammal, e.g. from the blood or other fluid of the mammal, and further purified using standard techniques that include, without limitation, precipitation using ammonium sulfate, gel filtration chromatography, ion exchange chromatography or affinity chromatography using protein A. In addition, an antibody-producing cell of the mammal can be isolated and used to prepare a hybridoma cell that secretes a monoclonal antibody of the invention. Techniques for preparing monoclonal antibody-secreting hybridoma cells are known in the art. See, for example, Kohler and Milstein, *Nature* 256:495-97 (1975) and Kozbor *et al. Immunol Today* 4: 72 (1983). A monoclonal antibody of the invention can also be prepared using other methods known in the art, such as, for example, expression from a recombinant DNA molecule, or screening of a recombinant combinatorial immunoglobulin library using an immunogenic molecular entity of the invention as discussed above.

Methods to generate chimeric and humanized monoclonal antibodies are also well known in the art and include, for example, methods involving

recombinant DNA technology. A chimeric antibody can be produced by expression from a nucleic acid that encodes a non-human variable region and a human constant region of an antibody molecule. See, for example, Morrison et al., *Proc. Nat. Acad. Sci. U.S.A.* 86: 6851 (1984). A humanized antibody can be produced by expression from a nucleic acid that encodes non-human antigen-binding regions (complementarity-determining regions) and a human variable region (without antigen-binding regions) and human constant regions. See, for example, Jones *et al.*, *Nature* 321:522-24 (1986); and Verhoeven *et al.*, *Science* 239:1534-36 (1988). Completely human antibodies can be produced by immunizing engineered transgenic mice that express only human heavy and light chain genes. In this case, therapeutically useful monoclonal antibodies can then be obtained using conventional hybridoma technology. See, for example, Lonberg & Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). Nucleic acids and techniques involved in design and production of antibodies are well known in the art. See, for example, Batra *et al.*, *Hybridoma* 13:87-97 (1994); Berdoz *et al.*, *PCR Methods Appl.* 4: 256-64 (1995); Boulianne *et al. Nature* 312:643-46 (1984); Carson *et al.*, *Adv. Immunol.* 38 :274-311 (1986) ; Chiang *et al.*, *Biotechniques* 7 :360-66 (1989) ; Cole *et al.*, *Mol. Cell. Biochem.* 62 :109-20 (1984) ; Jones *et al.*, *Nature* 321 : 522-25 (1986) ; Larrick *et al.*, *Biochem Biophys. Res. Commun.* 160 :1250-56 (1989) ; Morrison, *Annu. Rev. Immunol.* 10 :239-65 (1992) ; Morrison *et al.*, *Proc. Nat'l Acad. Sci. USA* 81 : 6851-55 (1984) ; Orlandi *et al.*, *Pro. Nat'l Acad. Sci. U.S.A.* 86:3833-37 (1989); Sandhu, *Crit. Rev. Biotechnol.* 12:437-62 (1992); Gavilondo & Larrick, *Biotechniques* 29: 128-32 (2000); Huston & George, *Hum. Antibodies.* 10:127-42 (2001); Kipriyanov & Le Gall, *Mol. Biotechnol.* 26: 39-60 (2004).

Examples of monoclonal antibodies and single chain variable fragments of the invention are shown below, as well as their coding nucleotide sequences.

Amino Acid Sequences of the Variable Heavy and Light Chains of Murine Monoclonal Antibodies

Antibody	Variable Heavy Chain	Variable Light Chain

AP1-15B4	EVHLVESGGDLVKPGGSLKLS CAASGFAFSDFAMSWVRQTPE KRLEWVAIIKSDDSYTYYPDS VRDRFTISRDNARNTLYLQMT SLRSEDTALYYCTKIYDAYFY AMDYWGQGTSVTVSS (SEQ ID NO: 19)	DIVRTQSPLSLSVSLGDQASISC RSSQSLLHSNGNTYLHWYLQKPG QSPKLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISILEAEDLGIYF CSQSTHFPTFGGGTKLEIK (SEQ ID NO: 147)
AP4-24H11	EVKPQESGPGLVKPSQSLSLT CTVTGYSITSNYAWNWIRQFP GNKLEWMGFISSYGTTTYNPS LKSRFSITRDTSKNQFFLQLH SVTIEDTGTYFCTREGDYWGQ GTTLTVSS (SEQ ID NO: 20)	DIVMTQATLSLPVSLGDQASISC RSSQRLVPSNGNIYLHWFLQKPG QSPKLLIYKLSSRFSGVPDRFSG SGSGTDFTLKISRVESEDLGIYF CSQTTHVPYTFGGGTKLEIK (SEQ ID NO: 148)
AP4-29E10-1	EVQLQQSGPELEKPGASVKIS CKASGHSFTGYNMNWVKQSND KSLEWIGNIAPYYGVTAYNQK FKGKATLTGDKSSSTAYMQLK SLASEDSAVYYCVLDTSGYAS WGQGTLVTVSA (SEQ ID NO: 21)	DIVMTQATASLTVSLGQRATISC RASKSVSTSGYSYMHWYQQKPGQ PPKLLIYLASNLESGVPARFSGS GSGTDFTLNIHPVEEEDAATYYC QHSREVPYTFGGGTKLELK (SEQ ID NO: 149)
AP4-29E10-2	QVQLQQSGPELEKPGASVKIS CKASGHSFTGYNMNWVKQSND KSLEWIGNIAPYYGVTAYNQK FKGKATLTGDKSSSTAYMQLK SLTSEDSAVYYCVLDTSGYAS WGQGTLVTVSA (SEQ ID NO: 22)	DIEMTQITASLTVSLGQRATISC RASKSVSTSGYSYMHWYQQKPGQ PPKLLIYLASNLESGVPARFSGS GSGTDFTLNIHPVEEEDAATYYC QHSREVPYTFGGGTKLELK (SEQ ID NO: 150)
AP1-15B4-Δ	GGDLVKPGGSLKLSCAASGFA FSDFAMSWVRQTPEKRLEWVA IIKSDDSYTYYPDSVRDRFTI SRDNARNTLYLQMTSLRSEDT ALYYCTKIYDAYFYAMDYWGQ GTS (SEQ ID NO: 23)	PLSLSVSLGDQASISCRSSQSLL HSNGNTYLHWYLQKPGQSPKLLI YKVSNRFSGVPDRFSGSGSGTDF TLKISILEAEDLGIYFCSQSTHF PTFGGGT (SEQ ID NO: 151)
AP4-24H11-Δ	GPGLVKPSQSLSLTCTVTGYS ITSNYAWNWIRQFPGNKLEWM GFISSYGTTTYNPSLKSRFSI TRDTSKNQFFLQLHSVTIEDT GTYFCTREGDYWGQGTT (SEQ ID NO: 24)	TLSLPVSLGDQASISCRSSQRLV PSNGNIYLHWFLQKPGQSPKLLI YKLSSRFSGVPDRFSGSGSGTDF TLKISRVESEDLGIYFCSQTTHV PYTFGGGT (SEQ ID NO: 152)
AP4-29E10-1-Δ	GPELEKPGASVKISCKASGHS FTGYNMNWVKQSNDKSLEWIG NIAPYYGVTAYNQKFKGKATL TGDKSSSTAYMQLKSLASEDS AVYYCVLDTSGYASWGQGTL (SEQ ID NO: 25)	TASLTVSLGQRATISCRASKSVS TSGYSYMHWYQQKPGQPPKLLIY LASNLESGVPARFSGSGSGTDFT LNIHPVEEEDAATYYCQHSREVP YTFGGGT (SEQ ID NO: 153)
AP4-29E10-2-Δ	GPELEKPGASVKISCKASGHS FTGYNMNWVKQSNDKSLEWIG NIAPYYGVTAYNQKFKGKATL TGDKSSSTAYMQLKSLTSEDS AVYYCVLDTSGYASWGQGTL (SEQ ID NO: 26)	TASLTVSLGQRATISCRASKSVS TSGYSYMHWYQQKPGQPPKLLIY LASNLESGVPARFSGSGSGTDFT LNIHPVEEEDAATYYCQHSREVP YTFGGGT (SEQ ID NO: 154)

Nucleic Acid Sequences Encoding the Variable Heavy and Light Chains of Murine Monoclonal Antibodies

Antibody	Variable Heavy Chain	Variable Light Chain
AP1-15B4	gaggtgcacctggtggagtctgggggagacttagtgaagcctgggggg tccctcaaactctcctgtgcagcctctggattcgcttcagtgacttt gccatgtcttgggttcgccagactccggagaaggctggagtgggtc gcaatcattaaaagtgatgattcttacacctactatccagacagtgtg agggaccgattcaccatctccagagacaatgccagggaacacccttac ctgcaaatgaccagtctgaggtctgaagacacggccttgtattactgt acaaaaatctatgatgcttacttctatggactactgggggtcaa ggaacctcagtcaccgtctcctcg (SEQ ID NO: 27)	gacattgtgaggacacagtctccactctccctgtctgtcagtcttggag atcaagcctccatcttcttgtagatctagtcagagccttatacacagtaa tggaaacacctatttacattggtacctgcagaagccaggccagtctcca aaactcctgatctacaaagtttccaaccgatttctgggtcccagaca ggttcagtggcagtggatcagggacagatttcacactcaagatcagcat attggaggctgaggatctgggaatttatttctgctctcaaagtacacat tttccgacgttcggtggaggcaccaagctggaaataaaa (SEQ ID NO: 155)
AP4-24H11	gaggtgaagcctcaggagtcaggacctggcctggtgaaaccttctcag tctctgtccctcacctgcactgtcactcaatcaccagtaat tatgcctggaactggatccggcagtttccaggaaacaaactggagtgg atgggcttcataagttcctatggaaccactacctacaacccttcctc aaaagtcgattctctatcactcgagacacatccaagaaccagttcttc ctgcaattgcattctgtgactattgaggacacaggcacatatttctgt acaagagagggtgactactgtgggccaaggcaccactctcacagtctcc tca (SEQ ID NO: 28)	gacattgtgatgactcaggctacactctccctgcctgtcagtcttggag accaagcctccatctcttgcagatccagtcagcgccttgttcccagtaa tggaaacatttatttacattggttcctgcagaagccaggccagtctcca aagctcctgatctacaaactttccagtcgatttttctgggtcccagaca ggttcagtggcagtggatcagggacagatttcacactcaagatcagcag agtggagtctgaggatctgggaatttatttctgctctcaaactacacat gttccatacacgttcggagggggggaccaagctggaaatcaaa (SEQ ID NO: 156)
AP4-29E10-1	gaggtccagctgcaacagtccggacctgagctggagaagcctggcgct tcagtgaagatatcctgcaaggcttctggttcattcactggctac aacatgaactgggtgaagcagagcaatgacaagagccttgagtggatt ggaaatattgctcctactatggtgttactgcctacaaccagaagttc aagggcaaggccacattgactggagacaaatcctccagcactgcctac atgcagctcaagagcctgccatctgaggactctgcagtctattactgt gtcctagacacctcgggctacgcttcctgggccaagggactctggta actgtctctgca (SEQ ID NO: 29)	gacattgtgatgactcaggctacactgcttccttaactgtatctctgggggc agagggccaccatcctcatgcagggccagcaaaagtgtcagtacatctgg ctatagttatatgcactggtaccaacagaaaccaggacagccacccaaa ctcctcatctatcttgcatccaacctagaatctgggtccctgccaggt tcagtggcagtgggtctgggacagacttcaccctcaacatccatcctgt ggaggaggaggatgctgcaacctattactgtcagcacagtagggaggtt ccgtacacgttcggagggggggaccaagctggagctgaaa (SEQ ID NO: 157)

AP4-29E10-2	caggtccagctgcagcagtctgggcctgagctggagaagcctggcgctt cagtgaagatatcctgcaaggcttctggtcatcattcactggctacaa catgaactgggtgaagcagagcaatgacaagagccttgagtggattgga aatattgctccttactatggtgttactgcctacaaccagaagttccaagg gcaaggccacattgactggagacaaatcctccagcactgcctacatgca gctcaagagcctgacatctgaggactctgcagtctattactgtgcccta gacacctcgggctacgcttcctgggggccaagggactctggtcactgtct ctgca (SEQ ID NO: 30)	gacattgagatgacccagattactgcttcctttaactgtatctctgggggc agagggccaccatcctcatgcagggccagcaaaagtgtcagtacatctgg ctatagttatatgcactggtaccaacagaaaccaggacagccacccaaa ctcctcatctatcttgcatccaacctagaatctgggtccctgccaggt tcagtggcagtgggtctgggacagacttcaccctcaacatccatcctgt ggaggaggaggatgctgcaacctattactgtcagcacagtagggaggtt ccgtacacgttcggagggggggaccaagctggagctgaaa (SEQ ID NO: 158)
AP1-15B4-Δ	ggggagacttagtgaagcctgggggtccctcaaactctcctgtgcca gcctctggattcgctttcagtgacttttgccatgtcttgggttcgccag actccggagaagaggctggagtgggtcgcaatcattaaaagtgatgat tcttacacctactatccagacagtgtgagggaccgattcaccatctcc agagacaatgccaggaaacaccctttacctgcaaatgaccagtctgagg tctgaagacacggccttgtattactgtacaaaaatctatgatgcttac ttctatgctatggactactggggtcaaggaacctca (SEQ ID NO: 31)	acactctccctgcctgtcagtcttggagaccaagcctccatctcttgca gatccagtcagcgccttgttcccagtaatggaaacatttatttacattg gttcctgcagaagccaggccagtcctccaaagctcctgatctacaaactt tccagtcgatttctcttgggtcccagacaggttcagtggcagtggatcag ggacagatttcacactcaagatcagcatattggaggctgaggatctggg aatttatttctgctctcaaagtacacatttttccgacgttcggtggaggc acc (SEQ ID NO: 159)
AP4-24H11-Δ	ggacctggcctggtgaaaccttctcagtctctgtcccctcacctgcact gtcactggctactcaatcaccagtaattatgcctggaactggatccgg cagtttccaggaaacaaactggagtgggatgggtcgcaatcattaaaagtgatgat ggaaccactacctacaaccctctctcaaaagtcgattctctatcact cgagacacatccaagaaccagttcttcctgcaattgcattctgtgact attgaggacacaggcacatatttctgtacaagagagggtgactactgg ggccaaggcaccact (SEQ ID NO: 32)	acactctccctgcctgtcagtcttggagaccaagcctccatctcttgca gatccagtcagcgccttgttcccagtaatggaaacatttatttacattg gttcctgcagaagccaggccagtcctccaaagctcctgatctacaaactt tccagtcgatttctcttgggtcccagacaggttcagtggcagtggatcag ggacagatttcacactcaagatcagcatattggaggctgaggatctggg aatttatttctgctctcaaactacacatgttccatacacgttcggaggg gggacc (SEQ ID NO: 160)
AP4-29E10-1-Δ	ggacctgagctggagaagcctggcgcttcagtgaagatatcctgcaag gcttctggtcattccattcactggctacaacatgaactgggtgaagcag agcaatgacaagagagccttgagtggattggaaatattgctccttactat ggtgttactgcctacaaccagaagttccaaggccaaggccacattgact ggagacaaatcctccagcactgcctacatgcagctcaagagcctgacg tctgaggactctgcagtctattactgtgtcctagacacctcgggctac gcttcctgggggccaagggactctg (SEQ ID NO: 33)	actgcttccttaactgtatctctgggcagagggccaccatctcatgca gggccagcaaaagtgtcagtacatctggctatagtttatatgcactggta ccaacagaaaccaggacagccacccaaactcctcatctatcttgcatcc aacctagaatctgggtccctgccaggttcagtggcagtgggtctggga cagacttcaccctcaacatccatcctgtggaggaggaggatgctgcaac ctattactgtcagcacagtagggaggttccgtacacgttcggagggggg acc (SEQ ID NO: 161)

AP4-29E10-2-Δ	gggcctgagctggagaagcctggcgcttcagtgaagatatcctgcaagg cttctggtcattcattcactggctacaacatgaactgggtgaagcagag caatgacaagagccttgagtggattggaaatattgctccttactatggt gttactgcctacaaccagaagttcaagggcaaggccacattgactggag acaaatcctccagcactgcctacatgcagctcaagagcctgacatctga ggactctgcagtctattactgtgtcctagacacctcgggctacgcttcc tggggccaagggactctg (SEQ ID NO: 34)	actgcttccttaactgtatctctgtgggcagagggccaccatctcatgca gggccagcaaaagtgtcagtacatcctggctatagttatatgcactggta ccaacagaaaccaggacagccacccaaactcctcatctatcttgcatcc aacctagaatctgtgggtccctgccaggttccagtggcagtgggtctggga cagacttcaccctcaacatccatcctgtggaggaggaggatgctgcaac ctattactgtcagcacagtagggaggttccgtacacgttcggagggggg acc (SEQ ID NO: 162)

Amino Acid Sequences of Human scFv Antibodies

AP1-2	QVQLVQSGAEVKKPGESLRISCKGSGYSFTSHWISWVRQMPGKGLEWMGRIDPSDSYSNYSPSFQGHVIISVDKSISTAYLQWSSLKASDTAIYY CARQLIVVVPAAPYYYYYYGMDVWGQGTLVTVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQTVNSYLAWYQKPGQAPRLL IYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSHPWTFGQGTKVEIK (SEQ ID NO: 35)
AP1-6	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAIYY CARVFGSESQDPSDIWSGYYGMEVWGQGTLVTVSSGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPK LLIYAASSLQSRVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPYTFGQGTKLEIK (SEQ ID NO: 36)
AP1-8	QVQLVESGAEAKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC ARAGITGTTAPPDYWGQGTLVTVSSGGGSGGGGSGGGGSVIWMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQRKPGKAPKLLIYAASSLQS GVTSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKLEIK (SEQ ID NO: 37)
AP1-11	QVQLVQSGSELKKPGASVKLSCRASGYTFTSYSMVWVRQAPGEGLEWMGGINTNTGNPTYAQGFTERFVFSFDSSVSTAYLQISSLKAEDTAVYY CARDWAYSGSWPLGQNPSDHWGQGTLVTVSSGGGSGGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSRNLAWYQQKPGQAPRLLIY DTSTRATGIPARFSGSGSGTEFTLTISSLQSEDSAVYYCQQYNIWPPLTFGGGTKVEIK (SEQ ID NO: 38)
AP1-15	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYRTWIRQSPVKGLEWIGEVNDRGSPNYNPSFKSRLTISIDTSKNLSLKLRFMTAADTAVYSCA RIRPRYGMDVWGQGTMVTVSSGGGSGGGGSGGGGSDIVMTQTPLSSPVTLGQPASISCRSSQSLVHSDGNTYLTWFHQRPGQPPRVLIHKVSNL FSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATQLYTFGQGTKVEIK (SEQ ID NO: 39)
AP1-16	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETISAQKFQGRVTMTEDTSTDTAYMDLSSLRSEDTAVYYC ATQRLCSGGRCYSHFDYWGQGTTVTVSSGGGSGGGGSGGGGSETTLTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQKPGSSPRLLIYDTSNV APGVPFRFSGSGSGTSYSLTINRMEAEDAATYYCQEWSGYPYTFGGGTKVEIK (SEQ ID NO: 40)

AP1-19	QMQLVQSGAEVKKPGSSVKVSCKASGGTFNTYVISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYY CARVWSPLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNMNYLAWYQQKPGQPPKLLIYWAST RESGVPDRFSGSGSGTDFTLTISSLQAEDAAVYYCQQYYSTPPTFGQGTKLEIK (SEQ ID NO: 41)
AP3-1	QVQLVQSGAEVKKPGASVKVSCKGSGYTFTGYYMHWVPQAPGQGLEWMGWINPNNGGTNYDQKFQGRVAMTRDTSISTAYMELSRLRSDDTAVYY CARDNGRVTTGGYWGQGTLVTVSSGGGGSGGGGSGGGSQSVLTQPPSLSGAPGQSVTISCAGTSSSIGAGYDVQWYQQLPGKTPKLLIYGNDNR PSGVPDRFSGSRSYTSASLVITRVQIEDEADYYCQSYDSSLIGPQFGGGTKLTVLG (SEQ ID NO: 42)
AP3-2	QVQLVQSGAEVKKPGESLKISCTASGYNFASYWIGWVRQMPGQGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTATYY CVRRVPLYTNNHYLDYWGQGTLVTVSSGGGGSGGGGSGGGGSAIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASS LQSGVPSKYSGSGSGTDFTLTISSLQPEDFATYYCQQYKSYPLTFGGGTKVEIK (SEQ ID NO: 43)
AP3-3	EVQLVQSGAEVKKPGASVKVSCKASGYTFSDYFMHWVRQAPGQGLEWMGVINPTGGSTTYAQSFQGRVTMTRDTSTSIVYMELSSLRSEDTAVYY CTRVGYYGMDVWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPSTLSASVGDRVTITCRASQSTSRFLNWYQQKPGQAPKLLIYAASSLHSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTSSYPLTFGGGTKVEIK (SEQ ID NO: 44)
AP3-5	QVQLVQSGGGVVQVGRSLRLSCAASGFTFTNFGMHWVRQAPGKGLEWVALISSDGYRQAYADSVKGRFTISGDNSKNTVYLQMNSLTSEDTAVYY CAIIPPVLRIFDWEFDYWGQGTLVTVSSGGGGSGGGGSGGGGSETTLTQSPGTLSLSPGERATLSCRASQSVSSPYLAWYQQKPGQAPRLLIYGA SNRATGIPDRFSGSGSGTDFTLTISSLQAEDEAVYYCQQYYNTPLTFGGGTKVEIK (SEQ ID NO: 45)
AP3-6	QVQLQQWGAGLLKPSETLSLTCAVYSGSFTRDYWGWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTTSVDKSKNQFSLKLTSVTAADTAVYYC ARRRLSSDLFMRGVGGMDVWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQTPGTLSSSPGERATLSCRASQGVSSNLAWYQQKPGQAPRLLIYD ASNRATGIPLRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYGSSPYTFGQGTKVEIK (SEQ ID NO: 46)
AP3-8	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQASGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYY CARVPRYFDWLLYGSDYFDYWGQGTLVTVSSGGGGSGGGGSGGGSDIQMTQSPSTLSVSVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKLEIK (SEQ ID NO: 47)
AP3-10	QVQLVQSGAEVKEPGSSVKVSCKASGGTFSSYAIYWVRQAPGQGLEWMGWIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYC ARAAGHSTNYYYYGMDVWGQGTLVTVSSGGGGSGGGGSGGGSQTVVTQEPSLTVSLGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAPRTLIYDT SNKHSWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLLSYSGTRVFGGGTKLTVLG (SEQ ID NO: 48)
AP3-13	EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYY CARDFKEYSRTGYFDYWGQGTLVTVSSGGGGSGGGGSGGGGSSYELMQPSSVSVSPGQTARITCSGDVLAKKCARWFQQKPGQAPVLVIYKDSER PSGIPERFSGSSSGTTVTLTISCAQVEDEADYYCYSAADNNLGVFGGGTKVTVLG (SEQ ID NO: 49)
AP3-20	QITLKESGPALVKPTQTLTLTCNFSGFSLSTYGGGVGWLRQPPGKALEWLAVIYWSDGKRYSPSVKNRLTITKDTSKNHVVLTMTNMDPVDTATY YCAHLMMDTSITTHWFDPWGQGTLVTVSSGGGGSGGGGSGGGGSAIRMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAA STLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPGTFGQGTKVEIK (SEQ ID NO: 50)

AP4-8	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYFIHWVRQAPGQGLEWMGLLNPTDSGTLYAQNFQGRITMTSDTSTNTVYMELSSLRSDDTAMYY CAREGGADTTRVHSSFDYWGQGTLVTVSSGGGGSGGGGSSGGGSQAVLTQPPSVSGSPGQSITISCTGTSSDVEAYNYVSWYQQHPGKAPKLMIY DVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSRTWVFGGGTKVIVL (SEQ ID NO: 51)
AP4-14	QVQLQESGGGLVQPGRSLRLSCAASGFTFDDYALHWVRQAPGKGLEWVSGISWNSVTVKYAVSVKGRFTISRDNAKNSLFLQMNALRSEDTALYYC AKARGALLEAADTPSDDWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPWTFGQGTKVDIK (SEQ ID NO: 52)
AP4-20	QVQLQQSGAGLLRPSETLSLTCGLYGGSFSGHYWNWIRQSPEKGLVWIGEITHSGTTNYNPSLKSRVITSVDTSKNQYSLKLSFVTPADTAVYYCA RGDYYGYWYFDLWGRGTLVTVSSGGGSGGGGSGGGGSQSVLTQPPSVPVAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDTNKRPSG IPDRFAGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGVFGGGTKLTVL (SEQ ID NO: 53)

Nucleotide Sequences Encoding the Heavy and Light Chains of Human scFv Antibodies

Antibody	Variable Heavy Chain	Variable Light Chain
AP1-2	caggtgcagctggtgcagtctggagcagaggtgaaaaagcccgggga gtctctgaggatctcctgcaagggttctggatacagctttaccagcc actggatcagctgggtgcgccagatgcccgggaaaggcctggagtgg atggggaggattgatcctagtgactcttatagcaactacagcccctc cttccaaggccacgtcatcatctcagttgacaagtccatcagcactg cctacttgcagtggagcagcctgaaggcctcggacaccgccatatat tactgtgcgagacagctcattgtagtagtaccagctgctccctatta ctactactacggtatggacgtctgggccaaggaaccctggtca ccgtctcctca (SEQ ID NO: 163)	gaaattgtgttgacgcagtctccaggcaccctgtctttgtctccagg ggaaagagccaccctctcctgcagggccagtcagactgttaacagct acttagcctggtaccagtagaaacctggccaggctcccaggctcctc atctatggtgcatccagcagggccactggcatcccagacaggttcag tggcagtgggtctgggacagacttcactctcaccatcagcagactgg agcctgaagattttgcagtgtattactgtcagcagtatggtagctca catccgtggacgttcggccaagggaccaaggtggagatcaaacgtgg cctcgggggcctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 73)

AP1-6	caggttcagctggtgcagtctgggctgaggtgaagaagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatcagctgggtgcgacaggccccctggacaagggcttgagtgg atgggagggatcatccctatctttggtacagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacgaatccacgagcacag cctacatggagctgagcagcctgagatctgaggacacggccatatat tactgtgcgagagtctttggttccgagtcgcaagatccgtccgatat ttggagtggttattacggtatggaagtctgggggccaaggaaccctgg tcaccgtctccctca (SEQ ID NO: 164)	gacatccagatgacccagtctccgtcttccgtctgcatctgtagg agacagagtcaccatcacttgtcgggcgagtcagggtattagcagct ggttagcctggtatcagcagaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtagggtcccatcaaggttcag cggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagatttttgcaactactattgtcaacaggctaacagtttc ccgtacactttttggccagggggaccaagctggagatcaaacgtgggct cgggggcctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 74)
AP1-8	caggtgcagctggtggagtctgggggctgaggcgaagaagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatcagctgggtgcgacaggccccctggacaagggcttgagtgg atgggagggatcatccctatctttggtacagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacgaatccacgagcacag cctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagccggtataactggaactacggctcccccagacta ctggggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 165)	gtcatctggatgacccagtctccatcctccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcaagtcagagcattagcagct atttaaattggtatcagcggaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtggggtcacatcaaggttcag tggcagtggatctgggacagatttcactctcaccatcagcagtctgc aacctgaagatttttgcaactactattgtcaacagagttacagtacc cctccgacgttcggccaagggaccaagctggagatcaaa (SEQ ID NO: 75)
AP1-11	caggtgcagctggtgcagtccggatctgagttaaagaagcctgggggc ctcagtgaagctttcctgcagggcttctggatacacattcactagtt attccatggtttgggtgcgacaggccccctggagaagggcttgagtgg atgggagggatcaacaccaacactgggaacccaacgtatgcccagggg cttcacagaacggtttgtctttcctcgacagctctgtcagcacgg catatctgcaaatcagcagcctaaaggctgaggacactgccgtgtat tactgtgcgagagattgggcgtatagcggcagctggccctttaggcca gaaccctttctgaccactgtgggccagggcaccctggtcaccgtctcct ca (SEQ ID NO: 166)	gaaatagtgatgacgcagtctccagccaccctgtctgtctccagg ggaaagagccaccctcctcctgcagggccagtcagagtgttagccgca acttagcctggtaccagcagaaacctggccaggctcccaggctcctc atctatgatacatccaccagggccactggtatcccagccaggttcag tggcagtgggtctgggacagagttcactctcaccatcagcagcctgc agtctgaagattttctgcagtttattactgtcagcagtatataatctgg cctccactcactttcggcggagggaccaaggtggagatcaaa (SEQ ID NO: 76)

AP1-15	caggtgcagctacagcagtggggcgcaggattgttgaagccttcgga gaccctgtccctcacctgcgctgtctatggtgggtccttcagtggtt actaccggacctggatccgccagtccccagtgaaggggctggagtgg attggggaagtcaatgatcgtggaagccccaactacaacccgtcctt caagagtcgactcaccatatcaatcgacacgtccaagaactagttat ccctgaagttgagatttatgaccgccgcggacacggctgtatattcg tgtgcgagaattaggcctaggtacggtatggacgtctggggccaggg gacaatggtcaccgtctcctcaggcggcggcggctct (SEQ ID NO: 167)	Gatattgtgatgacccagactccactctcctcacctgtcacccttgg acagccggcctccatctcctgcaggtctagtcaaagcctcgtacaca gtgatggaaacacctacttgacttggtttcaccagaggccaggccag cctccaagagtcctcattcataaggtttctaacctgttctctggggt cccagacagattcagtggcagtggggcagggacagatttcacactga aaatcagcagggtggaagctgaggatgtcggggtttattactgcatg caagctacacaattgtacacttttggccaggggaccaaggtggaaat caaa (SEQ ID NO: 77)
AP1-16	gaggtccagctggtacagtctggggctgaggtgaagaagcctggggc ctcagtgaaggtctcctgcaaggtttccggatacaccctcactgaat tatccatgcactgggtgcgacaggctcctggaaaagggcttgagtgg atgggaggttttgatcctgaagatggtgaaacaatctccgcgcagaa gttccagggcagagtcaccatgaccgaggacacatctacagacacag cctacatggatctgagcagcctgagatctgaggacacggccgtttat tactgtgcaacgcagcgcttgtgtagtggtggtcgctgctactccca ctttgactactggggccagggcaccacggtcaccgtctcctca (SEQ ID NO: 168)	gaaacgacactcacgcagtctccagcaatcatgtctgcatctccagg ggagagggtcaccatgacctgcagtgccagctcaagtatacgttaca tatattggtaccaacagaagcctggatcctcccccagactcctgatt tatgacacatccaacgtggctcctggagtccctttttcgcttcagtgg cagtgggtctgggacctcttattctctcacaatcaaccgaatggagg ctgaggatgctgccacttattactgccaggagtggagtggttatccg tacacgttcggaggggggaccaaggtggagatcaaa (SEQ ID NO: 78)
AP1-19	cagatgcagctggtgcagtctggggctgaggtgaagaagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcaacacct atgttatcagttgggtgcgacaggcccctggacaagggcttgagtgg atgggatggatcagcgcttacaatggtaacacaaactatgcacagaa gctccagggcagagtcaccatgaccacagacacatccacgagcacag cctacatggagctgaggagcctgagatctgacgacacggccgtgtat tactgtgcgagagtttggagtccccttgactactggggccagggcac cctggtcaccgtctcctca (SEQ ID NO: 169)	gacatcgtgatgacccagtctccagactccctggctgtgtctctggg cgagagggccaccatcaactgcaagtccagccagagtgttttataca gctccaacaatatgaactacttagcttggtaccagcagaaaccagga cagcctcctaagctgctcatttactgggcatctacccgggaatccgg ggtccctgaccgattcagtggcagcgggtctgggacagatttcactc tcaccatcagcagcctgcaggctgaagatgcggcagtttattactgt cagcagtattatagtactcctccgacgttcggccaagggaccaagct ggagatcaaa (SEQ ID NO: 79)
AP3-1	caggtgcagctggtgcaatctggggctgaggtgaagaagcctggggc ctcagtgaaggtctcctgcaagggttctggatacaccttcaccggct actatatgcactgggtgccacaggcccctggacaagggcttgagtgg atgggatggatcaaccctaacaatggtggcacaaactatgaccagaa gtttcagggcagggtcgccatgaccagggacacgtccatctccacag cctacatggagctgagcaggctgagatctgacgacactgccgtgtat tactgtgcgagagataatgggagggtgaccacaggggggctactgggg ccagggcaccctggtcaccgtctcctca (SEQ ID NO: 170)	cagtctgtgttgacgcagcctccctcattgtctggggccccgggaca gagtgtcaccatctcctgcgctgggaccagttccagcatcggggcag gttacgatgtacagtggtaccagcaacttccaggaaaaacccccaaa ctcctcatctacgggaatgataatcggccctcaggggtccctgaccg attctctggatccaggtcttacacctcagcctccctggtcatcacta gagtccagattgaggatgaggctgattattactgccagtcgtatgac agcagtctcattggtcctcaattcggcggg (SEQ ID NO: 80)

AP3-2	cagtgcagctggtgcaatctgggctgaggtgaaaaagcccgggga gtcctgaagatcctcctgtacggcctccggatacaacttttgccagct actggatcggctgggtgcgccagatgcccgggcaaggcctggagtgg atgggatcatctatcctggtgactctgataccagatacagtccgtc cttccaaggccaggtcaccatctcagccgacaagtccatcagcaccg cctacctgcagtggagcagcctgaaggcctcggacaccgccacgtat tactgtgtgagacgggtccccctctacactaacaaccactaccttga ctattgggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 171)	gccatccagatgacccagtctccatcctcactgtctgcatctgtagg agacagagtcaccatcacttgtcgggcgagtcagggcattagcaatt atttagcctggtttcagcagaaaccagggaaagcccctaagtccctg atctatgctgcatccagtttgcaaagtggggtcccatcaaagtacag cggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagattttgcaacttattactgccaacagtataagagttac ccctcactttcggcggagggaccaaggtggagatcaaa (SEQ ID NO: 81)	gacatcgtgatgacccagtctccatccaccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcaagtcagagcactagcaggt tttaaattggtatcagcagaaacctgggaaagcccctaaactcctg atctatgctgcatccagtttgcatagtggcgtcccatcaaggttcag tggcagtggatctgggacagatttcactctcaccatcagcagtctgc aacctgaagatttttgcaacttactactgtcaacagacttccagttac cctctcactttcggcggagggaccaaggtggaaatcaaa (SEQ ID NO: 82)
AP3-3	gaggtgcagctggtgcagtctggggctgaagtgaagaagcctggggc ctcagtgaaggtttcctgtaaggcatctggatacaccttcagcgact actttatgcactgggtgcgacaggcccctggacaagggcttgagtgg atggagtaatcaacccaactggtggttccacaacctacgcacagag cttccagggcagagtcaccatgaccgagacacgtccacgagcatag tctacatggagctgagcagcctgagatctgaagacacggccgtgtac tactgtacgcgagtcggctactacggtatggacgtctgggggccaagg caccctggtcaccgtctcctca (SEQ ID NO: 172)	gaaacgacactcacgcagtctccagccaccctgtctttgtctccagg ggaaagagccacctctcctgcagggccagtcagagtgtttccagcc cctacttagcctggtaccagcagaaacctggccaggctcccaggctc ctcatttatggtgcatctaacagggccactggcatcccagacaggtt cagtggcagtgggtctgggacagacttcactctcaccatcagcagcc tgcaggctgaagatgaggcagttttattactgtcagcaatactacaat actccgctcactttcggcggagggaccaaggtggaaatcaaa (SEQ ID NO: 83)
AP3-5	caggtccagctggtacagtctggggaggcgtggtccaggttgggag gtccctgagactttcctgtgcggcctctggattcaccttcacaaact ttggcatgcactgggtccgccaggctccagggcaagggctggagtgg gtggcactcatctcatctgatggatatagacaggcctatgcagactc cgtgaagggccggttcaccatctccggagacaactccaagaacacag tgtatctgcaaatgaacagcctgacaagtgaggacacggctgtttat tactgtgccatcataccccctgtattacggatttttgatgtggaatt tgactactggggccagggaaccctggtcaccgtctcctca (SEQ ID NO: 173)	

AP3-6	caggtgcagctacagcagtggggcgcaggcctgttgaagccttcgga gaccctgtccctcacctgcgctgtctatagtgggtctttttactcgtg actactgggctggatccgccagccccccgggaaggggctggagtgg attgggaaatcaatcatagtggaagcaccaactacaacccgtccct caagagtcgagtcaccacgtcggtagacaagtccaagaatcagttct ccctgaagttgacctctgtgaccgccgcggacacggctgtctattac tgtgcgagacgccggcttttctagcgacctcttcatgcgggggttgg cggtatggacgtctgggcaaggcaccctggtcaccgtctcctca (SEQ ID NO: 174)	gatattgtgatgacccagactccagcaccctgtcttcgtctccagg ggaaagagccaccctcctcctgcagggccagtcagggtgttagcagca acttagcctggtaccagcagaaacctggccaggctcccaggctcctc atctatgatgcatccaacagggccactggcatcccactcaggttcag tggcagtgggtctgggacagacttcactctcaccatcagcagactgg aacctgaagatttttgcagtgtattactgtcaccagtatggtagctca ccgtacaccttttggccagggggaccaagggtggaaatcaaa (SEQ ID NO: 84)
AP3-8	gaggtgcagctggtgcagtctggagctgaggtgaagaagcctggggc ctcagtgaaggtctcctgcaaggcttctggttacaccttaccagct atggtatcagctgggtgcgacaggcctctggacaagggcttgagtgg atgggatggatcagcgcttacaatggtaacacaaactatgcacagaa gctccagggcagagtcaccatgaccacagacacatccacgagcacag cctacatggagctgaggagcctgagatctgacgcacggccgtgtat tactgtgcgagagtaccccgatatttgactggttatatacgggag cgactactttgactactggggccagggaaccctggtcaccgtctcct ca (SEQ ID NO: 175)	gacatccagatgacccagtctccttccaccctgtctgtatctgtagg agacagagtcaccatcacttgtcgggcgagtcagggtattagcagct ggttagcctggtatcagcagaaaccagggaaagccctaagctcctg atctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag cggcagtggatctgggacagatttcactctcactatcagcagcctgc agcctgaagattttgcaacttactattgtcaacaggctaacagtttc ccgctcactttcggcggagggaccaagctggagatcaaa (SEQ ID NO: 85)
AP3-10	caggtgcagctggtgcaatctggagctgaggtgaaggagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatctactgggtgcgacaggccccctggacaagggcttgagtgg atgggatggatcatccctatcctttggtatagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacaaatccacgagcacag cctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagctgccggtcatagtactaactactactacgg tatggacgtctgggccaaggcaccctggtcaccgtctcctca (SEQ ID NO: 176)	cagactgtggtgacccaggagccctcactgactgtgtccctaggagg gacagtcactctcacctgtggctccagcactggagctgtcaccagtg gtcattatccctactgttccagcagaagcctggccaagccccccagg acactgatttatgatacaagcaacaaacactcctggacccctgcccg gtgcgcagcctgaggatgaggctgagtattactgcttgctctcctat agtggtactcgggtgttcggcggagggaccaagctgaccgtccta (SEQ ID NO: 86)

AP3-13	gaggtgcagctggtgcagtctgggggctgaggtgaagaagcctgggggc ctcagtgaaggtttcctgcaaggcatctggatacaccttcaccaact actatatgcactgggtgcgacaggccccctggacaagggcttgagtgg atgggaataatcaaccctagtggtggtagcacaagctacgcacagaaa gttccagggcagagtcaccatgactagggacacgtccacgagcacag tctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagatttcaaagagatagccgtacgggctactttga ctactgggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 177)
AP3-20	cagatcaccttgaaggagtctggtcctgcgctggtgaaacccacaca gaccctcacgctgacctgcaacttctctgggttctccctcagcactt atggagggggtgtgggctggctccgtcagccccaggaaaggccctg gagtggcttgccgtcatttattggagtgatggtaaacgctacagccc ctctgtaaagaaccggctcaccatcaccaaggacacctccaaaaacc acgtggtcctgacaatgaccaacatggaccctgtggacacagccacc tattattgtgcacaccttatgatggatacatctattactacccactg gttcgacccctggggccagggaaccctggtcaccgtctcctca (SEQ ID NO: 178)
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Antibody	Variable Heavy Chain
AP4-14	caggtgcagctgcaggagtcgggggaggcttggtacagcctggcag gtccctgagactctcctgtgcagcctctggattcacctttgatgatt atgccctccactgggtccggcaagctccagggaagggcctggagtgg gtctcaggtattagttggaatagtgttaccgtaaagtatgcggtcctc tgtgaagggccggttcaccatctccagagacaacgccaagaactccc tgtttctgcaaatgaacgctctgagatctgaggacacggccttatat tactgtgcaaaagccagagggccccctctagaagcagctgacacacc atctgacgactgggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 180)	gacatcgtgatgacccagtctccgtccctccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcaagtcagagcattagcagct atttaaattggtatcagcagaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag tggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagatgttgcaacttattactgtcaaaagtataacagtgcc ccgtggacgttcggccaagggaccaaagtggatatcaaa (SEQ ID NO: 90)
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Nucleic Acids Encoding the Human scFvs

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AP3-10	caggtgcagctggtgcaatctggagctgaggtgaagaagcctgggtcctcgtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatctactgggtgcgacaggccctggacaagggcttgagtgggatgggatcatccctatcctggtatagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacaaatccacgagcacagcctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagctgccggtcatagtactaactactactacggtatggacgtctgggccaaggcaccctggtcaccgtctcctcaggcg gcggcggctctggcggaggtggcagcagcggtggcggatcccagactgtggtgacccaggagccctcactgactgtgtccctaggagggacagt cactctcacctgtggctccagcactggagctgtcaccagtggttcatatccctactgattccagcagaagcctggccaagccccccaggacactg atttatgatacaagcaacaaacactcctggacccctgcccggttctcaggctccctccttgggggcaaagctgccctgaccctttcgggtgcgc agcctgaggatgaggctgagtattactgctgctctcctatagtggtactcgggtgttcggcggagggaccaagctgaccgtcctaggt (SEQ ID NO: 67)
AP3-13	gaggtgcagctggtgcagtctgggggctgaggtgaagaagcctgggggcctcagtgaaggtttcctgcaaggcatctggatacaccttcaccaact actatatgcactgggtgcgacaggccccctggacaagggcttgagtggatgggataataatcaaccctagtggtggtagcacaagctacgcacagaa gttccagggcagagtcaccatgactagggacacgtccacgagcacagtctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagatttcaaagagtatagccgtacgggctactttgactactgggggccagggcaccctggtcaccgtctcctcaggcggcggcg gctctggcggaggtggcagcagcggtggcggatcctctcctatgagctgatgcagccatcctcagtgtcagtgtctccgggacagacagccaggat cacctgctcaggagatgtactggcaaaaaaatgtgctcggtggttccagcagaagccaggccagggcccctgtgctggtgatttataaagacagt gagcggccctcagggatccctgagcgattctccggctccagctcagggaccacagtcaccttgaccatcagcgggggcccaggttgaggatgagg ctgactattactgttactctgcggctgacaacaacctgggggtgttcggcggagggaccaaggtcaccgtcctaggt (SEQ ID NO: 68)

AP3-20	cagatcaccttgaaggagtctggtcctgcgctggtgaaacccacacagaccctcacgctgacctgcaacttctctgggttctccctcagcactt atggagggggtgtgggctggctccgtcagcccccaggaaaggccctggagtggcttgccgtcatttattggagtgatggtaaacgctacagccc ctctgtaaagaaccggctcaccatcaccaaggacacctccaaaaaaccacgtggtcctgacaatgaccacaacatggacctgtggacacagccacc tattattgtgcacacctttatgatgatacatctattactacccactggttcgaccctgggccagggaaccctggtcaccgtctcctcaggcg gcggcggctctggcggaggtggcagcggcggtggcggatccggccatccggatgacccagtctcccatcctccctgtctgcatctgtaggagacag agtcaccatcacttgccgggcgagtcagggcattagcaattatttagcctggtatcagcagaaaccagggaaagttcctaagctcctgatctat gctgcatccactttgcaatcagggtcccatctcggttcagcggcagtggatctgggacagatttcactctcaccatcagcagcctgcagcctg aagatgttgcaacttattactgtcaaaagtataacagtgcccctgggacgttcggccaagggaccaaggtggagatcaaacgtggcctcgggggg cctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 69)
AP4-8	caggtgcagctggtgcaatctgggggctgaggtgaagaagcctgggtcctcggtgaaggtttcctgcaaggcatctggatacaccttcaccaact actttatacactgggtgcgacaggccctggacaagggcttgagtggatgggactactcaaccctactgatagtggcacacttctacgcacagaa ctccagggcagaatcaccatgaccagtgacacgtccacaaacacagtctacatggagctgagcagcctgagatctgacgacacggccatgtat tactgtgcaagagagggggggccgacactacccgggtccacttcttcgtttgactactgtgggccagggaaccctggtcaccgtctcctcaggcg gcggcggctctggcggaggtggcagcagcggtggcggatcccaggctgtgctgactcagccgccttccgtgtcgggtctcctggacagtcgat caccatctcctgcactggaaccagcagtgacgttgaagcttacaactatgtctcctggtatcaacaacacccaggcaaagcccccaaactcatg atttatgatgtcagtaatcggccctcagggttctctcgctttctctggctccaagtctggcaacacgggcctccctgaccatctctgggctcc aggctgaggacgaggctgattattactgcagctcatatacaagcagcagcactgggtgttcggcggagggaccaagggtcatcgtccta (SEQ ID NO: 70)
AP4-14	caggtgcagctgcaggagtcgggggaggcttggtacagcctggcaggtccctgagactctcctgtgcagcctctggattcaccttttgatgatt atgccctccactgggtccggcaagctccagggaagggcctggagtgggtctcaggtattagttggaatagtgttaccgtaaagtatgcggtctc tgtgaagggccggttcaccatctccagagacaacgccaagaactccctgtttctgcaaatgaacgctctgagatctgaggacacggccttatat tactgtgcaaaagccagaggggccctcttagaagcagctgacacaccatctgacgactgggggccagggcaccctggtcaccgtctcctcaggcg gcggcggctctggcggaggtggcagcggcggtggcggatccgacatcgtgatgacccagtctccgtctccctgtctgcatctgtaggagacag agtcaccatcacttgccgggcaagtcagagcattagcagctatttaaattggtatcagcagaaaccagggaaagcccctaagctcctgatctat gctgcatccagtttgcaaagtggggtcccatcaaggttcagtggcagtggatctgggacagatttcactctcaccatcagcagcctgcagcctg aagatgttgcaacttattactgtcaaaagtataacagtgccccgtggacgttcggccaagggaccaaggtggatatcaaa (SEQ ID NO: 71)

AP4-20	caggtacagctgcagcagtcaggcgcaggtctattgaggccttcggagaccctgtccctcacctgcggtctctatggtgggtccttcagtggtc actattggaactggatccgccagtccccagaaaagggctggtgtggattggggaaatcactcatagtggaaccaccaattacaacccgtccct caagagtcgagtcatcacatcagtagacacgtccaagaatcagtactccctgaagctgagctttgtgaccctgcggacacggccgtgtattac tgtgcgagaggtgattactatgggtactgacttcgatctctggggccgtggcaccctggtcaccgtctcctcaggcggcggcggctctggcg gaggtggcagcggcggtgggatcccagtctgtgttgacgcagccgccctcagttcctgtggccccaggacagaaggtcaccatctcctgctc tggaagcagctccaacaattgggaataattatgtatcctggtaccagcagctcccaggaacagcccccaaactcctcatttatgacactaataag cgaccctcagggattcctgaccgattcgctggctccaagtctggcacgtcagccaccctgggcatcaccggactccagactggggacgaggccg attattactgcggaacatgggatagcagcctgagtgctggcgtgttcggcggagggaccaagctgaccgtccta (SEQ ID NO: 72)

An antibody of the invention can be used to detect the presence, or determine the amount, of a cyclic signaling peptide in a biological sample. An antibody of the invention can also be used for a prophylactic purpose to prevent a mammal from becoming infected with a Gram positive bacterium or developing a disease or condition that is caused by a Gram positive bacterium.

Pharmaceutical Compositions of the Invention

The immunogenic molecular entity, supramolecular assembly including the immunogenic molecular entity or antibody of the invention, herein "active agents" of the invention, can be incorporated into a pharmaceutical composition for administration to a mammal. A pharmaceutical composition of the invention can include one or more active agents of the invention (e.g. one or more antibodies, immunogenic molecular entities, supramolecular assemblies or combinations thereof). A pharmaceutical composition of the invention can also include one or more active agents of the invention in combination with another polypeptide or antibody vaccine.

For example, a pharmaceutical composition of the invention may include one or more immunogenic molecular entities, the haptens of which include the lactone, lactam, carbamide or semicarbazide analogs of a *S. aureus* AIP-I, AIP-II, AIP-III or any combination thereof. Thus, a pharmaceutical composition of the invention may include a combination of two or more immunogenic molecular entities of the invention, each of which has a hapten that includes to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP cyclic peptide signaling molecule.

A pharmaceutical composition of the invention can include two different immunogenic molecular entities of the invention: (1) the first having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-I cyclic signaling peptide, and a second having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-II, III or IV; (2) the first having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-II cyclic signaling peptide, and a second having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-III or IV; or (3) the first having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-III cyclic signaling peptide, and a

second having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-IV.

A pharmaceutical composition of the invention can also include three different immunogenic molecular entities of the invention, for example: (1) a first having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-I cyclic signaling peptide, a second having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-II, and a third immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-III; (2) a first immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-I cyclic signaling peptide, a second immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-II, and a third immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-IV; (3) a first immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-I cyclic signaling peptide, a second immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-III, and a third immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-IV; (4) a first immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-II cyclic signaling peptide, a second immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-III, and a third immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-IV.

A pharmaceutical composition of the invention can also include four different immunogenic molecular entities of the invention, for example, a first immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-I cyclic signaling peptide, a second immunogenic molecular entity having a hapten that

corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-II, a third immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-III; and a fourth immunogenic molecular entity having a hapten that corresponds to the lactone, lactam, carbamide or semicarbazide analog of a *S. aureus* AIP-IV.

Similarly, a pharmaceutical composition of the invention can also include one or more antibodies that bind specifically one or more cyclic peptide signaling molecules. For example, a pharmaceutical composition of the invention can include an antibody that binds specifically to any one of the *S. aureus* AIP-I, AIP-II, AIP-III or AIP-IV cyclic signaling peptides. A pharmaceutical composition of the invention can include two or more antibodies that bind specifically to two or more cyclic signaling peptides, for example, any two, three or all four cyclic signaling peptides of the *S. aureus* AIP-I, AIP-II, AIP-III or AIP-IV cyclic signaling peptides.

A pharmaceutical composition of the invention can also include one or more immunogenic molecular entities having haptens that correspond to cyclic signaling peptides from one or more Gram positive bacteria, as well as one or more antibodies that bind specifically with one or more cyclic signaling peptides from one or more Gram positive bacteria that use quorum sensing.

A pharmaceutical composition of the invention can also include the active agent of the invention in combination with one or more vaccines directed against different infectious agents including, without limitation, hepatitis B, *Haemophilus influenzae* type b bacteria, diphtheria, measles, mumps, pertussis, polio, rubella, tetanus, tuberculosis, and varicella.

In addition to the above, a pharmaceutical composition of the invention includes a pharmaceutically-acceptable carrier. As used herein, the term "pharmaceutically-acceptable carrier" includes, without limitation, any one or more solvents, dispersion media, coatings, antibacterial or antifungal agents, antioxidants, stabilizers, isotonic agents, adjuvants and the like that are suitable for administration to a mammal. Pharmaceutically-acceptable carriers are well known in the art, and unless a conventional carrier is incompatible with the immunogenic molecular entity or antibody of the invention, or incompatible with

the route of administration, use thereof in a composition of the invention is contemplated.

A pharmaceutical composition of the invention is formulated to be compatible with a selected route of administration. Examples of route of administration include any route of parenteral administration including intravenous, intradermal, subcutaneous, inhalation, transdermal, transmucosal and rectal administration.

Solutions or suspensions used for parenteral, intradermal or subcutaneous application may include (1) a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; (2) antibacterial agents such as benzyl alcohol or methyl parabens; (3) antoxidants such as ascorbic acid or sodium bisulfite; (4) chelating agents such as ethylenediaminetetraacetic acid; (5) buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH may be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation may be enclosed in ampoules, disposable syringes or multiple dose vials.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions or dispersions and sterile powders for extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline. Compositions must be sterile and be stable under the conditions of manufacture and storage and must be preserved against contamination by microorganisms such as bacteria and fungi. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), and suitable mixtures thereof. The proper fluidity may be achieved, for example, by using a coating such as lecithin, by maintaining the required particle size in the case of dispersion and by using surfactants. Prevention of the action of microorganisms may be achieved using various antibacterial and antifungal agents such as, for example, parabens, chlorobutanol, phenol, ascorbic acid, and thimerosal. Other ingredients such as an isotonic agent or an agent that delays absorption (e.g. aluminum monostearate and gelatin) may be included.

Sterile injectable solutions may be prepared by incorporating the active agent in the required amount in an appropriate solvent with one or a combination of ingredients discussed above, as required, followed by filtered sterilization. Dispersions may be prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and other required ingredients discussed above. In the case of sterile powders for the preparation of injectable solutions, the preferred methods of preparation include vacuum drying and freeze-drying which yield a powder of the active ingredient and any additional desired ingredient from a previously sterile-filtered solution.

Oral compositions may include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions may also be prepared using a fluid carrier. Pharmaceutically compatible binding agents and /or adjuvant materials may be included as part of the composition. The tablets, pills, capsules, troches and the like may contain any of the following ingredients or compound of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose; a disintegrating agent such as alginic acid or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate or orange flavoring.

For administration by inhalation, the composition may be delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, for example, a gas such as carbon dioxide or a nebulizer.

For transmucosal or transdermal administration, penetrants known in the art to be appropriate to the barrier to be permeated may be used. These include detergents, bile salts and fusidic acid derivatives for transmucosal administrations, which may be accomplished using nasal sprays, for example. For transdermal administration, the active agents of the invention are formulated into ointments, salves, gels or creams as generally known in the art.

The compositions of the invention may be prepared with carriers that will protect against rapid elimination from the body. Controlled release formulations

such as implants and microencapsulated delivery systems, for example, permit sustained slow release of the active agents of the invention, and in some cases, release of immunostimulators as well. Examples of such formulations include active agents of the invention entrapped in liposomes, ethylene-vinyl acetate copolymer (EVAc) (see Niemi *et al.*, *Laboratory Animal Science* 35:609-612 (1985)), and degradable polymer. The biodegradable, biocompatible polymers used for encapsulation include, without limitation, poly(DL-lactide-co-glycolide) (see Eldridge *et al.*, *Molecular Immunology* 28: 287-294 (1991)). Additional examples of polymers that can be used include polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions, including those targeted to infected cells with monoclonal antibodies to viral antigens may also be used as pharmaceutically acceptable carriers. These may be prepared using methods known in the art.

Thus, compositions formulated to elicit an immune response can include adjuvants, as well as other carriers and vehicles. Non-limiting examples of adjuvants, carriers and vehicles include Freund's incomplete adjuvant; Freund's complete adjuvant; aluminum salts (e.g. potassium sulfate, aluminum phosphate, aluminum hydroxide); bacterial lipopolysaccharide; synthetic polynucleotides (poly IC/poly AU); Montanide ISA Adjuvants (Seppic, Paris, France); Ribi's Adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); Hunter's TiterMax (CytRx Corp., Norcross, GA); Nitrocellulose-Adsorbed Protein; Gerbu Adjuvant (Gerbu Biotechnik GmbH, Gaiberg, Germany/C-C Biotech, Poway, CA); saponin; muramyl di- and tripeptides; monophosphoryl lipid A; Bordetella pertussis; cytokines; bacterial toxoids; fatty acids; living vectors; mineral oil emulsions; biodegradable oil emulsions (e.g. those containing peanut oil, squalene, or squalane); nonionic block copolymer surfactants; liposomes and biodegradable polymer microspheres. See, for example, Eldridge *et al.*, *Mol Immunol.* 28:287-94 (1991)). Additional examples of vaccine delivery systems are discussed in Felnerova *et al.*, *Current Opinion in Biotechnology* 15:518-29 (2004); Saupe et al., Expert Opin. Drug Deliv. 3:345-54 (2006); Sakarellos-Daitsiotis *et al.*, *Current Topics in Medicinal Chemistry* 6:1715-1735 (2006); Chen & Huang, Advances in Genetics 54: 315-37 (2005); Westerfeld and Zurbriggen, *J. Peptide Sci* 11: 707-712 (2005); Shahiwala *et al.*, *Recent Patents*

on Drug Delivery & Formulation 1: 1-9 (2007); and McDermott *et al.*, *Immunology and Cell Biology* 76:256-62 (1998); the contents of which are incorporated by reference herein.

Compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. The phrase "dosage unit form" refers to physically discrete units suited as unitary dosages for the subject to be treated, each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms is dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

Kits and Articles of Manufacture

The active agents or pharmaceutical compositions of the invention may be included in a container, pack or dispenser together with instructions for their use. Such kits can include additional reagents as required for the intended use of the immunogenic molecular entities, antibodies or pharmaceutical compositions. For example, an antibody of the invention can be used for diagnostic purposes, in which case, one or more reagents that enable detection/visualization can be included in the kit, preferably in a separate container, pack or dispenser from that holding the antibody of the invention. The kit or article of manufacture can include instructions for its use in diagnostic, prophylactic and/or therapeutic purposes as described below.

Methods of the Invention

The invention provides a method for identifying a mammal susceptible to or having a disease or condition associated with a Gram-positive bacterial infection, as well as a method to prevent infection by a Gram positive bacterium or its associated disease or condition. The invention also provides a method of eliciting an immune response in the mammal and a method of preventing quorum sensing in a mammal.

In the context of the invention, a mammal is any warm-blooded vertebrate including, for example, a mouse, rat, hamster, rabbit, guinea pig, pig, cow, horse, sheep, monkey, and human. A Gram-positive bacterium is any bacterium that utilizes cyclic peptides as signaling molecules in quorum sensing and can be, for example, *Enterococcus faecalis* and a *Staphylococcus* species

including, for example, *S. aureus*, *S. epidermidis*, *S. auricularis*, *S. capitis*, *S. caprae*, *S. carnosus*, *S. arlettae*, *S. cohnii*, *S. epidermis*, *S. intermedius*, *S. lugdunensis*, *S. simulans*, *S. gallinarum*, *S. xylosus*, and *S. warneri*. The disease or condition associated with infection by such a bacterium includes, for example, food poisoning, toxic shock syndrome, scalded skin syndrome, surgical wound infection, urinary tract infection, sepsis, and pneumonia.

Diagnostic Methods

A diagnostic method of the invention can be used to identify a mammal in need of or that may benefit from treatment using an immunogenic molecular entity or antibody of the invention. A mammal in need of or that may benefit from treatment using an immunogenic molecular entity or antibody of the invention is one that has a Gram positive bacterial infection or is susceptible to the infection or to a disease or condition associated with a Gram-positive bacterial infection. To identify such a mammal, a biological sample from the mammal can be obtained. The biological sample can be a tissue sample, a cell sample or a sample of a biological fluid such as blood, urine, or lymph. An antibody of the invention can be used to determine whether a biological sample contains a cyclic peptide signaling molecule, the presence of which indicates that the mammal has a Gram positive bacterial infection or is susceptible to or has a disease or condition associated with a Gram-positive bacterial infection. For example, an antibody of the invention that binds specifically to the *S. aureus* AIP-IV signaling peptide can be used to detect the presence of *S. aureus* AIP-IV in a biological sample from a mammal suspected of being susceptible to or of having a disease or condition associated with a *S. aureus* infection. The presence of *S. aureus* AIP-IV in the sample indicates that the mammal has an *S. aureus* infection or susceptible to or has a disease or condition associated with the *S. aureus* infection. Thus, an antibody of the invention can be used diagnostically to detect the presence of and/or determine the amount of a cyclic peptide signaling molecule in a biological sample from a mammal.

The presence or amount of the cyclic peptide signaling molecule in a biological sample from a mammal can be detected in a competitive assay using a suitably-labelled antibody of the invention. For example, an immunogenic molecular entity of the invention, e.g. hapten linked to macromolecular carrier such as a polypeptide, can be immobilized on a surface. The binding of a

suitably-labelled antibody of the invention to the immobilized immunogenic molecular entity in the presence or absence of a biological sample from the mammal is determined. A decrease in binding of the labeled-antibody to the surface in the presence of the biological sample indicates the presence of a cyclic peptide signaling molecule. The biological sample can be a partially purified or processed sample in which unrelated mammalian cells have been removed. The antibody can be labeled with a detectable molecule, which can be an enzyme such as alkaline phosphatase, acetylcholinesterase, β-galactosidase or horseradish peroxidase; a prosthetic group such as streptavidin, biotin, or avidin; a fluorescent group such as dansyl chloride, dichlorotriazinylamine, dichlorotriazinylamine fluorescein, fluorescein, fluorescein isothiocyanate, phycoerythrin, rhodamine, umbelliferone; a luminescent group such as luminal; a bioluminescent group such as aequorin, luciferase, and luciferin; or a radioisotope such as ^{3}H, ^{125}I, ^{131}I, ^{35}S.

Therapeutic Methods

An immunogenic molecular entity or antibody of the invention can be used to prevent or treat infection of a mammal by a Gram positive bacterium such as, for example, a *Staphylococcus* species, that utilizes cyclic peptide signaling molecules in quorum sensing. Mammals that can benefit from treatment with an immunogenic molecular entity or antibody of the invention include: (1) a mammal at risk for or susceptible to infection by a Gram positive bacterium, (2) a mammal who has come into contact with an infectious Gram positive bacterium, or (3) a mammal who is infected by a Gram positive bacterium. To prevent or treat a Gram-positive bacterial infection, an immunogenic molecular entity of the invention can be administered to the mammal to elicit an immune response in the mammal. In addition, an antibody of the invention can be administered to inhibit the activity of a cyclic signaling peptide thereby preventing the production of virulence genes or toxins that aid in bacterial infection or development of the disease condition associated the bacterial infection.

A mammal that can benefit from treatment with the immunogenic molecular entity or antibody of the invention can be identified using the methods discussed above in which the presence and/or amount of a cyclic peptide signaling peptide is determined. Other methods of detecting the presence of a

Gram positive bacterial infection such as, for example, by culturing from a sample from the mammal, e.g. a blood culture, can be used. A mammal, such as a human, who can benefit from treatment with an immunogenic molecular entity or antibody of the invention can be an individual having a weakened immune system, an individual with a suppressed immune system, an individual who has undergone or will undergo surgery, an older individual or one who is very ill, an individual who has been hospitalized or has had a medical procedure. A mammal that can benefit from treatment with an immunogenic molecular entity or antibody of the invention can be a hospital patient at risk of developing nosocomial infection or a mammal known to be infected with or having been exposed to antibiotic resistant bacteria such as, for example, Methicillin-resistant *S. aureus*, Vancomycin-intermediary-sensible *S. aureus*, Vancomycin-resistant *S. aureus* and other antibiotic resistant enterococci including *Pneumococcus pneumoniae*.

The antibody or immunogenic molecular entity of the invention can be administered prior to infection, after infection but prior to the manifestation of symptoms associated with the infection, or after the manifestation of symptoms to prevent further bacterial multiplication and to prevent further expression of virulence genes thereby hindering development of the disease or its progression. When administered to a mammal, the immunogenic molecular entity of the invention elicits the production of antibodies that prevent the disease or condition or its progression by binding to and neutralizing the cyclic peptide signaling molecules produced by the bacteria thereby preventing the production of virulence genes or toxins that aid in development of the infection or the disease condition associated with the bacterial infection. In addition, a neutralizing antibody of the invention can also be administered to the mammal. The neutralizing antibody can bind to a cyclic peptide signaling molecule produced by the bacteria and prevent its binding to its cell-associated receptor and in doing so, prevent the production of virulence genes or toxins that aid in infection or development of the disease condition associated with the bacterial infection. Accordingly, a composition that includes an immunogenic molecular entity of the invention can be used as a live vaccine, while a composition that includes an antibody of the invention can be used as a passive vaccine, to

prevent the bacterial infection or a disease or condition associated with the bacterial infection.

The active agents of the invention can be administered by any route discussed herein. The dosage of the immunogenic molecular entity or supramolecular assembly to be administered to a mammal may be any amount appropriate to elicit an immune response against a cyclic signaling peptide. The dosage of the antibody to be administered to a mammal can be any amount appropriate to neutralize the activity of a cyclic signaling peptide.

The dosage may be an effective dose or an appropriate fraction thereof. This will depend on individual patient parameters including age, physical condition, size, weight, the condition being treated, the severity of the condition, and any concurrent treatment. Factors that determine appropriate dosages are well known to those of ordinary skill in the art and may be addressed with routine experimentation. For example, determination of the physicochemical, toxicological and pharmacokinetic properties may be made using standard chemical and biological assays and through the use of mathematical modeling techniques known in the chemical, pharmacological and toxicological arts. The therapeutic utility and dosing regimen may be extrapolated from the results of such techniques and through the use of appropriate pharmacokinetic and/or pharmacodynamic models.

The precise amount to be administered to a patient will be the responsibility of the attendant physician. An immunogenic molecular entity or antibody of the invention may be administered by injection at a dose of from about 0.05 to about 2000 mg/kg weight of the mammal, preferable from about 1 to about 200 mg/kg weight of the mammal. As certain agents of the invention are long acting, it may be advantageous to administer an initial dose of 80 to 4,000 mg the first day then a lower dose of 20 to 1,000 mg on subsequent days. A patient may also insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons. One or more booster doses of the immunogenic molecular entity or antibody could be administered at a selected time period after the first administration.

Treatment using an antibody or immunogenic molecular entity of the invention can be for a duration needed to elicit an effective neutralizing immune response.

Methods of Generating Antibodies of the Invention

An immunogenic molecular entity or supramolecular assembly of the invention can be used to generate antibodies directed to a cyclic signaling peptide.

An immunogenic molecular entity or supramolecular assembly of the invention can be used to screen a recombinant immunoglobulin library to identify an antibody that binds specifically with a selected cyclic signaling peptide. Methods and reagents for generating and screening a recombinant combinatorial immunoglobulin library are described in, for example, Barbas, C.F., 3rd, D.R. Burton, J.K. Scott, and G.J. Silverman, *Phage Display – A Laboratory Manual.* 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, and Kontermann, R., Dübel, S., Antibody Engineering, 2001, Berlin, Heidelberg: Springer-Verlag

An immunogenic molecular entity, or supramolecular assembly of the invention can also be used to elicit an immune response in a mammal, from which polyclonal or monoclonal antibodies can be obtained. An immunogenic molecular entity or supramolecular assembly of the invention can be administered to a mammal such as a goat, sheep, rat, mouse, or rabbit, for example. Polyclonal antibodies can be isolated from the blood of the mammal using methods known in the art. Monoclonal antibodies can be obtained by isolating antibody-producing cells from the mammal and generating antibody-producing hybridomas. Methods of producing and obtaining antibodies from a mammal are known in the art. See, for example, Harlow, D. and D. Lane, *Antibodies A laboratory manual.* Cold spring harbor laboratory, New York (1988), and Tramontano, A. and D. Schloeder, *Production of antibodies that mimic enzyme catalytic activity.* Methods Enzymol 178: p. 531-550 (1989).

The invention is further illustrated by the following non-limiting Examples.

EXAMPLES

Example 1 – Materials

RN4850 was obtained from Dr. Richard P. Novick (Skirball Institute, New York University Medical Center). Purified monoclonal antibodies were obtained from TSRI Antibody Production Core Facility. The clinical isolate NRS168 was obtained through the Network on Antimicrobial Resistance in

Staphylococcus aureus (NARSA) Program supported by NIAID/NIH (N01-AI-95359).

Example 2 – Synthesis of Native AIPs 1-4

The following general procedure was used to synthesize all natural products. Batch synthesis was carried out on 0.25 mmol of MBHA resin swollen in DMF following standard Boc solid-phase peptide synthesis protocols. A solution of *S*-trityl-3-mercaptoproprionic acid (2 eq), HBTU (3.9 eq), and DIEA (0.5 mL) in 4 mL DMF was prepared and allowed to sit for 3 minutes for pre-activation. The cocktail was added to the resin for coupling, which is generally complete in 1hour. The resin was then washed with DMF and subjected to trityl deprotection with 5 % TIS in TFA (2 × 10 minutes). Once washed with DMF, the peptide sequence was completed by sequential coupling reactions using 4 eq Boc amino acid, 3.9 eq HBTU, and 0.5 mL DIEA, with 3 minute preactivation. When the synthesis was complete, the resin was washed with DMF, then CH_2Cl_2, and finally with ether before it was placed in the desiccator.

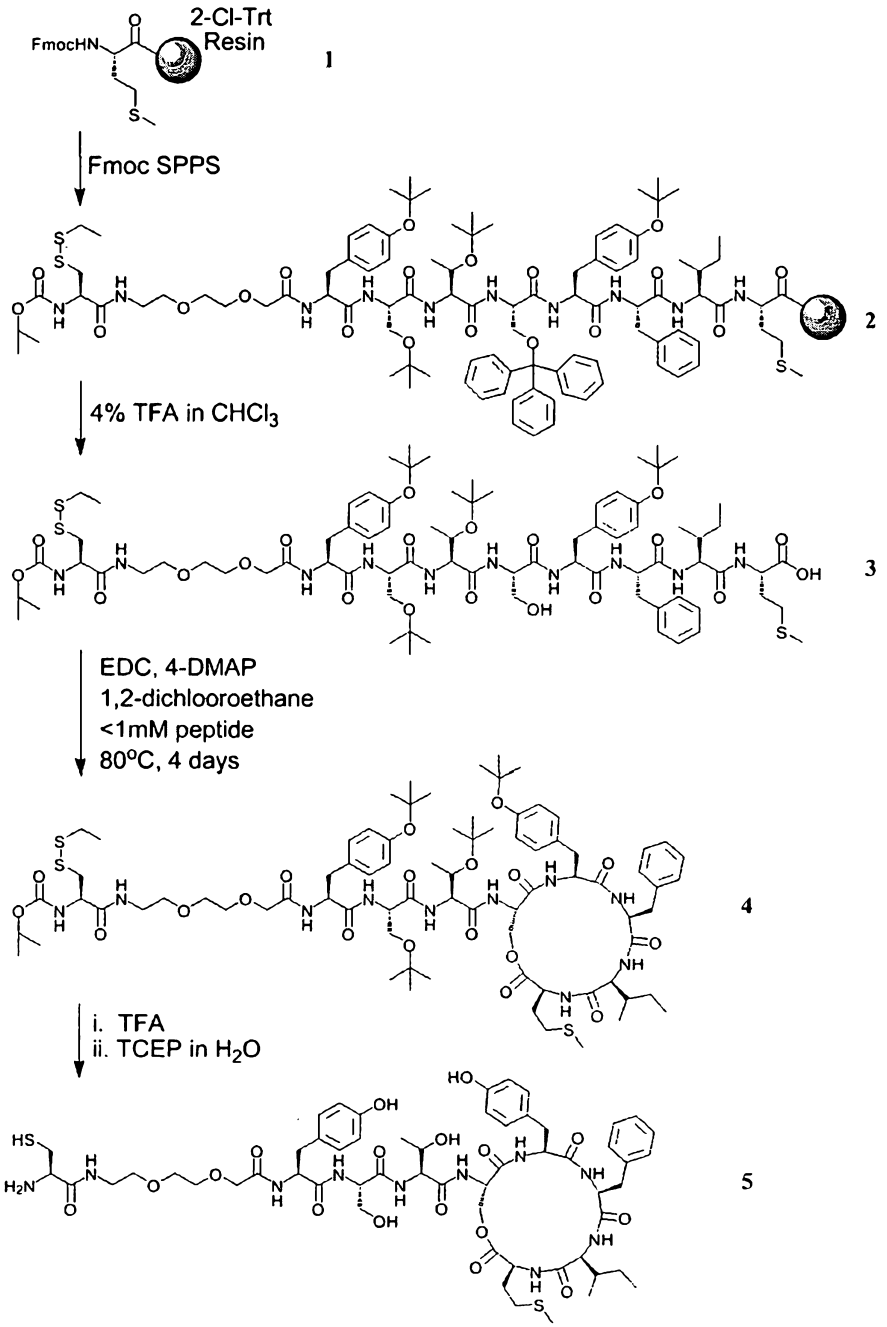
Cleavage: The resin was subjected to 5-10 mL of HF for 1 hour using anisole as a scavenger. The resulting mixture was washed with ether and extracted with 1:1 water/acetonitrile. This solution was frozen and lyophilized, and the resulting solid was purified by prep-HPLC. Pure fractions were pooled, frozen, and lyophilized.

Thiolactonization: Intramolecular thiolactonization was achieved by taking up the purified, solid linear peptide in a mixture of 80 % MOPS buffer (100 mM, pH 7.0) and 20 % acetonitrile, giving a peptide concentration of less than 1mM. The reaction was monitored by ESI-MS, and was usually complete in 24-48 hour. The product was purified by prep-HPLC. Pure fractions were pooled, frozen, and lyophilized. ESI-MS: *m/z* calcd for AIP-1, $C_{43}H_{60}N_8O_{13}S_2$ (M + H), 961.4; found 961.8: *m/z* calcd for AIP-2, $C_{38}H_{58}N_{10}O_{12}S$ (M + H), 879.4; found, 879.6: *m/z* calcd for AIP-3, $C_{38}H_{58}N_8O_{10}S$ (M + H), 819.4; found, 819.7: *m/z* calcd for AIP-4, $C_{48}H_{64}N_8O_{12}S_2$, 1009.4; found 1009.7. **See FIG. 2A-H.**

Example 3 – Synthesis of AIP4 Hapten 5 – AIP4 Lactone Analog

The scheme for the synthesis of AIP4 hapten 5 is depicted in Scheme 1 below. The linear peptide YSTSYFLM (SEQ ID NO: 1, not including protecting groups) was synthesized on 2-chlorotrityl resin preloaded with Fmoc-Methionine 1 using standard Fmoc chemistry employing DIC/HOBt as coupling reagents. The N-terminal pendant cysteine was incorporated for conjugation to a carrier protein and the short flexible linker was added between the hapten and the carrier protein as spacer. The protected linear peptide was released from the resin using 4% trifluoroacetic acid in chloroform, which also selectively removed the trityl protection group from the serine. Intramolecular lactonization under dilute conditions was performed using EDC/4-DMAP, and subsequent side chain de-protections afforded the AP4 hapten 5. The details of the synthetic procedure are described in the following text.

Scheme 1 Synthesis of the AP4 Hapten 5



SEQ ID NO: 1 (YSTSYFLM, not including protecting groups)

Synthesis of the Linear Protected Peptide (3)

All N-α-Fmoc protected amino acids, coupling reagents and the resins for peptide synthesis were purchased from EMD Biosciences, Inc. (San Diego, CA). All other chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO). ESI-MS analyses were performed with API150EX (PE SCIEX, Foster City, CA), and HITACHI L-7300 and SHIMADZU SCL-10A were used for analytical and preparative HPLC experiments, respectively.

The peptide was synthesized by Fmoc SPPS on 2-chlorotrityl resin preloaded with the Fmoc-Met **1**. An Fmoc-Ser(Trt)-OH was incorporated at the position of lactonization. All other residues were chosen with side chain protecting groups stable to dilute TFA and labile in 95% TFA. A short flexible linker was incorporated penultimate to the N-terminus by coupling Fmoc-8-amino-3,6-dioxaoctanoic acid. The N-terminal residue was Boc-Cys(Set)-OH for eventual use in conjugation to carrier proteins.

Specific Conditions: Batch synthesis was carried out on 1 mmol of resin swollen in DMF for at least 1 hour. A solution of the protected amino acid, DIC, and HOBt (4 equivalents each) in 5 mL DMF was prepared and allowed to sit for 5 minutes for pre-activation, followed by the addition of 0.5 mL sym-collidine. The cocktail was added to the resin for coupling, which was generally complete in 1 hour. The resin was then washed with DMF and subjected to Fmoc deprotection with 20 % (v/v) piperidine in DMF (2 × 7 min). The resin was then washed with DMF and the next coupling reaction was carried out. When synthesis was complete, the resin was washed with DMF, then CH_2Cl_2, and finally with ether before it was placed in the desiccator.

Cleavage (and Trityl Deprotection): The resin was added to a cocktail of 4 % TFA, 4 % triisopropylsilane (TIS) and 0.5 % H_2O in chloroform, and shaken for 6 hours. The mixture was filtered, allowing the filtrate to drip into cold ether to precipitate the peptide. The ether mixture was centrifuged and the supernatant was decanted. The peptide was then washed (2x) with ether by re-suspending the solid in ether, centrifugation, and decanting the supernatant. The resulting solid was placed in a desiccator.

Purification: The fully protected peptide **3** was dissolved in methylene chloride and purified by normal phase silica gel chromatography eluted with 5 % methanol in methylene chloride.

B. Lactonization of (3)

The protected linear peptide **3** was dissolved in 1,2-dichloroethane (previously dried over anhydrous $MgSO_4$) to give a final concentration of no greater than 1.0 mM. The solution was stirred and heated to 80 °C and 3 equivalents each of EDC and 4-DMAP were added; another equivalent each of EDC and 4-DMAP were added at both 24 and 48 hours into the reaction. The reaction was monitored by HPLC. After 4 days, the reaction mixture was cooled to room temperature, washed with 2 × 200 mL of 0.2 M $KHSO_4$ (aq), dried over anhydrous Na_2SO_4, and evaporated to dryness. The cyclized peptide **4** was purified by prep-HPLC. Yields range from 30-60 % as determined by analytical HPLC integration.

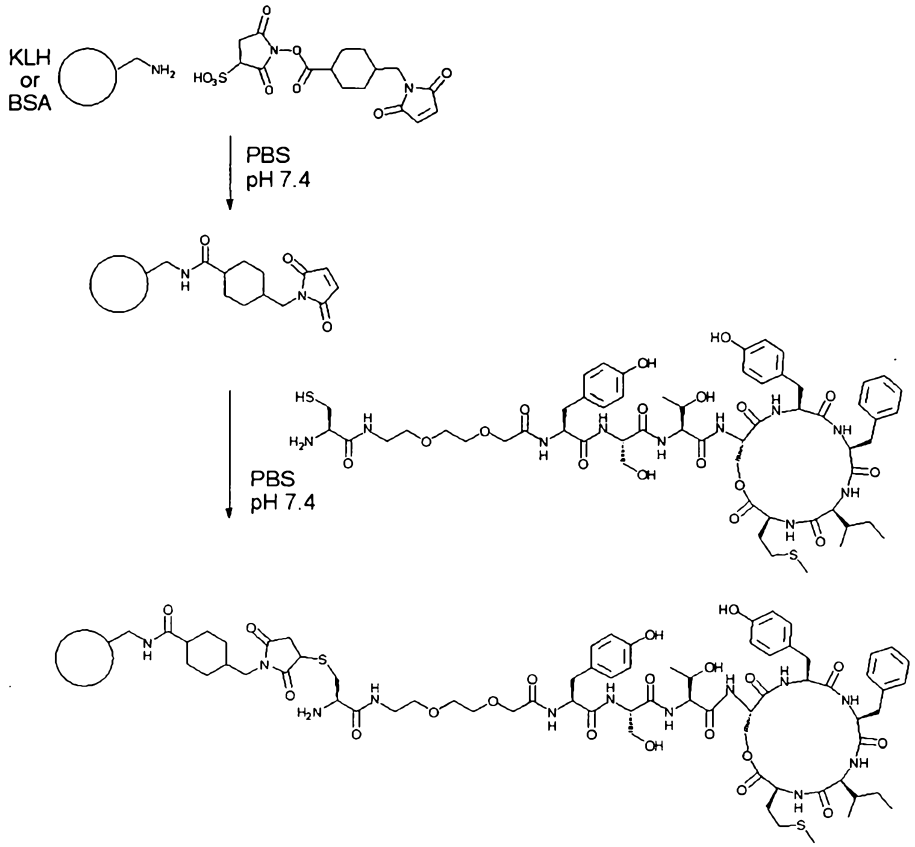
C. Global Deprotection and Disulphide Deprotection of (4)

The solid, purified peptide was dissolved in TFA containing 2 % TIS and stirred for 1 hour. The mixture was then evaporated to dryness. Water was added and the mixture was frozen and lyophilized. The lyophilized solid was then dissolved in H_2O with tris(2-carboxyethyl)phosphine hydrochloride (TCEP). The mixture was stirred for 1 hour and injected directly into the prep-HPLC for purification yielding AP4 hapten **5**. The collected pure fractions were pooled, frozen, and lyophilized. ESI-MS: *m/z* calcd for $C_{57}H_{80}N_{10}O_{17}S_2$ (M + H), 1241.5; found, 1242.2. See **FIG. 2I & J.**

D. Conjugation of (5) to KLH/BSA

The conjugation of hapten 5 to KLH/BSA was performed as depicted in Scheme 2 below. The details of the procedure is described in the following text.

Scheme 2 Conjugation of Hapten 5 to KLH/BSA



SEQ ID NO: 2 (INSDFLL, not including protecting groups)

Attachment of Sulpho-SMCC. 5 mg of the carrier protein were resuspended in 0.9 mL PBS, pH 7.4. To this solution was added 1 mg of the linker sulpho-SMCC (sulphosuccinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate). The solution was stirred for 6-8 hours and the protein-linker conjugate was purified by dialysis in PBS at 4 °C.

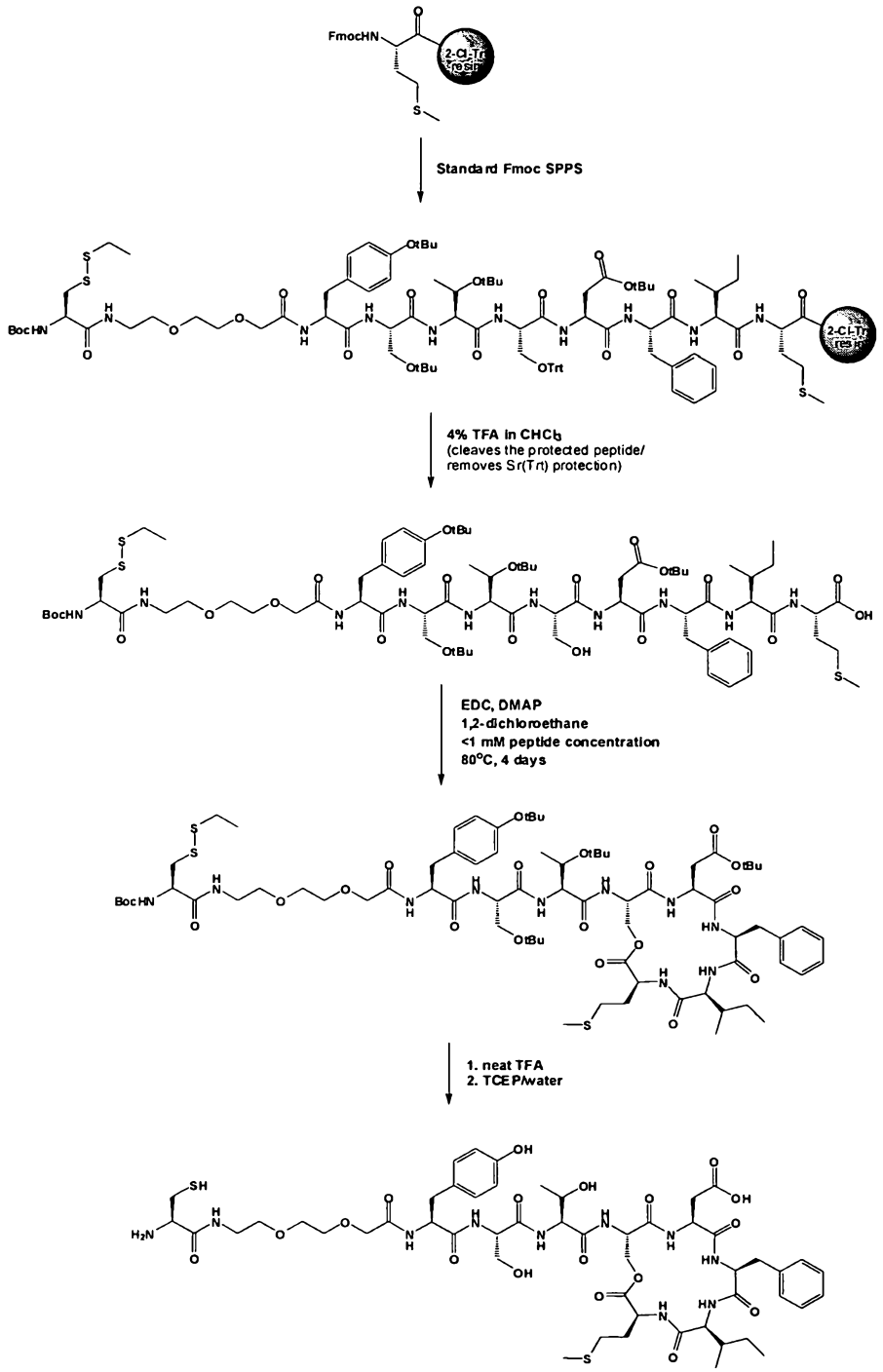
*Conjugation of the hapten **5**.* To the protein-linker conjugate in PBS were added 100 μL of DMF containing 2 mg of the hapten **5**. The solution was shaken overnight and the protein-hapten conjugate was purified by dialysis. MALDI-TOF analysis confirmed the attachment on average of ≈ 6 haptens per BSA molecule (molecular weight of BSA-AIP4 conjugate = 75581 Daltons; BSA = 67000 Daltons; and hapten = 1461.15 Daltons). See **FIG. 2K**.

Example 4 – Preparation of the AP1, AP2, AP3 and AP4 Lactone Analogs as Synthetic Haptens and Hapten-Protein Carrier Conjugates

For immunization and elicitation of an immune response, active vaccine, and generation of monoclonal antibodies, synthetic haptens in the form of AP1, AP2, AP3 and AP4 lactone analogs and hapten-protein carrier conjugates were prepared using procedures as described for the preparation of the AP4 hapten 5 described above. The preparation schemes are as follows.

Scheme 3

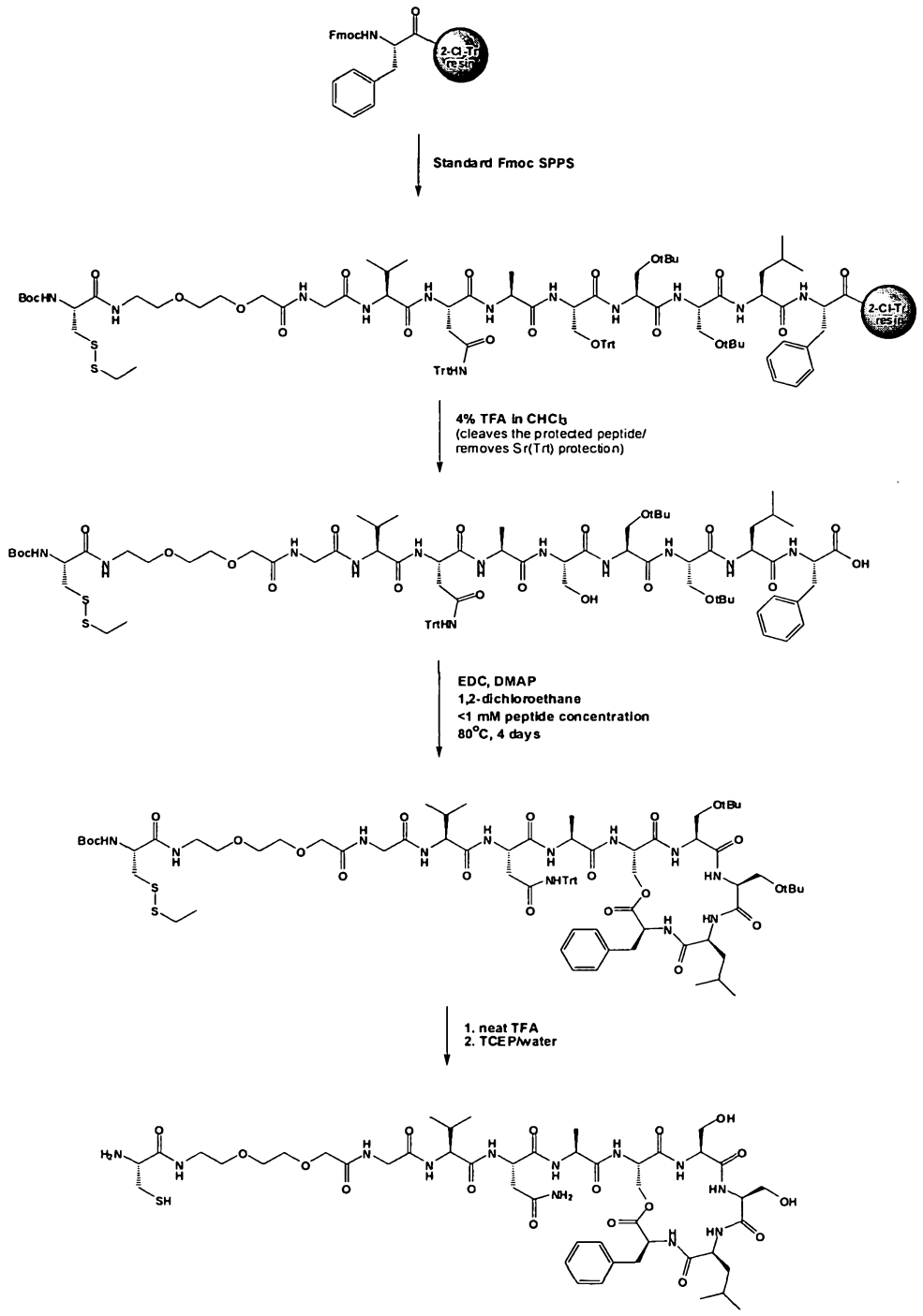
Preparation of a Synthetic Hapten (AP1) for Immunization and Elicitation of an Immune Response/Active Vaccine/Generation of Monoclonal Antibodies to AIP-1



SEQ ID NO: 3 (YSTSDFIM, not including protecting groups)

Scheme 4

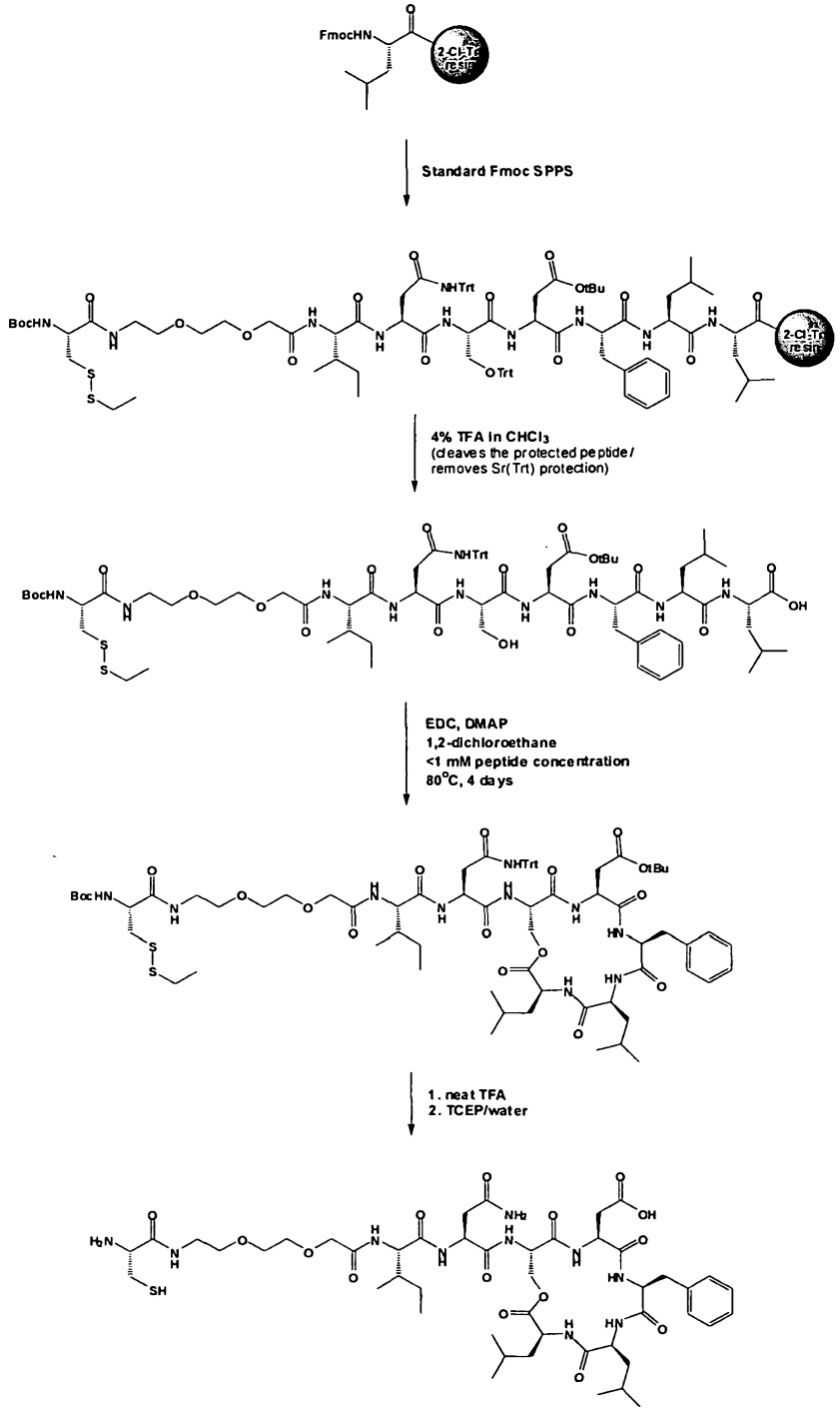
Preparation of a Synthetic Hapten (AP2) for Immunization and Elicitation of an Immune Response/Active Vaccine/Generation of Monoclonal Antibodies to AIP-2



SEQ ID NO: 4 (GVNASSSLY, not including protecting groups)

Scheme 5

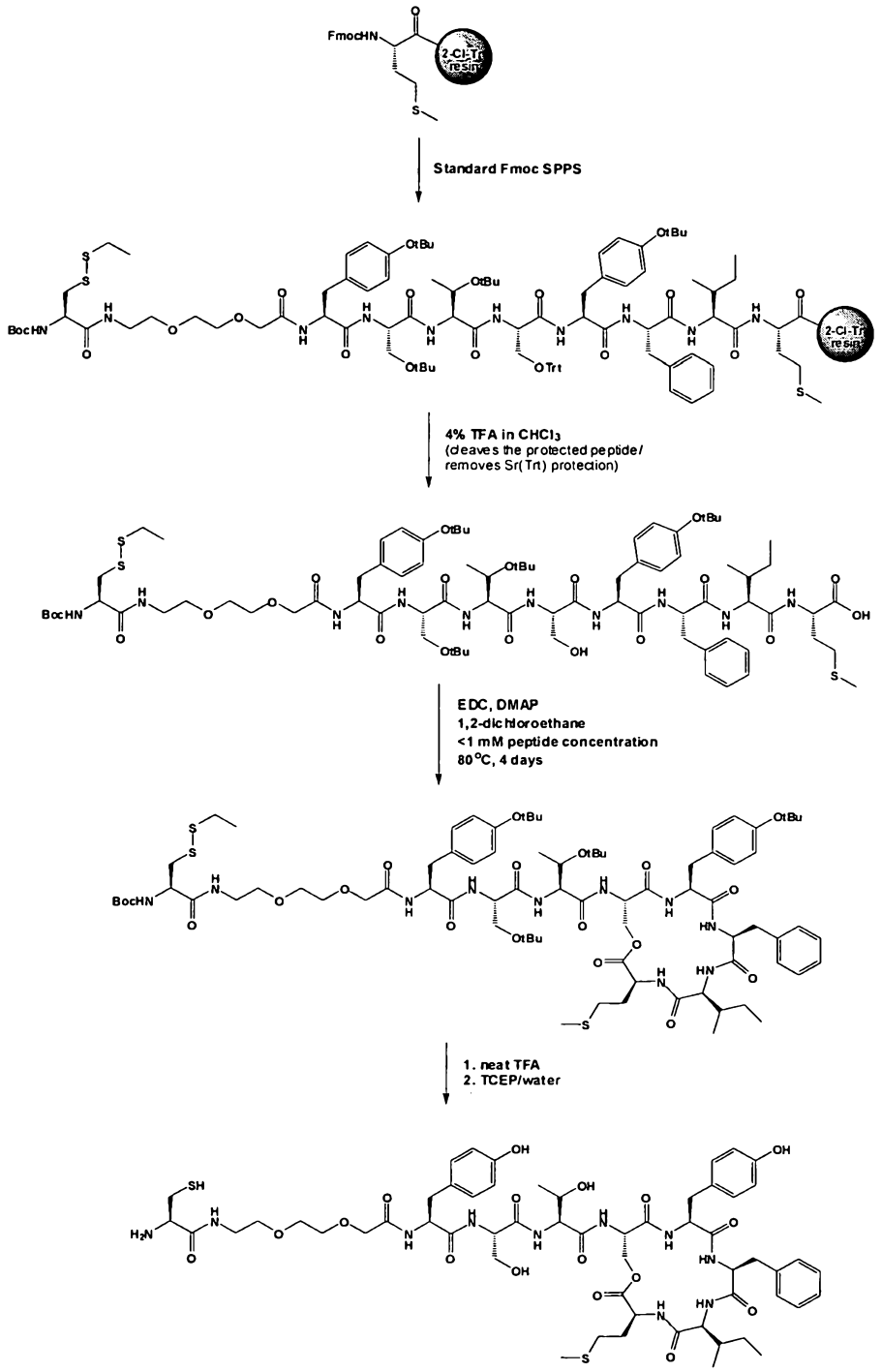
Preparation of a Synthetic Hapten (AP3) for Immunization and Elicitation of an Immune Response/Active Vaccine/Generation of Monoclonal Antibodies to AIP-3



SEQ ID NO: 2 (INSDFLL, not including protecting groups)

Scheme 6

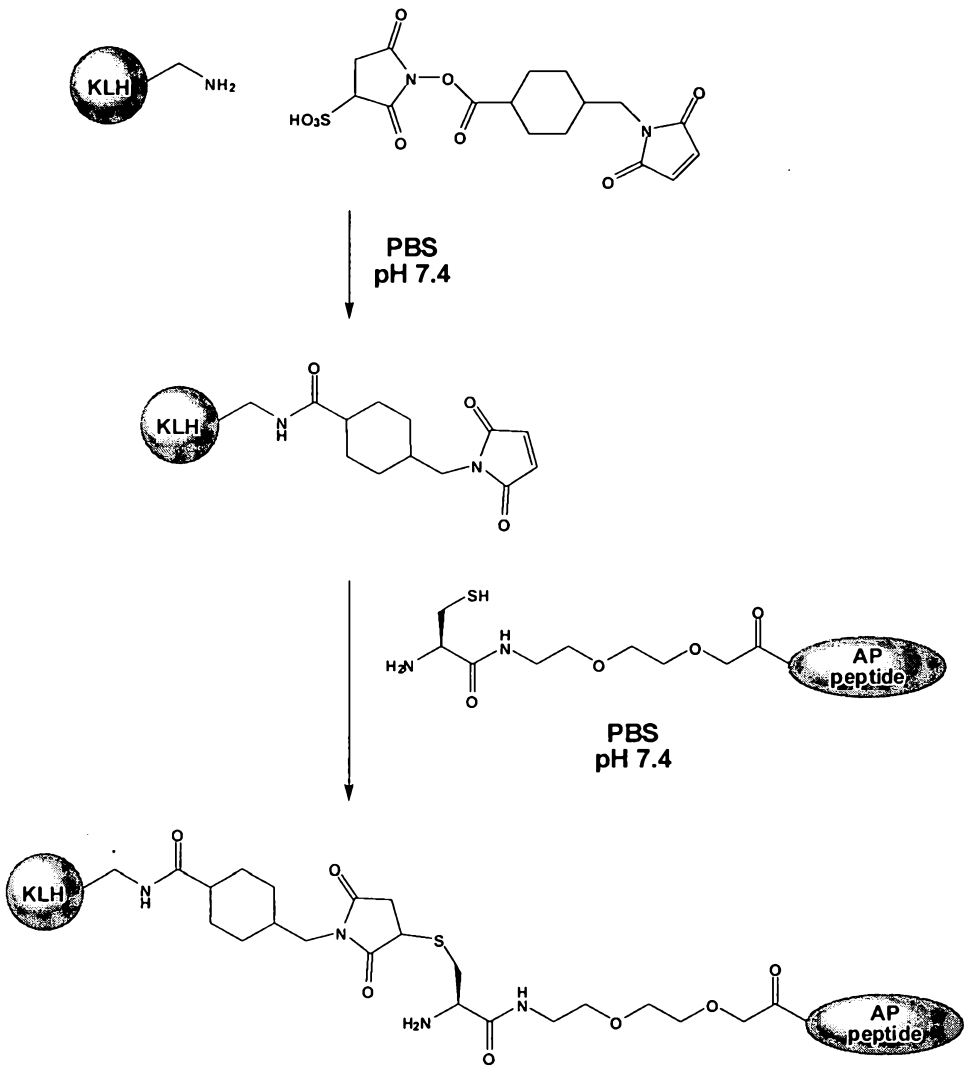
Preparation of a Synthetic Hapten (AP4) for Immunization and Elicitation of an Immune Response/Active Vaccine/Generation of Monoclonal Antibodies to AIP-4



SEQ ID NO: 1 (YSTSYFLM, not including protecting groups)

Scheme 7

Preparation of Hapten-Protein Carrier Conjugates for Immunization and Elicitation of an Immune Response/Active Vaccine/Generation of Monoclonal Antibodies to AIP-1-AIP-4



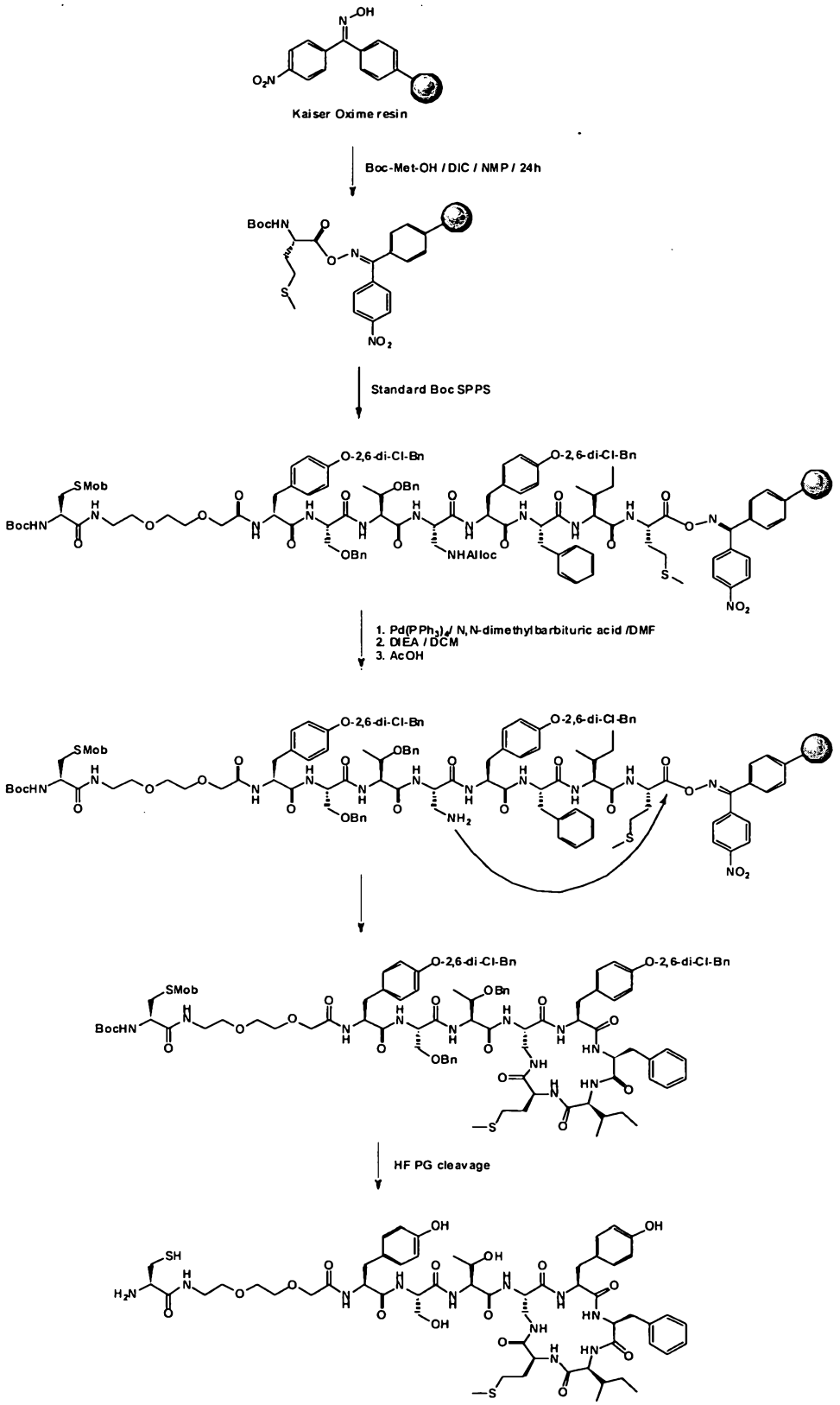
5

Example 5 – Preparation of the AP4 Lactam, Carbamide and Semicarbazide Analogs as Synthetic Haptens

The proteolytically stable cyclic lactam, carbamide and semicarbazide AIP peptide haptens are prepared using the well documented methodology of peptide cyclization on base-labile Kaiser oxime resin. See DeGrado et al., *J. Org. Chem.* 1980, *45*, 1295-1300; DeGrado et al., *J. Org. Chem.* 1982, *47*, 3258-3261; Nakagawa et al., *J. Org. Chem.* 1983, *48*, 678-685; Nakagawa et al., *J. Am. Chem. Soc.* 1985, *107*, 7087-7092; Kaiser et al., *Science* 1989, *243*, 187-192. This synthetic approach is based on Boc-based solid phase peptide synthesis, where the peptide cyclization coincides with the cleavage of the cyclized peptide off the solid support. Osapay et al., *J. Am. Chem. Soc.* 1992, *114*, 6966-6973; Taylor et al., *Biopolymers* 2002, *66*, 49-75; and Li et al., *Curr. Org. Chem.* 2002, *6*, 411-440. Synthesis of the cyclic carbamide peptides requires the retro-inverso motif, as described in the literature. Chorev et al., *Biopolymers* 2005, *80*, 67-84. The pre-requisite 1-*N*-Boc-4-(methylthio)butane-1,2-diamine building block is synthesized from the commercially available Boc-methioninol and then coupled onto the peptide chain *via* the nitrophenyl carbamate protocol according to a literature precedent. Vince et al., *Bioorg. Med. Chem. Lett.* 1999, *9*, 853-856. The following schemes outline syntheses of proteolytically stable cyclic lactam, carbamide and semicarbazide analogs of AIP-4 peptide. These synthetic methodologies can be applied to the preparation of other cyclic peptide haptens, e.g. AIP-1, AIP-2, AIP-3 as well as other Staphylococcal quorum sensing peptides.

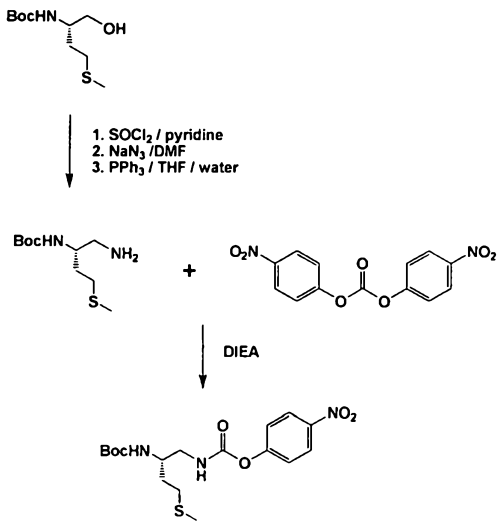
The synthesis of the cyclic lactam AIP4 hapten is summarized in scheme 8. Schemes 9 and 10 outline the syntheses of the intermediates 1-*N*-Boc- 4-(methylthio)butane-1,2-diamine *p*-nitrophenylcarbamate and *N*-Fmoc- Met-hydrazide *p*-nitrophenylcarbamate used in the syntheses of the cyclic carbamide AIP4 hapten and the cyclic semicarbazide AIP4 hapten, respectively. Synthesis of the carbamide and semicarbazide AIP4 haptens is shown in schemes 11 and 12, respectively.

Scheme 8

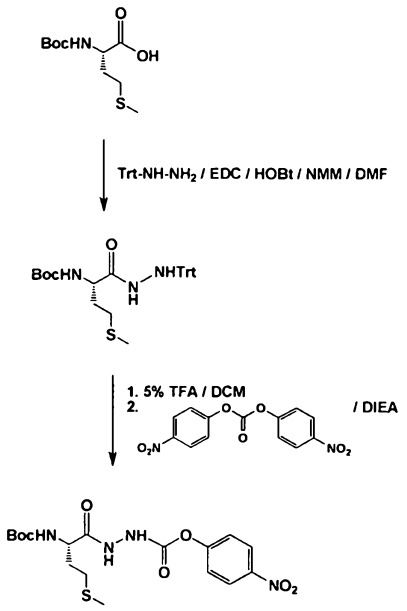


SEQ ID NO: 1 (YSTSYFLM, not including protecting groups)

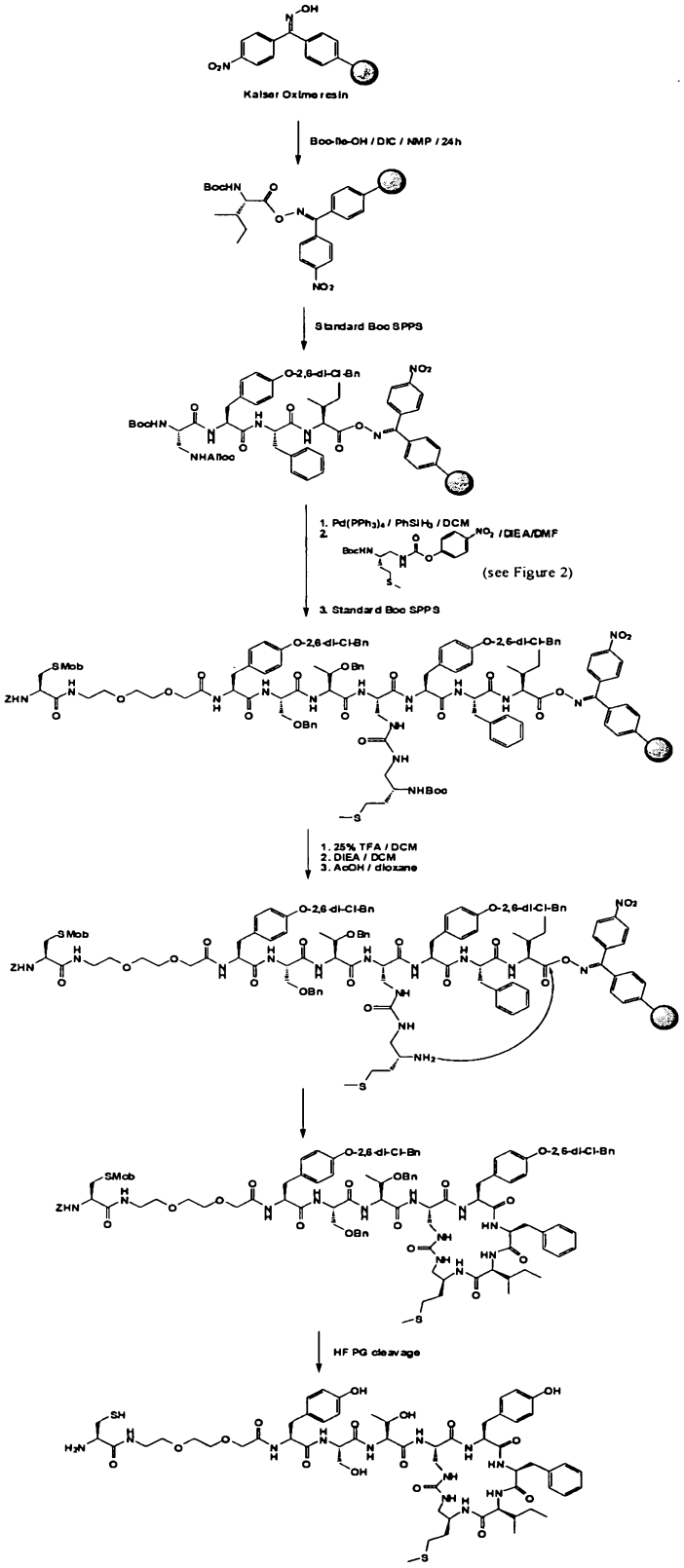
Scheme 9



Scheme 10

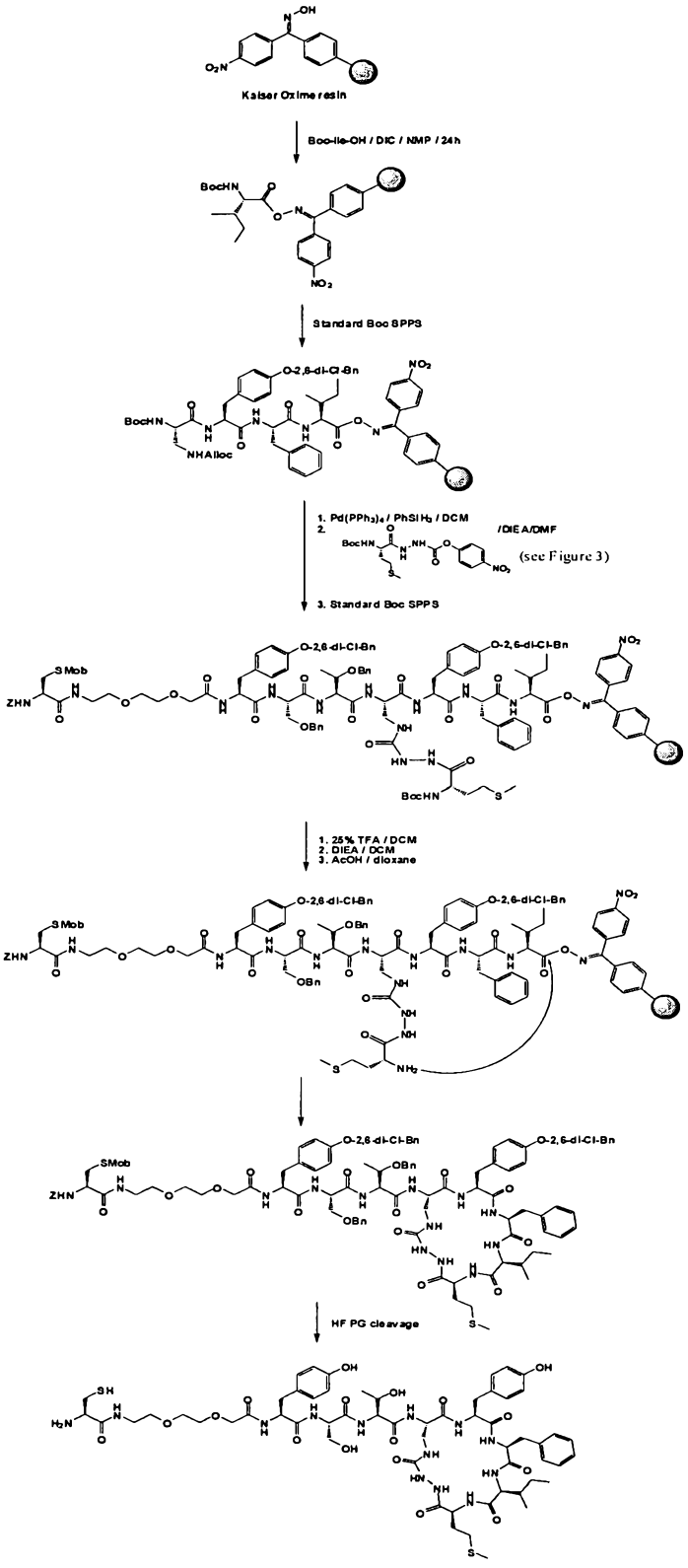


Scheme 11



SEQ ID NO: 1 (YSTSYFLM, not including protecting groups)

Scheme 12



SEQ ID NO: 1 (YSTSYFLM, not including protecting groups)

Example 6 – Analysis of Exoprotein Secretion in S. aureus

After overnight growth on an agar plate at 37 °C, a single colony of *S. aureus* (RN4850 or Wood 46) was inoculated into 3 mL CYGP medium and grown for overnight (18 hours) (see Novick, *Methods Enzymol* 204:587-636 (1991)). The overnight cultured cells were diluted to $OD_{600} \approx 0.03$ in fresh CYGP medium, and distributed to 5 mL polystyrene cell-culturing tube, where each tube contained 0.5 mL of the diluted cells and the appropriate antibody (0.2 mg/mL). After growth for 20-24 hours at 37 °C in a humid incubator without agitation, the samples were transferred to the microcentrifuge tubes (1.5 mL) and centrifuged at 13,000 rpm for 5 minutes. The supernatants were sterilized by filtration through a Millex®-GV filter unit (0.22 μm; Millipore, Ireland), and analyzed by SDS-PAGE (10 % Bis-Tris gel, Invitrogen, Carlsbad CA). To confirm α-hemolysin and protein A expression, Western blot analyses were performed using the HRP conjugated sheep polyclonal α-hemolysin antibody (abcam Inc., Cambridge MA) and anti-Protein A mouse monoclonal antibody (Sigma-Aldrich, St. Louis MO) and murine mAb SP2-6E11 (Park and Janda, unpublished data) was used as a control antibody. To test hemolytic activity, the *S. aureus* supernatants (75 μL × 3) were applied onto the sheep blood agar plate, and the plates were incubated at 37 °C for 18 hours and at room temperature for another 24 hours.

Example 7 – Static Biofilm Analysis

The biofilm assay was conducted by following a literature procedure with a few modifications (see O'Toole, *Methods Enzymol* 310:91-109 (1999)). After *S. aureus* cells (200 μL) were grown in tryptic soy broth (TSB) medium containing 0.2 % glucose with or without the antibody (0.2 mg/mL) in the polystyrene 96-well plate for 20-24 hours without agitation, the plate was washed by submersion in water and dried. A crystal violet solution (200 μL, aq. 0.1 %) was added to stain the biofilm, and then the plate was washed vigorously with water followed by adding acetic acid (250 μL, aq. 30 %) to solubilize the remaining crystal violet. Absorbance was measured at 570 nm with Spectramax 250 (Molecular Devices, Sunnyvale CA).

Example 8 – Real Time-PCR Analysis

Overnight cultured *S. aureus* RN4850 cells were diluted to $OD_{600} \approx 0.03$ in fresh CYGP medium (1 mL) containing the antibody and grown for 20-24 hours ($OD_{600} \approx 2$) at 37 °C without shaking. RNA from the cells was isolated using Rneasy® Mini Kit (QIAGEN Inc., Valencia CA) according to the manufacturer's instructions. Isolated RNA was further purified by treating with Rnase-Free Dnase (QIAGENE Inc.) for 30 minutes at room temperature. The first-strand DNA was synthesized using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen) using ≈ 300 ng of purified RNA. RT-PCR experiments were performed with at least two independent samples, and each experiment was set up in duplicate using LightCycler® FastStart DNA Master^{PLUS} SYBR Green I (Roche Applied Science, Indianapolis, IN). Generic SYBR Green Protocol (Roche) was used for the PCR conditions, and relative quantification analyses were performed with LightCycler® 2.0 system (Roche Applied Science) using the housekeeping GyrA gene was a reference. The sequences of the primers used are as follows:

gyrA F: 5'–TGGCCCAAGACTTTAGTTATCGTTATCC–3' (SEQ ID NO: 5);
gyrA R: 5'–TGGGGAGGAATATTTGTAGCCATACCTAC–3' (SEQ ID NO: 6);
rnaIII F: 5'–GCACTGAGTCCAAGGAAACTAACTC–3' (SEQ ID NO: 7);
rnaIII R: 5'–GCCATCCCAACTTAATAACCATGT–3' (SEQ ID NO: 8);
hla F: 5'–CTGAAGGCCAGGCTAAACCACTTT–3' (SEQ ID NO: 9);
hla R: 5'–GAACGAAAGGTACCATTGCTGGTCA–3' (SEQ ID NO: 10);
spa F: 5'–GCGCAACACGATGAAGCTCAACAA–3' (SEQ ID NO: 11);
spa R: 5'–ACGTTAGCACTTTGGCTTGGATCA–3' (SEQ ID NO: 12);
eta F: 5'–GTTCCGGGAAATTCTGGATCAGGT–3' (SEQ ID NO: 13);
eta R: 5'–GCGCTTGACATAATTCCCAATACC–3' (SEQ ID NO: 14);
sarA F: 5'–CTGCTTTAACAACTTGTGGTTGTTTG–3' (SEQ ID NO: 15)
sarA R: 5'–CGCTGTATTGACATACATCAGCGA–3' (SEQ ID NO: 16);
saeR F: 5'–CGCCTTAACTTTAGGTGCAGATGAC–3' (SEQ ID NO: 17);
saeR R: 5'–ACGCATAGGGACTTCGTGACCATT–3' (SEQ ID NO: 18).

Example 9 – Dermal Infection Model in Mice

All experiments on mice were performed in accordance with TSRI guidelines and regulations. SKH1 euthymic hairless mice, 6-8 weeks old were obtained from Charles River Laboratories and housed in the biocontainment vivarium for one week before use in experiments. Brain heart infusion agar was from BBL (#211065) and CYGP broth contained 1% casamino acids (Fisher BP1424) 1% yeast extract (EMD 1.03753) 0.59% sodium chloride, 0.5% dextrose and 60 mM β-glycerol phosphate disodium salt (Fluka 50020) as described by Novick, *Methods Enzymol* 204: 587-636 (1991). Cytodex 1 beads (GE Healthcare 17-0448-01) were suspended (1 gram in 50 mL) in Dulbecco's Phosphate Buffered Saline without calcium/magnesium (Gibco) overnight at 20°C. The supernatant was decanted and the beads washed three times by suspension in DPBS and 1G sedimentation followed by autoclaving (121°C, 15 psi, 15 minutes). *Staphylococcus aureus* RN4850 (AIP4) was grown from frozen stock (BHI + 20% glycerol) on brain heart infusion agar plates 35°C overnight. Three representative colonies were combined to inoculate 2 mL CYGP broth, and after overnight incubation without shaking, 0.25 mL of the culture was used to inoculate 5 mL of CYGP followed by incubation at 35°C, 200 rpm for 3 hours. The culture was centrifuged 1,300 × G at 4 °C for 20 minutes, the supernatant poured off, and the bacterial pellet was suspended in 1 mL DPBS without calcium/magnesium. The SKH1 received 200 μL intradermal flank injections containing *S. aureus* (1×10^7 or 1×10^8 bacteria), 4 μL packed volume Cytodex beads, DPBS, anti-AIP4 antibody or control IgG (0.6 or 0.06 mg). Additional control animals received 200 μL intradermal injections containing Cytodex beads or beads plus antibody. After injections were made the mice were monitored at least three times each day over a period of 4-7 days. At the conclusion of the monitoring period the mice were euthanized and tissues harvested for bacteriologic and histologic analysis.

Example 10 – Passive Immunization of Mice With AP4-24H11

S. aureus RN4850 were stored at -80 °C in 20 % glycerol/BHI medium, thawed and grown on BHI-agar plates overnight, and three separate colonies sampled to inoculate 2 mL CYGP medium. The inoculum culture was maintained 1 hour at 35 °C without shaking, followed by shaking at 200 rpm for 3 hours. Aliquots of the freshly grown inoculum culture were transferred to 5

mL CYGP medium in 50 mL conical polypropylene tubes (1/20 dilution) followed by shaking at 200 rpm, 35 °C for 3 hours. The bacteria were pelleted by centrifugation at 3,000 rpm (1300 × G) for 10 minutes, 4 °C. The bacterial pellets were resuspended in Dulbecco's phosphate buffered saline without calcium or magnesium (DPBS$^-$), and enumerated using a Petroff-Hausser counting chamber. Final dilutions were made in DPBS$^-$ so that 3×10^8 bacteria were administered i.p. in 0.5 mL. To maintain viability bacteria were administered within two hours of harvest.

Mab AP4-24H11, isotype-matched control IgG (1 mg each) or DPBS was administered i.p. in DPBS to SKH1 mice (6-9 weeks old; 6 animals per treatment group) followed two hours later by 0.5 mL DPBS$^-$ i.p. containing 3 x 10^8 *S. aureus*. The mice were monitored several times on the day of injection and twice each day on subsequent days, observing ambulation, alertness, response to handling and skin temperature measured by infrared thermometry (Raytek MiniTemp MT4) using a 1 cm diameter infrasternal skin site. Animals showing surface temperature consistently below 30 °C and also diminished response to handling and weakened righting reflex were considered moribund and were euthanized.

Example 11 – Competition ELISA Analysis

The optimal concentrations of the AP4-BSA conjugate as well as of each mAb were determined. 96 well ELISA plates were coated with the appropriate amount of AP4-BSA conjugate respectively. The plates were blocked with 4% skim milk, washed and mAbs were added at the predetermined optimal concentration. The plates were washed and free antigen, i.e. the native AIPs 1-4, was added to the wells in a concentration series starting at 100 μM. The plate was incubated for 1hour at 37 °C, thoroughly washed, and goat anti-mouse-horseradish peroxidase (HRP) conjugate (Pierce, Rockford, IL) was added. After an incubation period of 1 hour at RT, the plate was thoroughly washed again and the HRP substrate (TMB substrate kit; Pierce) was added, the reaction was allowed to develop for 15 minute and stopped by the addition of 2 M H_2SO_4. The absorbance at 450 nm was read and the values plotted using GraFit (Erithacus Software Ltd). The free antigen concentration at which the

absorbance value is 50 % of the maximum absorbance was considered the K_d of the antibody for its antigen.

Example 12 – Generation of Anti-AP4 Monoclonal Antibodies

Based on the reported structural information of AIP-4 (Mayville *et al.*, *Proc. Natl. Ncad. Sci. U.S.A.* 96:1218-1223 (1999)), the hapten AP4-**5** was designed and synthesized to elicit an anti-AIP-4 antibody immune response in mice (**Scheme 1**). The rationale for the chemical switch from the native thiolactone to a lactone-containing hapten was based on a lactone's greater aminolytic stability. This strategy ensured that the hapten conjugates remained structurally intact during the immunization process and subsequent immune response; thus, avoiding the generation of degradation products with unknown chemical and biological properties as previously uncovered for other QS molecules. This substitution was also prevented a possible intramolecular thiol exchange between the conserved thiolactone and the pendant cysteine thiol. Therefore, Fmoc-Serine(Trt)-OH was incorporated at position 4 in place of the native cysteine residue.

The hapten **5** was conjugated to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) via a bifunctional linker (**Scheme 2**). Balb/c mice were immunized with the KLH conjugate using standard protocols (see Kaufmann *et al.*, *J. Am. Chem. Soc.* 128:2802-03 (2006)). Overall, the immunizations resulted in moderate titers (1600 – 3200), and based on ELISA analysis, 20 monoclonal antibodies (mAbs) were selected.

Of these, the binding affinities of three AP4-mAbs were determined. Their binding affinities, shown in the following table, were determined against all four natural AIPs using competition ELISA methodology.

Binding Constants of Selected AP4 Monoclonal Antibodies as Measured by Competition ELISA

AP4-mAb	AIP-1	AIP-2	AIP-3	AIP-4
23E6	≈ 6 μM	> 25 μM	> 25 μM	≈ 390 nM
24H11	≈ 5 μM	> 25 μM	> 25 μM	≈ 90 nM
29E10	≈ 3 μM	> 25 μM	> 25 μM	≈ 24 nM

All binding constants were measured at least twice, and the average values are shown. While AP4-29E10 possessed a higher affinity for AIP-4, it

was not selected for further biological evaluation due to technical difficulties encountered during the protein production phase.

AP4-24H11, possessed strong binding affinity ($K_{d\ AIP-4} \approx 90$ nM) and high specificity to AIP-4, while displaying little cross reactivity for the other AIPs ($K_{d\ AIP-1} \approx 5$ μM, $K_{d\ AIP-2} = >25$ μM, $K_{d\ AIP-3} = >25$ μM). The ability of AP4-24H11 to discriminate between AIP1 and AIP4 is noteworthy as these two oligopeptides differ only at position 5 with an aspartic acid residue in AIP-1, and a tyrosine moiety in AIP-4. AP4-24H11 was selected for further biological evaluation.

***Example 13 – AP4-24H11 Alters Expression of Virulent Factors in* S. aureus**

α-Hemolysin and protein A are two major virulence factors in *S. aureus*, and expression of these proteins is tightly regulated by *S. aureus* signaling networks including the AIP-based *agr* QS system. The *agr* QS system positively regulates expression of α-hemolysin, while protein A production is down-regulated by QS signaling.

To determine whether anti-AIP antibodies are able to interfere with QS signaling in *S. aureus*, whether the anti-AIP-4 mAb AP4-24H11 could modulate the expression of α-hemolysin and protein A in *agr* group IV strains, RN4850 and NRS168, was examined. Results in FIG 3A indicate that AP4-24H11 affects the expression and/or secretion of *S. aureus* exoproteins, some of which might also be regulated by the *agr* QS circuits. As seen in FIG 4A, mAb AP4-24H11 can successfully reduce the α-hemolysin expression in *S. aureus*, and no hemolytic activity was observed on blood agar plates with the AP4-24H11 treated supernatant as shown in FIG. 3B. In contrast, protein A expression was significantly increased by mAb AP4-24H11 in RN4850, which is also consistent with *agr* QS inhibition.

The only structural difference between AIP-1 and AIP-4 is position 5, and the data suggest that AP4-24H11 is able to bind to AIP-1 with moderate affinity (≈ 5 μM). Therefore, whether AP4-24H11 could affect QS signaling in an *agr* group I strain, namely Wood 46 was investigated. AP4-24H11 was not able to block α-hemolysin expression in Wood 46 as effectively as in RN4850. However, a notable decrease in α-hemolysin production in Wood 46 grown in the presence of AP4-24H11 was evident (FIG. 4A). These data suggest that it is

possible to generate cross-reactive mAbs that suppress *S. aureus* QS signaling of two or more different *agr* groups.

It is possible that the decrease in toxin production and overall protein secretion is caused by an antibody-mediated growth defect, results indicate that no significant growth changes of *S. aureus* were observed over a 24-hour growth period in the presence of AP4-24H11 (Fig. 4B). In addition, no discernable growth effects were observed with mAb SP2-6E11, an unrelated isotype control ($\kappa\gamma_{2a}$) for AP4-24H11.

One of the important bacterial virulent factors regulated by QS is biofilm formation. In *S. aureus*, biofilm formation is negatively regulated by *agr* QS signaling, which is one of the problems in controlling *S. aureus* virulence through *agr* QS inhibition. Consistent with previous studies, AP4-24H11-mediated QS inhibition led to increased biofilm formation in RN4850 (FIG. 4C). Although the increase of biofilm formation poses a significant problem in chronic infection of *S. aureus*, it represent a lesser predicament in acute infections and thus, mAb AP4-24H11 can be an effective way to control such *S. aureus* infections.

Example 14 – AP4-24H11 Alters Expression of Virulent Factors by Interfering with the agr QS System

To further examine *agr* QS inhibition by AP4-24H11, real time-polymerase chain reaction (RT-PCR) analysis was performed to evaluate if the observed changes in virulent factor expression were indeed caused by interference with the *agr* QS system, i.e. whether the presence of AP4-24H11 affects the transcription of *rnaIII*, the immediate product of *agr* autoinduction and the main QS effector in *S. aureus*. As expected, the *rnaIII* transcriptional level in RN4850 during stationary growth phase was reduced significantly (> 50 fold), by AP4-24H11. Thus, the alteration of α-hemolysin and protein A expression is a direct result of the interference of AIP-4-mediated QS signaling by AP4-24H11 (**FIG. 4D**). Yet, the subtle changes in overall exoprotein expression (see **FIG. 3**) might be misconstrued to mean that AP4-24H11 does not block the QS signaling efficiently. However, the RT-PCR analysis provides evidence that AP4-24H11 significantly inhibits AIP4-based QS in *S. aureus* RN4850.

To analyze the specificity of antibody-based QS interference in *S. aureus*, the transcriptional levels of two additional virulence regulators, namely *sarA* (*s*taphylococcal *a*ccessory *r*egulator) and *saeR* (*s*taphylococcal *a*ccessory protein *e*ffector), which control the response to environmental stresses as well as virulence factor expression in *S. aureus*, were investigated. Importantly, no significant changes ($\leq$ 2-fold) were observed in either *sarA* or *saeR* transcription, indicating that AP4-24H11 only affects *agr* QS system (**FIG. 4D**).

The transcription of α-hemolysin and protein A was analyzed by RT-PCR as described above. As stated, (vide supra), significant changes were seen in protein expression level. In terms of transcription, the *hla* and *spa* genes were suppressed and elevated respectively $\approx$ 3 to 5 fold, again confirming that *rnaIII* affects not only transcription but also translation of these proteins. Finally, exofoliatin A (*eta*) transcription was investigated. Exofoliatin is another *agr* QS regulated toxin exclusively produced by AIP-4-utilizing *S. aureus* strains. The data indicated that AP4-24H11 also decreased *eta* transcription by $\approx$ 10 fold (**FIG. 4D**).

Example 15 – Inactivation of AP4-24H11 by the Synthetic AIP-4

To determine whether AP4-24H11 inhibited *agr* QS through binding to AIP-4 and sequestering it from the cell growing medium, or whether AP4-24H11 affected other signaling systems in *S. aureus* including the linear peptide RNAIII-inhibiting peptide (RAP), which in turn affect *agr* QS network, the following experiment was conducted to determine whether external addition of AIP-4 could restore the *agr* QS signaling network in *S. aureus* RN4850 in the presence of AP4-24H11. Briefly, AP4-24H11 was treated with an equimolar amount of synthetic AIP-4 before addition to the *S. aureus* growth medium to assure saturation of the antibody binding sites with the AIP-4 peptide. As seen in **FIG. 4E**, the addition of synthetic AIP-4 efficiently reduced the quorum quenching effect of AP4-24H11, and as a result, fully restored expression of α-hemolysin in *S. aureus* RN4850. This finding provides additional confirmation that AP4-24H11 sequesters AIP-4 in *S. aureus* growth medium and inhibits AIP-dependent QS signaling in *S. aureus* in a strictly AIP-4-dependent manner.

Example 16 – AP4-24H11 Inhibits S. aureus-induced Apoptosis in Mammalian Cells

Recent studies have shown that incubation of Jurkat T cells with supernatant of *S. aureus* culture results in induction of apoptosis. Jurkat cells were treated with the supernatants of *S. aureus* (RN4850 and Wood 46) cultures grown in the presence or absence of AP4-24H11. After incubation for 4 hours with the supernatant, the cleavage of poly(ADP-ribose) polymerase (PARP), a biochemical marker indicative of apoptosis induction, was evaluated in Jurkat cell protein extracts. As shown in FIG. 5, AP4-24H11 prevented RN4850 supernatant (1 %)-induced PARP cleavage in Jurkat cells, and also partially inhibited the effect of Wood 46 supernatant. The results (**FIG. 4A** and **FIG. 5**) indicate a positive correlation between expression of α-hemolysin and *S. aureus*-induced apoptosis.

Example 17 – AP4-24H11 Blocks S. aureus-induced Dermal Injury in Mice

Next, the potential of mAb AP4-24H11 to mitigate *S. aureus*-induced injury *in vivo* was investigated by employing a murine subcutaneous infection model. Freshly grown log phase *S. aureus* RN4850 were suspended in PBS containing Cytodex beads, and where indicated, AP4-24H11 or control IgG. Subcutaneous injections of bacterial suspension or vehicle control were made in the flank of SKH1 hairless mice followed by close monitoring over seven days. Doses administered were 10^7 or 10^8 bacteria (colony forming units; cfu) and 0.6 or 0.06 mg AP4-24H11 or control IgG. Mice receiving 10^7 cfu developed minimal hyperemia/edema followed by limited induration over 7 days (see **FIG. 6**). However, as early as six hours after injection, mice receiving 10^8 cfu suspended in saline or control IgG showed early-stage hyperemia / redness at the injection site and extending 3-5 mm horizontally and 5-10 mm vertically in a diagonal pattern along the flank (**FIG. 7A**). Upon reexamination at 18 hours, the same areas surrounding the injection site were devitalized, and the skin was transformed to a brittle, reddish-brown scab. Over the 7-day observation period, the hardened scab began to detach from the surrounding relatively normal appearing skin, and small amounts of purulent exudate were observed at the normal/necrotic junction. In contrast, skin injury was abrogated in mice that received 10^8 bacteria with 0.6 mg AP4-24H11 (**FIG. 7C**). As anticipated, the lower dose of AP4-24H11 (0.06 mg) was not protective (**FIG. 7B**), and control

mice receiving 10^8 cfu with 0.6 mg control IgG were not protected (see **FIG. 6**). Mice that received an injection of PBS/Cytodex alone or containing 0.6 mg AP4-24H11 remained normal over the observation period with the exception of occasional local induration (**FIG. 7D**). Animals that had received the protective dose of 0.6 mg AP4-24H11 in combination with *S. aureus* RN4850 did not develop any significant lesions over the 7 day observation period.

Example 18 – Passive Immunization with AP4-24H11 Protected Mice From S. aureus-induced Fatality

To evaluate the effectiveness of a passive immunization approach using AP4-24H11 against a lethal challenge with *S. aureus*, SKH1 hairless mice received a 1 ml i.p. injection of AP4-24H11, control IgG or vehicle (DPBS) followed 2 hours later by 0.5 mL DPBS$^-$ containing 3×10^8 *S. aureus* RN4850. As shown in **FIG. 8**, all of the mice receiving AP4-24H11 (6/6) survived through the 8-day observation period. In contrast, only one of the DPBS-treated control mice (1/6) and none of the control IgG-treated mice (0/6) survived longer than 24 hours. These data further validated our immunopharmcaothereutic approach for combating acute *S. aureus* infections.

Example 19 – Competition ELISA Analysis of Monoclonal Antibodies Against AP-1, AP-3 and AP-4

The AP-1, AP-3 and AP-4 haptens and monoclonal antibodies specific for these haptens were prepared as described in Examples 4 and 12 above.

For the competition ELISA analysis, the optimal concentrations of the AP1-BSA, AP3-BSA, or AP4-BSA conjugate, as well as of each mAb were determined. 96 well ELISA plates were coated with the appropriate amount of AP1-BSA, AP3-BSA, or AP4-BSA conjugate respectively. The plates were blocked with 4v% skim milk, washed and mAbs were added at the predetermined optimal concentration. The plates were washed and free antigen, i.e. the native AIPs 1-4, was added to the wells in a concentration series starting at 100 μM. The plate was incubated for 1 hour at 37 °C, thoroughly washed, and goat anti-mouse-horseradish peroxidase (HRP) conjugate (Pierce, Rockford, IL) was added. After an incubation period of 1hour at RT, the plate was thoroughly washed again and the HRP substrate (TMB substrate kit; Pierce) was added, the

reaction was allowed to develop for 15 minutes and stopped by the addition of 2 M H_2SO_4. The absorbance at 450 nm was read and the values plotted using GraFit (Erithacus Software Ltd). The free antigen concentration at which the absorbance value is 50 % of the maximum absorbance was considered the K_d of the antibody for its antigen.

The affinity and crossreactivity data are shown in the following tables. These data demonstrate that using the hapten design strategy disclosed herein, monoclonal antibodies (mAbs) were obtained against the lactone analog of the native thiolactone peptide as hapten. The affinities of the mAbs range from low nanomolar to high micromolar, and some but not all mAbs showed crossreactivity, i.e. they recognize the native AIP based on which their original hapten was designed, as well as one or two of the other naturally-occurring AIPs.

AP1 Sups	AIP1 wt	AIP2 wt	AIP3 wt	AIP4 wt
1H11	> 25 μM	> 100 μM	> 25 μM	> 100 μM
2A9	> 25 μM	> 100 μM	> 25 μM	> 100 μM
2C2	**~ 800 nM**	> 100 μM	~ 3 μM	> 100 μM
2C10	> 25 μM	> 100 μM	> 25 μM	> 100 μM
2H9	**~ 6 μM**	> 100 μM	> 25 μM	~ 12 μM
3B1	~ 6 μM	> 100 μM	> 25 μM	> 100 μM
3B11	~ 6 μM	> 100 μM	> 25 μM	> 100 μM
3E11	> 25 μM	> 100 μM	> 25 μM	> 100 μM
4D3	~ 6 μM	> 100 μM	> 25 μM	> 100 μM
6H10	> 25 μM	> 100 μM	> 25 μM	> 100 μM
9A9	~ 6 μM	> 100 μM	~ 12 μM	> 100 μM
9B2	~ 25 μM	> 100 μM	> 25 μM	> 100 μM
9B9	No Data	No Data	No Data	No Data
9C3	~ 6 μM	> 100 μM	> 25 μM	> 100 μM
9C4	~ 6 μM	> 100 μM	> 25 μM	> 100 μM
9F9	**~ 3 μM**	> 100 μM	~ 3 μM	> 100 μM
10D6	> 25 μM	> 100 μM	> 25 μM	> 100 μM
10F4	> 25 μM	> 100 μM	> 25 μM	> 100 μM
11B10	**~ 3 μM**	> 100 μM	> 25 μM	> 100 μM
12A10	> 25 μM	> 100 μM	> 25 μM	> 100 μM
13A11	~ 12 μM	> 100 μM	> 25 μM	> 100 μM
13H3	> 25 μM	> 100 μM	> 25 μM	> 100 μM
15B4	**~ 800 nM**	> 100 μM	~ 1 μM	> 100 μM
15G12	> 25 μM	> 100 μM	> 25 μM	> 100 μM
16E11	> 25 μM	> 100 μM	> 25 μM	> 100 μM
16F4	~ 25 μM	> 100 μM	> 25 μM	> 100 μM
16G9	~ 12 μM	> 100 μM	> 25 μM	> 100 μM
17F5	~ 12 μM	> 100 μM	> 25 μM	> 100 μM

AP3 Sups	AIP1 wt	AIP2 wt	AIP3 wt	AIP4 wt
18A7	> 625 μM	> 625 μM	> 625 μM	> 625 μM
21C4	156 μM	> 625 μM	> 625 μM	> 625 μM
21E10	> 625 μM	> 625 μM	> 625 μM	> 625 μM
21H11	> 625 μM	> 625 μM	> 625 μM	> 625 μM
22B3	> 625 μM	> 625 μM	> 625 μM	> 625 μM
22D1	156-312 μM	> 625 μM	78 μM	> 625 μM
22E12	> 625 μM	> 625 μM	> 625 μM	> 625 μM
22H10	156 μM	> 625 μM	312 μM	> 625 μM
23C9	> 625 μM	> 625 μM	> 625 μM	> 625 μM
23H1	> 625 μM	> 625 μM	> 625 μM	> 625 μM
24H9	> 625 μM	> 625 μM	> 625 μM	> 625 μM
25A3	> 625 μM	> 625 μM	> 625 μM	> 625 μM
25E2	> 625 μM	> 625 μM	> 625 μM	> 625 μM
25E9	156 μM	> 625 μM	> 625 μM	> 625 μM
25F5	625-312 μM	> 625 μM	156-312 μM	> 625 μM
26A2	> 625 μM	> 625 μM	> 625 μM	> 625 μM
26G3	> 625 μM	> 625 μM	> 625 μM	> 625 μM
26G11	> 625 μM	> 625 μM	> 625 μM	> 625 μM
27E1	> 625 μM	> 625 μM	> 625 μM	> 625 μM
28H8	> 625 μM	> 625 μM	> 625 μM	> 625 μM
29A2	~ 9.8 μM	> 625 μM	**~ 612 nM**	> 625 μM
29B8	> 625 μM	> 625 μM	> 625 μM	> 625 μM
29D5	> 625 μM	> 625 μM	> 625 μM	> 625 μM
30C9	156 μM	> 625 μM	> 625 μM	> 625 μM
30H8	> 625 μM	> 625 μM	> 625 μM	> 625 μM
30H11	156 μM	156 μM	**4.9-2.5 μM**	> 625 μM

AP4 Sups	AIP1 wt	AIP2 wt	AIP3 wt	AIP4 wt
9G2	> 25 μM	> 25 μM	> 25 μM	**~ 700 nM**
12A2	> 25 μM	> 25 μM	> 25 μM	> 25 μM
13G5	> 25 μM	> 25 μM	> 25 μM	> 25 μM
15B3	> 25 μM	> 25 μM	> 25 μM	> 25 μM
15C3	> 25 μM	> 25 μM	> 25 μM	> 25 μM
15E8	> 25 μM	> 25 μM	> 25 μM	> 25 μM
16D1	> 25 μM	> 25 μM	> 25 μM	> 25 μM
17G2	> 25 μM	> 25 μM	> 25 μM	> 25 μM
18D3	> 25 μM	> 25 μM	> 25 μM	> 25 μM
18G10	> 25 μM	> 25 μM	> 25 μM	> 25 μM
22B8	> 25 μM	> 25 μM	> 25 μM	> 25 μM
22D9	> 25 μM	> 25 μM	> 25 μM	> 25 μM
22F2	> 25 μM	> 25 μM	> 25 μM	> 25 μM
22G7	> 25 μM	> 25 μM	> 25 μM	> 25 μM
23C4	> 25 μM	> 25 μM	> 25 μM	> 25 μM

23E6	~ 6 μM	> 25 μM	> 25 μM	**~ 390 nM**
24H11	~ 5 μM	> 25 μM	> 25 μM	**~ 98 nM**
26E8	> 25 μM	> 25 μM	> 25 μM	> 25 μM
27E9	> 25 μM	> 25 μM	> 25 μM	> 25 μM
29E10	~ 3 μM	> 25 μM	> 25 μM	**~ 24 nM**

All hybridomas competing were re-tested and the average is shown. AP4-29E10 was tested 5 different times showing variability ranging from 2 nM – 110 nM, but most hovered around 24 nM.

The amino acid and nucleotide sequences were determined for selected monoclonal antibodies, and their sequences are shown in the Tables below.

Amino Acid Sequences of the Variable Heavy and Light Chains of Murine Monoclonal Antibodies

Antibody	Variable Heavy Chain	Variable Light Chain
AP1-15B4	EVHLVESGGDLVKPGGSLKLS CAASGFAFSDFAMSWVRQTPE KRLEWVAIIKSDDSYTYYPDS VRDRFTISRDNARNTLYLQMT SLRSEDTALYYCTKIYDAYFY AMDYWGQGTSVTVSS (SEQ ID NO: 19)	DIVRTQSPLSLSVSLGDQASISC RSSQSLLHSNGNTYLHWYLQKPG QSPKLLIYKVSNRFSGVPDRFSG SGSGTDFTLKISILEAEDLGIYF CSQSTHFPTFGGGTKLEIK (SEQ ID NO: 147)
AP4-24H11	EVKPQESGPGLVKPSQSLSLT CTVTGYSITSNYAWNWIRQFP GNKLEWMGFISSYGTTTYNPS LKSRFSITRDTSKNQFFLQLH SVTIEDTGTYFCTREGDYWGQ GTTLTVSS (SEQ ID NO: 20)	DIVMTQATLSLPVSLGDQASISC RSSQRLVPSNGNIYLHWFLQKPG QSPKLLIYKLSSRFSGVPDRFSG SGSGTDFTLKISRVESEDLGIYF CSQTTHVPYTFGGGTKLEIK (SEQ ID NO: 148)
AP4-29E10-1	EVQLQQSGPELEKPGASVKIS CKASGHSFTGYNMNWVKQSND KSLEWIGNIAPYYGVTAYNQK FKGKATLTGDKSSSTAYMQLK SLASEDSAVYYCVLDTSGYAS WGQGTLVTVSA (SEQ ID NO: 21)	DIVMTQATASLTVSLGQRATISC RASKSVSTSGYSYMHWYQQKPGQ PPKLLIYLASNLESGVPARFSGS GSGTDFTLNIHPVEEEDAATYYC QHSREVPYTFGGGTKLELK (SEQ ID NO: 149)
AP4-29E10-2	QVQLQQSGPELEKPGASVKIS CKASGHSFTGYNMNWVKQSND KSLEWIGNIAPYYGVTAYNQK FKGKATLTGDKSSSTAYMQLK SLTSEDSAVYYCVLDTSGYAS WGQGTLVTVSA (SEQ ID NO: 22)	DIEMTQITASLTVSLGQRATISC RASKSVSTSGYSYMHWYQQKPGQ PPKLLIYLASNLESGVPARFSGS GSGTDFTLNIHPVEEEDAATYYC QHSREVPYTFGGGTKLELK (SEQ ID NO: 150)

AP1-15B4-Δ	GGDLVKPGGSLKLSCAASGFA FSDFAMSWVRQTPEKRLEWVA IIKSDDSYTYYPDSVRDRFTI SRDNARNTLYLQMTSLRSEDT ALYYCTKIYDAYFYAMDYWGQ GTS (SEQ ID NO: 23)	PLSLSVSLGDQASISCRSSQSLL HSNGNTYLHWYLQKPGQSPKLLI YKVSNRFSGVPDRFSGSGSGTDF TLKISILEAEDLGIYFCSQSTHF PTFGGGT (SEQ ID NO: 151)
AP4-24H11-Δ	GPGLVKPSQSLSLTCTVTGYS ITSNYAWNWIRQFPGNKLEWM GFISSYGTTTYNPSLKSRFSI TRDTSKNQFFLQLHSVTIEDT GTYFCTREGDYWGQGTT (SEQ ID NO: 24)	TLSLPVSLGDQASISCRSSQRLV PSNGNIYLHWFLQKPGQSPKLLI YKLSSRFSGVPDRFSGSGSGTDF TLKISRVESEDLGIYFCSQTTHV PYTFGGGT (SEQ ID NO: 152)
AP4-29E10-1-Δ	GPELEKPGASVKISCKASGHS FTGYNMNWVKQSNDKSLEWIG NIAPYYGVTAYNQKFKGKATL TGDKSSSTAYMQLKSLASEDS AVYYCVLDTSGYASWGQGTL (SEQ ID NO: 25)	TASLTVSLGQRATISCRASKSVS TSGYSYMHWYQQKPGQPPKLLIY LASNLESGVPARFSGSGSGTDFT LNIHPVEEEDAATYYCQHSREVP YTFGGGT (SEQ ID NO: 153)
AP4-29E10-2-Δ	GPELEKPGASVKISCKASGHS FTGYNMNWVKQSNDKSLEWIG NIAPYYGVTAYNQKFKGKATL TGDKSSSTAYMQLKSLTSEDS AVYYCVLDTSGYASWGQGTL (SEQ ID NO: 26)	TASLTVSLGQRATISCRASKSVS TSGYSYMHWYQQKPGQPPKLLIY LASNLESGVPARFSGSGSGTDFT LNIHPVEEEDAATYYCQHSREVP YTFGGGT (SEQ ID NO: 154)

Nucleotide Sequences of the Variable Heavy and Light Chains of Murine Monoclonal Antibodies

Antibody	Variable Heavy Chain	Variable Light Chain
AP1-15B4	gaggtgcacctggtggagtctgggggagacttagtgaagcctgggggg tccctcaaactctcctgtgcagcctctggattcgctttcagtgacttt gccatgtcttgggttcgccagactccggagaagaggctggagtgggtc gcaatcattaaaagtgatgattcttacacctactatccagacagtgtg agggaccgattcaccatctccagagacaatgccaggaacacccctttac ctgcaaatgaccagtctgaggtctgaagacacggccttgtattactgt acaaaaatctatgatgcttacttctatgctatggactactggggtcaa ggaacctcagtcaccgtctcctcg (SEQ ID NO: 27)	gacattgtgaggacacagtctccactctccctgtctgtcagtcttggag atcaagcctccatctcttgtagatctagtcagagccttttacacagtaa tggaaacacctatttacattggtacctgcagaagccaggccagtctcca aaactcctgatctacaaagtttccaaccgattttctggggtcccagaca ggttcagtggcagtggatcagggacagatttcacactcaagatcagcat attggaggctgaggatctgggaatttatttctgctctcaaagtacacat tttccgacgttcggtggaggcaccaagctggaaataaaa (SEQ ID NO: 155)
AP4-24H11	gaggtgaagcctcaggagtcaggacctggcctggtgaaaccttctcag tctctgtccctcacctgcactgtcactggctactcaatcaccagtaat tatgcctggaactggatccggcagtttccaggaaacaaactggagtgg atgggcttcataagttcctatggaaccactacctacaacccttctctc aaaagtcgattctctatcactcgagacacatccaagaaccagttcttc ctgcaattgcattctgtgactattgaggacacaggcacatatttctgt acaagagagggtgactactggggccaaggcaccactctcacagtctcc tca (SEQ ID NO: 28)	gacattgtgatgactcaggctacactctccctgcctgtcagtcttggag accaagcctccatctcttgcagatccagtcagcgccttgttcccagtaa tggaaacatttatttacattggttcctgcagaagccaggccagtctcca aagctcctgatctacaaactttccagtcgattttctggggtcccagaca ggttcagtggcagtggatcagggacagatttcacactcaagatcagcag agtggagtctgaggatctgggaatttatttctgctctcaaactacacat gttccatacacgttcggaggggggaccaagctggaaatcaaa (SEQ ID NO: 156)
AP4-29E10-1	gaggtccagctgcaacagtccggacctgagctggagaagcctggcgct tcagtgaagatatcctgcaaggcttctggtcattcattcactggctac aacatgaactgggtgaagcagagcaatgacaagagccttgagtggatt ggaaatattgctccttactatggtgttactgcctacaaccagaagttc aagggcaaggccacattgactggagacaaatcctccagcactgcctac atgcagctcaagagcctggcatctgaggactctgcagtctattactgt gtcctagacacctcgggctacgcttcctggggccaagggactctggta actgtctctgca (SEQ ID NO: 29)	gacattgtgatgactcaggctactgcttccttaactgtatctctggggc agagggccaccatctcatgcagggccagcaaaagtgtcagtacatctgg ctatagttatatgcactggtaccaacagaaaccaggacagccacccaaa ctcctcatctatcttgcatccaacctagaatctggggtccctgccaggt tcagtggcagtgggtctgggacagacttcaccctcaacatccatcctgt ggaggaggaggatgctgcaacctattactgtcagcacagtagggaggtt ccgtacacgttcggaggggggaccaagctggagctgaaa (SEQ ID NO: 157)

AP4-29E10-2	caggtccagctgcagcagtctgggcctgagctggagaagcctggcgctt cagtgaagatatcctgcaaggcttctggtcattcattcactggctacaa catgaactgggtgaagcagagcaatgacaagagccttgagtggattgga aatattgctccttactatggtgttactgcctacaaccagaagttcaagg gcaaggccacattgactggagacaaatcctccagcactgcctacatgca gctcaagagcctgacatctgaggactctgcagtctattactgtgtccta gacacctcgggctacgcttcctggggccaagggactctggtcactgtct ctgca (SEQ ID NO: 30)	gacattgagatgacccagattactgcttccttaactgtatctctggggc agagggccaccatctcatgcagggccagcaaaagtgtcagtacatctgg ctatagttatatgcactggtaccaacagaaaccaggacagccacccaaa ctcctcatctatcttgcatccaacctagaatctggggtccctgccaggt tcagtggcagtgggtctgggacagacttcaccctcaacatccatcctgt ggaggaggaggatgctgcaacctattactgtcagcacagtagggaggtt ccgtacacgttcggaggggggaccaagctggagctgaaa (SEQ ID NO: 158)
AP1-15B4-Δ	gggggagacttagtgaagcctgggggtccctcaaactctcctgtgca gcctctggattcgctttcagtgactttgccatgtcttgggttcgccag actccggagaagaggctggagtgggtcgcaatcattaaaagtgatgat tcttacacctactatccagacagtgtgagggaccgattcaccatctcc agagacaatgccaggaacacccttacctgcaaatgaccagtctgagg tctgaagacacggccttgtattactgtacaaaaatctatgatgcttac ttctatgctatggactactggggtcaaggaacctca (SEQ ID NO: 31)	ccactctccctgtctgtcagtcttggagatcaagcctccatctcttgta gatctagtcagagccttttacacagtaatggaaacacctatttacattg gtacctgcagaagccaggccagtctccaaaactcctgatctacaaagtt tccaaccgattttctggggtcccagacaggttcagtggcagtggatcag ggacagatttcacactcaagatcagcatattggaggctgaggatctggg aatttatttctgctctcaaagtacacattttccgacgttcggtggaggc acc (SEQ ID NO: 159)
AP4-24H11-Δ	ggacctggcctggtgaaaccttctcagtctctgtccctcacctgcact gtcactggctactcaatcaccagtaattatgcctggaactggatccgg cagtttccaggaaacaaactggagtggatgggcttcataagttcctat ggaaccactacctacaacccttctctcaaaagtcgattctctatcact cgagacacatccaagaaccagttcttcctgcaattgcattctgtgact attgaggacacaggcacatatttctgtacaagagagggtgactactgg ggccaaggcaccact (SEQ ID NO: 32)	acactctccctgcctgtcagtcttggagaccaagcctccatctcttgca gatccagtcagcgccttgttcccagtaatggaaacatttatttacattg gttcctgcagaagccaggccagtctccaaagctcctgatctacaaactt tccagtcgattttctggggtcccagacaggttcagtggcagtggatcag ggacagatttcacactcaagatcagcagagtggagtctgaggatctggg aatttatttctgctctcaaactacacatgttccatacacgttcggaggg gggacc (SEQ ID NO: 160)
AP4-29E10-1-Δ	ggacctgagctggagaagcctggcgcttcagtgaagatatcctgcaag gcttctggtcattcattcactggctacaacatgaactgggtgaagcag agcaatgacaagagccttgagtggattggaaatattgctccttactat ggtgttactgcctacaaccagaagttcaagggcaaggccacattgact ggagacaaatcctccagcactgcctacatgcagctcaagagcctggca tctgaggactctgcagtctattactgtgtcctagacacctcgggctac gcttcctggggccaagggactctg (SEQ ID NO: 33)	actgcttccttaactgtatctctggggcagagggccaccatctcatgca gggccagcaaaagtgtcagtacatctggctatagttatatgcactggta ccaacagaaaccaggacagccacccaaactcctcatctatcttgcatcc aacctagaatctggggtccctgccaggttcagtggcagtgggtctggga cagacttcaccctcaacatccatcctgtggaggaggaggatgctgcaac ctattactgtcagcacagtagggaggttccgtacacgttcggaggggggg acc (SEQ ID NO: 161)

AP4-29E10-2-Δ	gggcctgagctggagaagcctggcgcttcagtgaagatatcctgcaagg cttctggtcattcattcactggctacaacatgaactgggtgaagcagag caatgacaagagagccttgagtggattggaaatattgctccttactatggt gttactgcctacaaccagaagttcaagggcaaggccacattgactggag acaaatcctccagcactgcctacatgcagctcaagagcctgacatctga ggactctgcagtctattactgtgtcctagacacctcgggctacgcttcc tggggccaagggactctg (SEQ ID NO: 34)	actgcttccttaactgtatctctggggcagagggccaccatctcatgca gggccagcaaaagtgtcagtacatctggctatagttatatgcactggta ccaacagaaaccaggacagccacccaaactcctcatctatcttgcatcc aacctagaatctggggtccctgccaggttcagtggcagtgggtctggga cagacttcaccctcaacatccatcctgtggaggaggaggatgctgcaac ctattactgtcagcacagtagggaggttccgtacacgttcggagggggg acc (SEQ ID NO: 162)

Example 20 – Evaluation of Other Anti-AIP Antibodies

The quorum quenching ability of some of the newly obtained anti-AIP antibodies, *e.g.* anti-AP1 and anti-AP3 antibodies were evaluated. For the group I strains (RN6390B and Wood46), two monoclonal antibodies, AP1-2C2 and AP1-15B4, which showed high affinity toward AIP-1 in competition ELISA assay, were tested. FIG 9 shows that the anti-AP1 antibodies also efficiently inhibit quorum sensing of the group I strains resulting in changes in the virulent factors expression. In addition, the anti-AP3 antibodies against one of the group III strains, RN8465 were also tested. Due to low exoprotein expression in RN8465, the quorum quenching effects were not determined precisely.

Example 21 – Therapeutic Effects of Cyclic Peptide–based Vaccines

To evaluate the effectiveness of cyclic peptide-based vaccines, the following experiments are conducted. Active and passive vaccination schedules are as follows:

Active Vaccination Schedule

Initial titer:	day -1	
Initial immunization:	day 0	50-200 μg protein
Titer pre-boost 1:	day 6	
Boost 1:	day 7-14 (1-2 weeks after initial immunization)	50-200 μg protein
Titer pre-boost 2:	day 20	
Boost 2:	day 21-28 (1-2 weeks after boost 1)	50-200 μg protein
Titer pre-challenge:	day X (1 day before challenge)	
Challenge	day X (1 week after boost 2)	

Passive Vaccination Schedule

Initial titer:	day -1	
Immunization:	day 0	100-1000 μg IgG/mouse
Titer pre-challenge:	day 1	
Challenge	day 2	

The vaccines are administration by intravenous, intramuscular, intraperitoneal or subcutaneous injection to male Balb/c rats of 25-30g and between 8-12 weeks of age. Twenty animals are included in each treatment group.

To determine whether the vaccine protects the animal from a lethal system challenge, *S. aureus* strain of any with known agr group is used. About 10^8-10^9

C.F.U. of the bacteria is administered by intraperitoneal injection. Body temperatures and survival every 12 hours are determined. Death or survival after 10 days represents the end point of the study. Additional details are described above.

To determine whether the vaccine protects the animal from sepsis, *S. aureus* strain of any with known agr group is used. About 10^7-10^8 C.F.U. of the bacteria is administered by intravenous injection. Thus, *S. aureus* is administered directly into the blood stream and will spread hematogenously through the body. Body temperatures and survival every 12 hours are determined. Death or survival after 10 days represents the end point of the study.

To determine whether the vaccine protects the animal from septic arthritis, *S. aureus* strain LS-1 (a mouse-adapted strain belonging to agr group 1), or any strain with a known agr group that is capable of spontaneously causing arthritis, is used. About 10^6-10^7 C.F.U. of the bacteria is administered by intravenous injection. Body temperature, survival every 12 hours, joint swelling (scoring), redness, changes in moving patterns and morbidity are determined. Death or survival at 28 days represents the end point of the study.

To determine whether the vaccine protects the animal from renal abscess, *S. aureus* strain of any known agr group is used. About 10^6-10^7 C.F.U. of the bacteria is administered by intravenous injection. The animals are evaluated based on activity, alertness, and coat condition (scored 0-2 for normal, slightly abnormal, very abnormal). In addition, kidneys are removed aseptically and histologically evaluated (abscess formation; 0 – no visible abscesses; 1 – 1 small abscess; 2 – several abscesses; and 3 – severely abscessed kidneys), and C.F.U. counts are recovered from homogenized kidneys. Death or survival at 7 days marks the end point of the study.

The same model can be used to determine whether the vaccines can block renal abscess formation, in which case, general behavior and renal abscess based on histological evaluation of the kidneys are considered.

To determine whether the vaccine protects the animal from spreading throughout the body, as well as colonize a catheter, the foreign body model is used. A piece of catheter is implanted in a subcutaneous space on the mice. After 24 hours, a suspension of *S. aureus* strain of any known agr group is administered by subcutaneous injection of about 10^6-10^8 C.F.U. in the catheter bed. The ability of the bacteria to spread through the body and to colonize the catheter are evaluated by

determining body temperature, survival every 12 hours, subcutaneous abscess formation and C.F.U. count recovered from catheter at various time points. Death or survival at 7 days marks the end point of the study. Alternatively, a colonized catheter could be used in this model.

To determine whether the vaccine protects the animal from mastitis, lactating CD1 mice are administered by intramammary injection of about 10^2-10^4 C.F.U. of a *S. aureus* strain from any known agr group. C.F.U. counts from mammary glands are determined at various time points and expressed in C.F.U./gland or C.F.U./gram of mammary tissue. The amount of milk present in the gland and survival are also evaluated, and death or survival at 5 days marks the end point of the study. This is an established model of bovine mastitis caused by caused microbial intramammary infection that induces inflammation of the mammary gland. *S. aureus* provokes clinical mastitis, but more frequently causes subclinical infections that tend to become chronic and difficult to eradicate by conventional antimicrobial therapies.

Example 22 – Active Vaccination with AP4-KLH Protects Mice from a Lethal Systemic S. aureus Challenge

Mice were immunized i.p. with 100 μg of the immunoconjugate together with bacterial DNA containing unmethylated cytosine-guanosine dinucleotide motif-containing oligodeoxynucleotides (CpG-ODNs) as adjuvants. Chuang et al., *J Leukoc Biol* 71: 538-44 (2002). The animals received booster immunizations 7 days and 21 days after the initial vaccination. Serum samples were withdrawn for anti-AIP 4 antibody titer analysis from all animals prior to the infection experiment.

Results illustrating the protective effects of the vaccination in SKH1 hairless mice that had received 0.5 mL PBS i.p. containing 3×10^8 *S. aureus* RN4850 (Park et al., *Chem Biol* 14: 1119-1127 (2007)) are summarized in the following table..

Active Vaccination Against AIP4 Protects Mice From a Lethal *S. aureus* Challenge

Vaccine	Survivors
AIP4-KLH	4/6
KLH	1/6
PBS	2/6

As shown above, 4 of the 6 mice that received the AP4-KLH conjugate survived through the 8-day observation period. In contrast, only one of the KLH-

vaccinated control mice (1/6) and 2 of the PBS mock immunized mice (2/6) survived the observation period.

Analysis of the antibody titers revealed that the conjugates and immunization protocol elicited an immune response with titers in the range of 1:1000, i.e. the dilution at which 50% of the maximum signal strength is still observed as tested using standard ELISA methodology. This analysis also showed that the immunization induced an AIP4-specific immune response with cross-reactivities to AIP1 and AIP3 (anti-AIP4 titers: up to 1:6400; anti-AIP1 titers: up to 1:6400; anti-AIP3 titers: up to 1:3200).

Example 23 – Evaluation of Anti-AIP1 Antibodies

All anti-AIP1 mAbs obtained were tested against the group I *S. aureus* strain RN6390B. Results in **FIG. 10B** show that a number of anti-AP1 antibodies efficiently inhibited quorum sensing of group I *S. aureus* resulting in changes in hemolysin expression. The mAb AP1-15B4 (#4) exhibited the most potent activity in the immunization experiments.

Biofilm formation by *S. aureus* strain RN6390B was also evaluated in the presence of mAb AP1-15B4, as an increase in biofilm formation has been described in response to *agr* QS-signaling inhibition in *S. aureus*. Results in **FIG.10B** show an increase in biofilm formation by *S. aureus* strain RN6390B in the presence of mAb AP1-15B4.

Example 24 – Selection of Human scFv Antibodies Using Phage Display Technology

A phage display library generated using the method described by Gao et al. (Proc Natl Acad Sci U S A. 99:12612-6 (2002)) was screened using the AP1-BSA, AP3-BSA and AP4-BSA conjugates to identify human anti-AIP-1, AIP-3 and anti-AIP-4 scFv antibodies. The antibody-displaying phage particles were subtracted against BSA first to eliminate BSA-specific clones, as well as unspecific binders. After 4 rounds of panning, selected clones were analyzed by DNA sequencing and ELISA against BSA and AP1-BSA, AP3-BSA and AP4-BSA. The amino acid sequences of the scFv antibodies, the DNA sequences encoding the variable heavy and variable light chains, as well as the DNA sequences encoding the scFv antibodies are shown in the following tables.

Amino Acid Sequences of Human scFv Antibodies

AP1-2	QVQLVQSGAEVKKPGESLRISCKGSGYSFTSHWISWVRQMPGKGLEWMGRIDPSDSYSNYSPSFQGHVIISVDKSISTAYLQWSSLKASDTAIYY CARQLIVVVPAAPYYYYYYGMDVWGQGTLVTVSSGGGGSGGGGSGGGSEIVLTQSPGTLSLSPGERATLSCRASQTVNSYLAWYQKPGQAPRLL IYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSHPWTFGQGTKVEIK (SEQ ID NO: 35)
AP1-6	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAIYY CARVFGSESQDPSDIWSGYYGMEVWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPK LLIYAASSLQSRVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPYTFGQGTKLEIK (SEQ ID NO: 36)
AP1-8	QVQLVESGAEAKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC ARAGITGTTAPPDYWGQGTLVTVSSGGGGSGGGGSGGGGSVIWMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQRKPGKAPKLLIYAASSLQS GVTSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKLEIK (SEQ ID NO: 37)
AP1-11	QVQLVQSGSELKKPGASVKLSCRASGYTFTSYSMVWVRQAPGEGLEWMGGINTNTGNPTYAQGFTERFVFSFDSSVSTAYLQISSLKAEDTAVYY CARDWAYSGSWPLGQNPSDHWGQGTLVTVSSGGGGSGGGGSGGGGSEIVMTQSPATLSVSPGERATLSCRASQSVSRNLAWYQQKPGQAPRLLIY DTSTRATGIPARFSGSGSGTEFTLTISSLQSEDSAVYYCQQYNIWPPLTFGGGTKVEIK (SEQ ID NO: 38)
AP1-15	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYRTWIRQSPVKGLEWIGEVNDRGSPNYNPSFKSRLTISIDTSKNLSLKLRFMTAADTAVYSCA RIRPRYGMDVWGQGTMVTVSSGGGGSGGGGSGGGGSDIVMTQTPLSSPVTLGQPASISCRSSQSLVHSDGNTYLTWFHQRPGQPPRVLIHKVSNL FSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCMQATQLYTFGQGTKVEIK (SEQ ID NO: 39)
AP1-16	EVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETISAQKFQGRVTMTEDTSTDTAYMDLSSLRSEDTAVYYC ATQRLCSGGRCYSHFDYWGQGTTVTVSSGGGGSGGGGSGGGGSETTLTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQKPGSSPRLLIYDTSNV APGVPFRFSGSGSGTSYSLTINRMEAEDAATYYCQEWSGYPYTFGGGTKVEIK (SEQ ID NO: 40)
AP1-19	QMQLVQSGAEVKKPGSSVKVSCKASGGTFNTYVISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYY CARVWSPLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNMNYLAWYQQKPGQPPKLLIYWAST RESGVPDRFSGSGSGTDFTLTISSLQAEDAAVYYCQQYYSTPPTFGQGTKLEIK (SEQ ID NO: 41)
AP3-1	QVQLVQSGAEVKKPGASVKVSCKGSGYTFTGYYMHWVPQAPGQGLEWMGWINPNNGGTNYDQKFQGRVAMTRDTSISTAYMELSRLRSDDTAVYY CARDNGRVTTGGYWGQGTLVTVSSGGGGSGGGGSGGGGSQSVLTQPPSLSGAPGQSVTISCAGTSSSIGAGYDVQWYQQLPGKTPKLLIYGNDNR PSGVPDRFSGSRSYTSASLVITRVQIEDEADYYCQSYDSSLIGPQFGGGTKLTVLG (SEQ ID NO: 42)
AP3-2	QVQLVQSGAEVKKPGESLKISCTASGYNFASYWIGWVRQMPGQGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTATYY CVRRVPLYTNNHYLDYWGQGTLVTVSSGGGGSGGGGSGGGGSAIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWFQQKPGKAPKSLIYAASS LQSGVPSKYSGSGSGTDFTLTISSLQPEDFATYYCQQYKSYPLTFGGGTKVEIK (SEQ ID NO: 43)
AP3-3	EVQLVQSGAEVKKPGASVKVSCKASGYTFSDYFMHWVRQAPGQGLEWMGVINPTGGSTTYAQSFQGRVTMTRDTSTSIVYMELSSLRSEDTAVYY CTRVGYYGMDVWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPSTLSASVGDRVTITCRASQSTSRFLNWYQQKPGKAPKLLIYAASSLHSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTSSYPLTFGGGTKVEIK (SEQ ID NO: 44)

AP3-5	QVQLVQSGGGVVQVGRSLRLSCAASGFTFTNFGMHWVRQAPGKGLEWVALISSDGYRQAYADSVKGRFTISGDNSKNTVYLQMNSLTSEDTAVYY CAIIPPVLRIFDWEFDYWGQGTLVTVSSGGGGSGGGGSGGGGSETTLTQSPGTLSLSPGERATLSCRASQSVSSPYLAWYQQKPGQAPRLLIYGA SNRATGIPDRFSGSGSGTDFTLTISSLQAEDEAVYYCQQYNTPLTFGGGTKVEIK (SEQ ID NO: 45)
AP3-6	QVQLQQWGAGLLKPSETLSLTCAVYSGSFTRDYWGWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDKSKNQFSLKLTSVTAADTAVYYC ARRRLSSDLFMRGVGGMDVWGQGTLVTVSSGGGSGGGGSGGGGSDIVMTQTPGTLSSSPGERATLSCRASQGVSSNLAWYQQKPGQAPRLLIYD ASNRATGIPLRFSGSGSGTDFTLTISRLEPEDFAVYYCHQYGSSPYTFGQGTKVEIK (SEQ ID NO: 46)
AP3-8	EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQASGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYY CARVPRYFDWLLYGSDYFDYWGQGTLVTVSSGGGSGGGGSGGGGSDIQMTQSPSTLSVSVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIY AASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKLEIK (SEQ ID NO: 47)
AP3-10	QVQLVQSGAEVKEPGSSVKVSCKASGGTFSSYAIYWVRQAPGQGLEWMGWIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYC ARAAGHSTNYYYYGMDVWGQGTLVTVSSGGGSGGGGSGGGGSQTVVTQEPSLTVSLGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAPRTLIYDT SNKHSWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLLSYSGTRVFGGGTKLTVLG (SEQ ID NO: 48)
AP3-13	EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYYMHWVRQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYY CARDFKEYSRTGYFDYWGQGTLVTVSSGGGSGGGGSGGGGSSYELMQPSSVSVSPGQTARITCSGDVLAKKCARWFQQKPGQAPVLVIYKDSER PSGIPERFSGSSSGTTVTLTISGAQVEDEADYYCYSAADNNLGVFGGGTKVTVLG (SEQ ID NO: 49)
AP3-20	QITLKESGPALVKPTQTLTLTCNFSGFSLSTYGGVGWLRQPPGKALEWLAVIYWSDGKRYSPSVKNRLTITKDTSKNHVVLTMTNMDPVDTATY YCAHLMMDTSITTHWFDPWGQGTLVTVSSGGGSGGGGSGGGGSAIRMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAA STLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPGTFGQGTKVEIK (SEQ ID NO: 50)
AP4-8	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTNYFIHWVRQAPGQGLEWMGLLNPTDSGTLYAQNFQGRITMTSDTSTNTVYMELSSLRSDDTAMYY CAREGGADTTRVHSSFDYWGQGTLVTVSSGGGSGGGGSGGGGSQAVLTQPPSVSGSPGQSITISCTGTSSDVEAYNYVSWYQQHPGKAPKLMIY DVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSSRTWVFGGGTKVIVL (SEQ ID NO: 51)
AP4-14	QVQLQESGGGLVQPGRSLRLSCAASGFTFDDYALHWVRQAPGKGLEWVSGISWNSVTVKYAVSVKGRFTISRDNAKNSLFLQMNALRSEDTALYYC AKARGALLEAADTPSDDWGQGTLVTVSSGGGSGGGGSGGGGSDIVMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASS LQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPWTFGQGTKVDIK (SEQ ID NO: 52)
AP4-20	QVQLQQSGAGLLRPSETLSLTCGLYGGSFSGHYWNWIRQSPEKGLVWIGEITHSGTTNYNPSLKSRVITSVDTSKNQYSLKLSFVTPADTAVYYCA RGDYYGYWYFDLWGRGTLVTVSSGGGSGGGGSGGGGSQSVLTQPPSVPVAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDTNKRPSG IPDRFAGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGVFGGGTKLTVL (SEQ ID NO: 53)

Nucleotide Sequences Encoding the Heavy and Light Chains of Human scFv Antibodies

Antibody	Variable Heavy Chain	Variable Light Chain
AP1-2	caggtgcagctggtgcagtctggagcagaggtgaaaaagcccggggga gtctctgaggatctcctgcaagggttctggatacagctttaccagcc actggatcagctgggtgcgccagatgcccgggaaaggcctggagtgg atggggaggattgatcctagtgactcttatagcaactacagcccctc cttccaaggccacgtcatcatctcagttgacaagtccatcagcactg cctacttgcagtggagcagcctgaaggcctcggacaccgccatatat tactgtgcgagacagctcattgtagtagtaccagctgctccctatta ctactactacggtatggacgtctgggggccaaggaaccctggtca ccgtctcctca (SEQ ID NO: 54)	gaaattgtgttgacgcagtctccaggcaccctgtctttgtctccagg ggaaagagccaccctctcctgcagggccagtcagactgttaacagct acttagcctggtaccagtagaaacctggccaggctcccaggctcctc atctatggtgcatccagcagggccactggcatcccagacaggttcag tggcagtgggtctgggacagacttcactctcaccatcagcagactgg agcctgaagatttttgcagtgtattactgtcagcagtatggtagctca catccgtggacgttcggccaagggaccaaggtggagatcaaacgtgg cctcgggggcctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 55)
AP1-6	caggttcagctggtgcagtctgggggctgaggtgaagaagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatcagctgggtgcgacaggcccctggacaagggcttgagtgg atgggagggatcatccctatctttggtacagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacgaatccacgagcacag cctacatggagctgagcagcctgagatctgaggacacggccatatat tactgtgcgagagtctttggttccgagtcgcaagatccgtccgatat ttggagtggttattacggtatggaagtctgggggccaaggaaccctgg tcaccgtctcctca (SEQ ID NO: 56)	gacatccagatgacccagtctccgtctttccgtctgtctgcatctgtagg agacagagtcaccatcacttgtcgggcgagtcagggtattagcagct ggttagcctggtatcagcagaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtagggtcccatcaaggttcag cggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagatttttgcaacttactattgtcaacaggctaacagtttc ccgtacacttttggccaggggaccaagctggagatcaaacgtggcct cgggggcctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 57)
AP1-8	caggtgcagctggtggagtctgggggctgaggcgaagaagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatcagctgggtgcgacaggcccctggacaagggcttgagtgg atgggagggatcatccctatctttggtacagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacgaatccacgagcacag cctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagccggtataactggaactacggctcccccagacta ctggggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 58)	gtcatctggatgacccagtctccatcctccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcaagtcagagcattagcagct atttaaattggtatcagcggaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtggggtcacatcaaggttcag tggcagtggatctgggacagatttcactctcaccatcagcagtctgc aacctgaagatttttgcaacttactactgtcaacagagttacagtacc cctccgacgttcggccaagggaccaagctggagatcaaa (SEQ ID NO: 59)

AP1-11	caggtgcagctggtgcagtccggatctgagttaaagaagcctgggggc ctcagtgaagctttcctgcagggcttctggatacacattcactagtt attccatggtttgggtgcgacaggcccctggagaagaggcttgagtgg atgggagggatcaacaccaacactgggaacccaacgtatgcccagggg cttcacagaacggtttgtctttcctttcgacagctctgtcagcacgg catatctgcaaatcagcagcctaaaggctgaggacactgccgtgtat tactgtgcgagagatgggcgtatagcggcagctggcccttaggcca gaacccttctgaccactgggccagggcaccctggtcaccgtctcct ca (SEQ ID NO: 60)	gaaatagtgatgacgcagtctccagccaccctgtctgtctccagg ggaaagagccacccttcctcctgcagggccagtcagagtgttagccgca acttagcctggtaccagcagaaacctggccaggctcccaggctcctc atctatgatacatccaccagggccactggtatcccagccaggttcag tggcagtgggtcttgggacagagttcactctcaccatcagcagcctgc agtctgaagattctgcagtttattactgtcagcagtataatatctgg cctccactcacttttcggcggagggaccaaggtggagatcaaa (SEQ ID NO: 61)
AP1-15	caggtgcagctacagcagtggggcgcaggattgttgaagccttcgga gaccctgtccctcacctgcgctgtctatggtgggtccttcagtggtt actaccggacctggatccgccagtccccagtgaagggctggagtgg attgggggaagtcaatgatcgtgggaagcccccaactacaacccgtcctt caagagtcgactcaccatatcaatcgacacgtccaagaactagttat ccctgaagttgagatttatgaccgccgcggacacggctgtatattcg tgtgcgagaattaggcctaggtacggtatggacgtctgggccaggg gacaatggtcaccgtctcctcaggcggcggcggctct (SEQ ID NO: 62)	Gatattgtgatgacccagactccactcctcacctgtcacccttgg acagccggcctccatctcctgcaggtctagtcaaagcctcgtacaca gtgatggaaacacctacttgacttggtttcaccagaggccaggccag cctccaagagtcctcattcataaggtttctaacctgttctctgggct cccagacagattcagtggcagtgggggcagggacagatttcacactga aaatcagcagggtggaagctgaggatgtcgggggtttattactgcatg caagctacacaattgtacactttggccagggggaccaaggtggaaat caaa (SEQ ID NO: 63)
AP1-16	gaggtccagctggtacagtctgggggctgaggtgaagaagcctggggc ctcagtgaaggtctcctgcaaggtttccggatacaccctcactgaat tatccatgcactgggtgcgacaggctcctggaaaagggcttgagtgg atgggaggtttttgatcctgaagatggtgaaacaatctccgcgcagaa gttccagggcagagtcaccatgaccgaggacacatctacagacacag cctacatggatctgagcagcctgaggacacggccgtttttat tactgtgcaacgcagcgcttgtagtggtgctgctactcccca ctttgactactgggggccagggcaccacggtcaccgtctcctca (SEQ ID NO: 64)	gaaacgacactcacgcagtctccagcaatcatgtctgcatctccagg ggagagggtcaccatgacctgcagtgccagctcaagtatacgttaca tatattggtaccaacagaagcctggatcctccccagactcctgatt tatgacacatccaacgtggctcctggagtccctttcgcttcagtgg cagtgggtctgggacctcttattctctcacaatcaaccgaatggagg ctgaggatgctgccacttattactgccaggagtggagtggttatccg tacacgttcggagggggggaccaagtggagatcaaa (SEQ ID NO: 65)
AP1-19	cagatgcagctggtgcagtctgggctgaggtgaagaagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcaacacct atgttatcagttgggtgcgacaggcccctggacaagggcttgagtgg atgggatggatcagcgcttacaatggtaacacaaactatgcacagaa gctccagggcagagtcaccatgaccacagacacatccacgagcacag cctacatggagctgaggagcctgagatctgacgacacggccgtgtat tactgtgcgagagtttggagtcccccttgactactgggggccagggcac cctggtcaccgtctcctca (SEQ ID NO: 66)	gacatcgtgatgacccagtctccagactccctggctgtgtctctggg cgagagggccaccatcaactgcaagtccagccagagtgttttataca gctccaacaatatgaactacttagcttggtaccagcagaaaccagga cagcctcctaagctgctcatttactgggcatctacccgggaatccgg ggtccctgaccgattcagtggcagcgggtctgggacagatttcactc tcaccatcagcagcctgcaggctgaagatgcggcagtttattactgt cagcagtattatagtactcctccgacgttcggccaagggaccaagct ggagatcaaa (SEQ ID NO: 67)

AP3-1	caggtgcagctggtgcaatctgggggctgaggtgaagaagcctgggggc ctcagtgaaggtctcctgcaagggttctggatacaccttcaccggct actatatgcactgggtgccacaggccccttggacaagggcttgagtgg atgggatggatcaaccctaacaatggtggcacaaactatgaccagaa gtttcagggcagggtcgccatgaccagggacacgtccatctccacag cctacatggagctgagcaggctgagatctgacgcacactgccgtgtat tactgtgcgagagataatgggagggtgaccacaggggggctactgggg ccagggcaccctggtcaccgtctcctca (SEQ ID NO: 68)	cagtctgtgttgacgcagcctcccctcattgtctgggggccccgggaca gagtgtcaccatctcctgcgctgtgggaccagttccagcatcgggggcag gttacgatgtacagtggtaccagcaacttccaggaaaaaccccaaa ctcctcatctacgggaatgataatcggccctcagggggtccctgaccg attctctggatccaggtcttacacctcagcctccctggtcatcacta gagtccagattgaggatgaggctgattattactgccagtcgtatgac agcagtctcattggtcctcaattcggcgggg (SEQ ID NO: 69)
AP3-2	caggtgcagctggtggctgaggtgaaaaagcccgggga gtctctgaagatctcctgtacggcctccggatacaactttgccagct actggatcggctgggtgcgccagatgcccgggcaaggcctggagtgg atgggggatcatcctatcctgtgactctgatacagatacagtccgtc cttccaaggccaggtcaccatctcagccgacaagtccatcagcaccg cctacctgcagtggagcagcctgaaggcctcggacaccgccacgtat tactgtgtgagacgggtcccctctacactaacaaccactaccttga ctattgggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 70)	gccatccagatgacccagtcctccatcctcactgtctgcatctgtagg agacagagtcaccatcacttgtcgggcgagtcagggcattagcaatt atttagcctggtttcagcagaaaccagggaaagcccctaagtccctg atctatgctgcatccagtttgcaaagtgggtcccatcaaagtacag cggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagattttgcaacttattactgccaacagtatagagttac cccctcactttcggcggagggaccaaggtggagatcaaa (SEQ ID NO: 71)
AP3-3	gaggtgcagctggtgcagtctgggctgaagtgaagaagcctggggc ctcagtgaaggtttcctgtaaggcatctggatacacctcagcgact actttatgcactgggtgcgacaggccccttggacaagggcttgagtgg atggggagtaatcaacccaactggtggttccacaacctacgcacagag cttccagggcagagtcaccatgaccagagacacgtccacgagcatag tctacatggagctgagcagcctgagatctgaagacacggccgtgtac tactgtacgcgagtcggctactacggtatggacgtctgggccaagg caccctggtcaccgtctcctca (SEQ ID NO: 72)	gacatcgtgatgacccagtctccatccaccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcaagtcagagcactagcaggt tttaaattggtatcagcagaaacctgggaaagccccttaaactcctg atctatgctgcatccagtttgcatagtggcgtcccatcaaggttcag tggcagtggatctgggacagatttcactctcaccatcagcagtctgc aacctgaagattttgcaacttactactgtcaacagacttccagttac cctctcactttcggcggagggaccaagggtggaaatcaaa (SEQ ID NO: 73)
AP3-5	caggtccagctggtacagtctgggggaggcgtggtccagggttgggag gtccctgagactttcctgtgcggcctctggattcaccttcacaaact ttggcatgcactgggtccgccaggctccagggcaaggggctggagtgg gtggcactcatctcatctgatggatatagacaggcctatgcagactc cgtgaaggccggttcaccatctccggagacaactccaagaacacag tgtatctgcaaatgaacagcctgacaagtgaggacacggctgtttat tactgtgccatcatacccccctgtattacgatttttgattgggaatt tgactactggggccagggaaccctggtcaccgtctcctca (SEQ ID NO: 74)	gaaacgacactcacgcagtctccaggcaccctgtctttgtctccagg ggaaagagccacctctcctgcagggccagtcagagtgtttccagcc cctacttagcctggtaccagcagaaacctggccaggctcccaggctc ctcatttatggtgcatctaacagggccactggcatcccagacaggtt cagtggcagtgggtctgggacagacttcactctcaccatcagcagcc tgcaggctgaagatgaggcagtttattactgtcagcaatactacaat actccgctcacttttcggcggagggaccaaggtggaaatcaaa (SEQ ID NO: 75)

AP3-6	caggtgcagctacagcagtggggcgcaggcctgttgaagccttcgga gaccctgtccctcacctgcgctgtctatagtgggtctttttactcgtg actactgggctggatccgccagccccccgggaaggggctggagtgg attggggaaatcaatcatagtggaagcaccaactacaacccgtccct caagagtcgagtcaccacgtcggtagacaagtccaagaatcagttct ccctgaagttgacctctgtgaccgccgcggacacggctgtctattac tgtgcgagacgccggctttctagcgacctcttcatgcgggggggttgg cggtatggacgtctgggccaaggcaccctggtcaccgtcctcctca (SEQ ID NO: 76)	gatattgtgatgacccagactccaggcaccctgtcttcgtctccagg ggaaagagccaccctcctcctgcagggccagtcagggtgttagcagca acttagcctggtaccagcagaaacctggccaggctcccaggctcctc atctatgatgcatccaacagggccactggcatcccactcaggttcag tggcagtgggtctgggacagacttcactctcaccatcagcagactgg aacctgaagatttgcagtgtattactgtcaccagtatggtagctca ccgtacacctttggccagggacccaaggtggaaatcaaa (SEQ ID NO: 77)
AP3-8	gaggtgcagctggtgcagtctggagctgaggtgaagaagcctgggggc ctcagtgaaggtctcctgcaaggcttctggttacacctttaccagct atggtatcagctgggtgcgacaggcctctggacaagggcttgagtgg atgggatggatcagcgcttacaatggtaacacaaactatgcacagaa gctcccagggcagagtcaccatgaccacagacacatccacgagcacag cctacatggagctgaggagcctgagatctgacgcacacggccgtgtat tactgtgcgagagtaccccgatatttgactggttattatacgggag cgactactttgactactggggccagggaaccctggtcaccgtctcct ca (SEQ ID NO: 78)	gacatccagatgacccagtctccttccaccctgtctgtatctgtagg agacagagtcaccatcacttgtcgggcgagtcagggttattagcagct ggttagcctggtatcagcagaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag cggcagtggatctgggacagatttcactctcactatcagcagcctgc agcctgaagattttgcaacttactattgtcaacaggctaacagtttc ccgctcactttcggcggagggaccaagctggagatcaaa (SEQ ID NO: 79)
AP3-10	caggtgcagctggtgcaatctggagctgaggtgaaggagcctgggtc ctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagct atgctatctactgggtgcgacaggcccctggacaagggcttgagtgg atgggatggatcatccctatcctggtatagcaaactacgcacagaa gttccagggcagagtcacgattaccgcggacaaatccacgagcacag cctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagctgccggtcatagtactaactactactactacgg tatggacgtctggggccaaggcaccctggtcaccgtcctcctca (SEQ ID NO: 80)	cagactgtggtgacccaggagccctcactgactgtgtccctaggagg gacagtcactctcacctgtggctccagcactggagctgtcaccagtg gtcattatccctactggttccagcagaagcctggccaagccccagg acactgatttatgatacaagcaacaaacactcctggaccccctgcccg gttctcaggctccctccttgggggcaaagctgccctgaccctttcgg gtgcgcagcctgaggatgaggctgagtattactgcttgctcctcctat agtggtactcgggtgttcggcggagggaccaagctgaccgtccta (SEQ ID NO: 81)

AP3-13	gaggtgcagctggtgcagtctgggctgaggtgaagaagcctggggc ctcagtgaaggtttcctgcaaggcatctggatacaccttcaccaact actatatgcactgggtgcgacaggccctggacaagggcttgagtgg atgggaataatcaaccctagtggtagcacaagctacgcacagaa gttccagggcagagtcaccatgactagggacacgtccacgagcacag tctacatggagctgagcagcctgagatctgaggacacggccgtgtat tactgtgcgagagatttcaaagagtatagccgtacgggctactttga ctactgggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 82)	tcctatgagctgatgcagccatcctcagtgtcagtgtctccgggaca gacagccaggatcacctgctcaggagatgtactggcaaaaaatgtg ctcggtggttccagcagaagccaggccaggccctgtgctggtgatt tataaagacagtgagcggccctcagggatccctgagcgattctccgg ctccagctcagggaccacagtcaccttgaccatcagcgggcccagg ttgaggatgaggctgactattactgttactctgcggctgacaacaac ctgggggtgttcggcgagggaccaaggtcaccgtccta (SEQ ID NO: 83)
AP3-20	cagatcaccttgaaggagtctggtcctgcgctggtgaaacccacaca gaccctcacgctgacctgcaactttctctgggttctccctcagcactt atggagggggtgtgggctggctccgtcagccccccaggaaaggccctg gagtggcttgccgtcatttattggagtgatggtaaacgctacagccc ctctgtaaagaaccggctcaccatcaccaaggacacctccaaaaacc acgtggtcctgacaatgaccaacatggaccctgtggacacagccacc tattattgtgcacaccttatgatggatacatctattactacccactg gttcgaccctgggccagggaaccctggtcaccgtctcctca (SEQ ID NO: 84)	gccatccggatgacccagtctccatcctccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcgagtcagggcattagcaatt atttagcctggtatcagcagaaaccagggaaagttcctaagctcctg atctatgctgcatccactttgcaatcagggtcccatctcggttcag cggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagatgttgcaacttattactgtcaaaagtataacagtgcc cctgggacgttcggccaagggaccaaggtggagatcaaa (SEQ ID NO: 85)
AP4-8	caggtgcagctggtgcaatctgggctgaggtgaagaagcctgggtc ctcggtgaaggtttcctgcaaggcatctggatacaccttcaccaact actttatacactgggtgcgacaggccctggacaagggcttgagtgg atgggactactcaaccctactgatagtggcacactctacgcacagaa cttccagggcagaatcaccatgaccagtccacacgacagaacacag tctacatggagctgagcagcctgagatctgacgacacggccatgtat tactgtgcaagagagggggggccgacactacccgggtccactcttc gttgactactggggccagggaaccctggtcaccgtctcctca (SEQ ID NO: 86)	caggctgtgctgactcagccgccttccgtgtcgggggtctcctggaca gtcgatcaccatctcctgcactggaaccagcagtgacgttgaagctt acaactatgtctcctggtatcaacaacacccaggcaaagccccaaa ctcatgatttatgatgtcagtaatcggccctcagggggtttctaatcg cttctctggctcccaagtctggcaacacggcctccctgaccatctctg ggctccaggctgaggacgaggctgattattactgcagctcatataca agcagcagcacttgggtgttcggcggagggaccaaggtcatcgtcct a (SEQ ID NO: 87)

AP4-14	caggtgcagctgcaggagtcgggggaggcttggtacagcctggcag gtccctgagactctcctgtgcagcctctggattcacctttgatgatt atgccctccactgggtccggcaagctccagggaagggcctggagtgg gtctcaggtattagttggaatagtgttaccgtaaagtatgcggtctc tgtgaagggccggttcaccatctccagagacaacgccaagaactccc tgtttctgcaaatgaacgcctgagatctgaggacacggccttatat tactgtgcaaaagccagagggccctcttagaagcagctgacacacc atctgacgactggggccagggcaccctggtcaccgtctcctca (SEQ ID NO: 88)	gacatcgtgatgacccagtctccgtcctccctgtctgcatctgtagg agacagagtcaccatcacttgccgggcaagtcagagcattagcagct atttaaattggtatcagcagaaaccagggaaagcccctaagctcctg atctatgctgcatccagtttgcaaagtggggtcccatcaaggttcag tggcagtggatctgggacagatttcactctcaccatcagcagcctgc agcctgaagatgttgcaacttattactgtcaaaagtataacagtgcc ccgtggacgttcggccaagggaccaaagtggatatcaaa (SEQ ID NO: 89)
AP4-20	caggtacagctgcagcagtcaggcgcaggtctattgaggccttcgga gaccctgtccctcacctgcggtctctatggtgggtccttcagtggtc actattggaactggatccgccagtcccagaaaagggctggtgtgg attggggaaatcactcatagtggaaccaccaattacaacccgtccct caagagtcgagtcatcacatcagtagacacgtccaagaatcagtact ccctgaagctgagctttgtgaccctgcggacacggccgtgtattac tgtgcgagaggtgattactatgggtactgttcgatcctctgggg ccgtggcaccctggtcaccgtctcctca (SEQ ID NO: 90)	cagtctgtgttgacgcagccgccctcagttcctgtggcccaggaca gaaggtcaccatctcctgctctggaagcagctccaacattgggaata attatgtatcctggtaccagcagctcccaggaacagcccccaaactc ctcatttatgacactaataagcgaccctcagggattcctgaccgatt cgctggctccaagtctggcacgtcagccaccctgggcatcaccggac tccagactgggggacgaggccgattattactgcggaacatgggatagc agcctgagtgctggcgtgttcggcggagggaccaagctgaccgtcct a (SEQ ID NO: 91)

Nucleic Acids Encoding the Human scFvs

Antibody	Variable Heavy Chain
AP1-2	caggtgcagctggtgcagtctggagcagaggtgaaaaagcccggggagtctctgaggatctcctgcaagggttctggatacagctttaccagcca ctggatcagctgggtgcgccagatgcccgggaaaggcctggagtggggaggattgatcctagtgactctatagcaactacagccgctcct tccaaggccacgtcatcatctcagttgacaagtccatcagcactgcctacttgcagtggagcagcctgaaggcctcggacaccgccatatattac tgtgcgagacagctcattgtagtagtaccagctgctccctattactactactacggtatggacgtctgggccaagggaaccctggtcaccgt ctcctcaggcggcggctctggcggaggtggcagcggtggcggatccgaaattgttgttgacgcagtctccaggcaccctgtctttgtctcc agggggaaagagccacccctcctcctgcagggccagtcagactgttaacagctacttagcctggtaccagtagaaacctggccaggctcccagggctc ctcatctatggtgcatccagcagggccactggcatcccagacaggttcagtggcagtgggtctgggacagacttcactctcaccatcagcagact ggagcctgaagattttgcagtgtattactgtcagcagtatggtagctcacatccgtggacgttcggccaagggaccaaggtggagatcaaacgtg gcctcgggggcctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 54)

AP1-6	caggttcagctggtgcagtctgggggctgaggtgaagaagcctgggtcctcggtgaaggtctcctgcaaggcttctggaggcaccttcagcagcta tgctatcagctgggtgcgacaggccccctggacaagggcttgagtggatgggagggatcatccctatctttggtacagcaaactacgcacagaagt tccagggcagagtcacgattaccgcggacgaatccacgagcacagcctacatggagctgagcagcctgagatctgaggcacggccatatattac tgtgcgagagtctttggttccgagtcgcaagatccgtccgatatttggagtggtattacggtatggaagtctgggccaaggaaccctggtcac cgtctcctcaggcggtggcggctctggcggaggtggcagcggcggtggcggatccgacatccagatgacccagtctccgtctttccgtgtctgcat ctgtaggagacagagtcaccatcacttgtcgggcgagtcagggttattagcagctggttagcctggtatcagcagaaaccagggaaagccccctaag ctcctgatctatgctgcatccagtttgcaaagtagggtcccatcaaggttcagcggcagtggatctgggacagatttcactctcaccatcagcag cctgcagcctgaagattttgcaacttactattgtcaacaggctaacagtttcccgtacacttttggccaggggaccaagctggagatcaaacgtg gcctcgggggcctggtcgactacaaagatgacgatgacaaa (SEQ ID NO: 55)
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Example 25 – Suppression of Hemolysin Expression in RN4850 by an Anti-AIP4 Human scFv, AP4-4-20

Of the 20 clones obtained by panning an antibody-phage display library, the most potent clone AP4-4-20 was expressed as scFv antibody in *E. coli.* The expressed scFv antibody was purified, and evaluated for its ability to suppress hemolysins expression in *S. aureus* RN 4850 as follows.

S. aureus RN4850 was incubated in the presence of scFv AP4-4-20 (2.7 μM) in CYGP medium for 24 hours, and α-hemolysin expression was evaluated by western analysis using *S. aureus* culture supernatants. Results are shown in **FIG. 11**. The mAb AP4-24H-11 (1.3 μM) and an unrelated scFv antibody control (10 μM) were used as positive and negative controls, respectively. In the presence of the AIP-4 specific antibodies 4-20 and AP4-24H11, a clear reduction in hemolysins secretion is detectable, strongly indicative of inhibition of AIP-dependent QS in *S. aureus*.

Example 26 - Anti-AIP1 mAb AP1-15B4 protects mice from lethal systemic MRSA USA300 challenge in postexposure therapy

The effectiveness of our passive immunization approach was demonstrated in a postexposure scenario using mAb AP1-15B4 in a lethal *S. aureus* challenge mouse model. C57BL/6 mice received 1 mg of AP1-15B4 (i.p.), isotype control IgG or PBS 2 hours *after* they had been infected with at least 1×10^8 *S. aureus* USA300, an *agr I* MRSA strain. See Diep et al., *Lancet* 2006, 367, (9512), 731-739. USA300 is in fact one of the most common community-acquired MRSA (CA-MRSA) strains and represents an increasing threat for civilians and military personnel. Hageman et al., *Diagn Microbiol Infect Dis* 2008; James et al., *Arch Dis Child Fetal Neonatal Ed* 2008, 93, (1), F40-4; Tenover et al., *J Clin Microbiol* 2006, 44, (1), 108-18; Beilman et al., *Surg Infect (Larchmt)* 2005, 6, (1), 87-92. As shown in **FIG. 12**, 4 out of 6 mice receiving AP1-15B4 survived through the 48-hour observation period. In contrast, only two of the PBS treated control mice (2/6) and 2 of the control IgG treated mice (2/6) survived longer than 24 hours. These data for the first time demonstrate the existence of a therapeutic window for a quorum quenching strategy in *S. aureus*. This further validates our immunopharmacotherapeutic approach for preventing *S. aureus* infections as it shows that our quorum quenching antibodies can be administered after the infection of the patient.

All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an antibody" includes a plurality (for example, a solution of antibodies or a series of antibody preparations) of such antibodies, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described

or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

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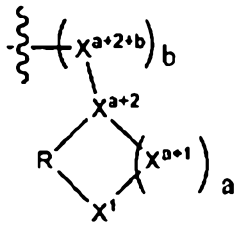
CLAIMS

1. An immunogenic molecular entity comprising at least one hapten covalently linked to a macromolecular carrier, wherein the hapten comprises a cyclic peptide having a structure represented by Formula I:



wherein the cyclic peptide comprises an amino acid sequence selected from the group consisting of YST(X^{a+2})DFIM (SEQ ID: 92), YST(X^{a+2}) YFIM (SEQ ID: 93), IN(X^{a+2})DFLL (SEQ ID: 94), and GVNA(X^{a+2})SSLF (SEQ ID: 95);

X in the cyclic peptide is any amino acid residue;

X^1 is an amino acid residue that is covalently bonded to R by a carbonyl group;

X^{a+2} is an internal amino acid, a respective carbon atom of which is covalently bonded to R;

R is selected from the group consisting of -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, and -$(CH_2)_nNH$-,

wherein

n is an integer from 1 to 4;

a is an integer from 1 to 9;

b is an integer from 1 to 8; and

a bond transected by a wavy line indicates a point of attachment of an N- terminal amino acid residue of the cyclic peptide to the macromolecular carrier.

2. The immunogenic molecular entity of claim 1, wherein the macromolecular carrier is selected from the group consisting of a protein, a polymer and a nanoparticle.

3. An immunogenic molecular entity having the structure:

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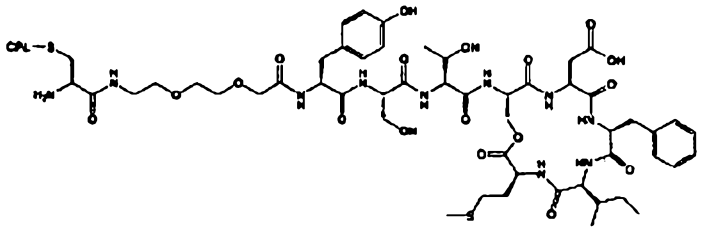
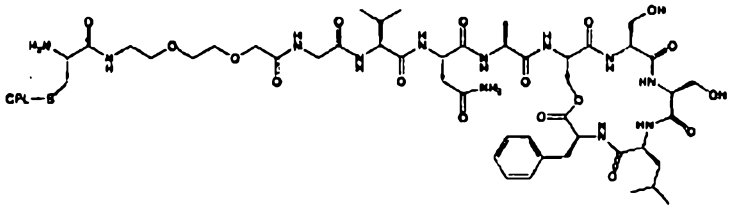
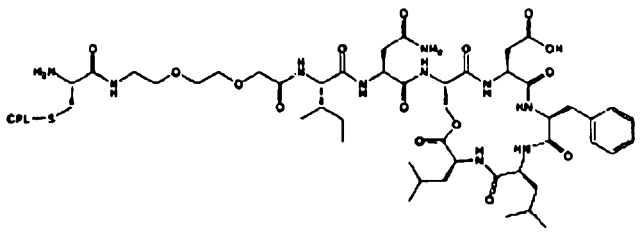


SEQ ID NO: 3 (YSTSDFIM, not including protecting groups),

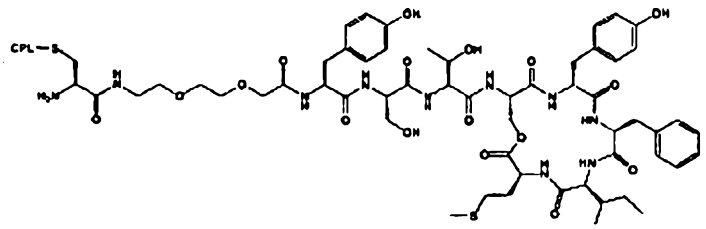


SEQ ID NO: 4 (GVNASSSLF, not including protecting groups),



SEQ ID NO: 2 (INSDFLL, not including protecting groups),

or



SEQ ID NO: 1 (YSTSYFIM, not including protecting groups), wherein CPL is a macromolecular carrier with optional linker covalently bonded to a cysteine thiol group.

4. An antibody that binds specifically with the immunogenic molecular entity of any one of claims 1-3.

5. A pharmaceutical composition comprising at least one antibody of claim 4, and a pharmaceutically-acceptable carrier.

6. A vaccine composition comprising at least one immunogenic molecular entity of any one of claims 1-3 and a pharmaceutically-acceptable carrier.

7. A method of eliciting an immune response in a mammal comprising administering to the mammal a composition comprising the immunogenic molecular entity of any one of claims 1-3, in an amount effective to elicit an immune response in the mammal;

wherein the mammal is susceptible to infection by *Staphlocuccus aureus*, or

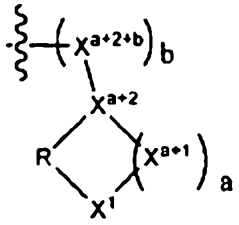
wherein the mammal is susceptible to a disease condition associated with *Staphlocuccus aureus*.

8. A method of inhibiting quorum sensing in a mammal comprising administering to the mammal a composition comprising at least one antibody of claim 4 in an amount effective to inhibit quorum sensing in the mammal.

9. A method of inhibiting quorum sensing in a mammal comprising administering to the mammal the immunogenic molecular entity of any one of claims 1-3, in an amount effective to elicit an immune response and inhibit the quorum sensing in the mammal.

10. A method for preventing or treating infection of a mammal by *Staphlocuccus aureus* comprising administering to the mammal, the immunogenic molecular entity of any one of claims 1-3, or at least one antibody of claim 4, in an amount effective to prevent or treat infection of the mammal by *Staphlocuccus aureus*.

11. An immunogenic molecular entity comprising at least one hapten covalently linked to a macromolecular carrier, wherein the hapten comprises a cyclic peptide having a structure represented by Formula I:



wherein the cyclic peptide comprises an amino acid sequence selected from the group consisting of YST(X^{a+2})DFIM (SEQ ID: 92), YST(X^{a+2}) YFIM (SEQ ID: 93), IN(X^{a+2})DFLL (SEQ ID: 94), and GVNA(X^{a+2})SSLF (SEQ ID: 95);

X in the cyclic peptide is any amino acid residue;

X^1 is an amino acid residue that is covalently bonded to R by a carbonyl group;

X^{a+2} is an internal amino acid, a respective carbon atom of which is covalently bonded to R;

R is selected from the group consisting of -CH_2O-, -CH_2CH_2O-, -$CH_2CH(CH_3)O$-, -CH_2-phenyl-O-, and -$(CH_2)_nNH$-,

wherein

n is an integer from 1 to 4;

a is an integer from 1 to 9;

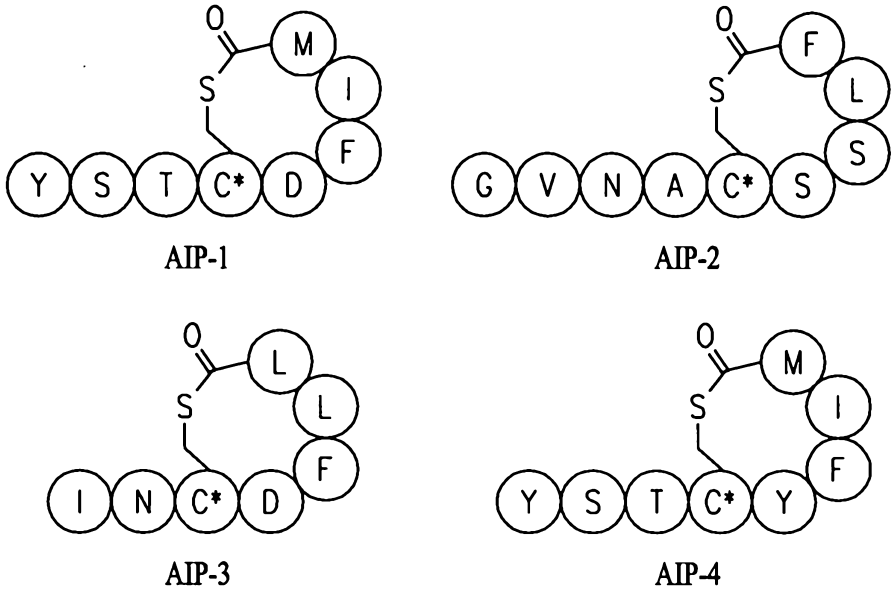
b is an integer from 1 to 8; and

a bond transected by a wavy line indicates a point of attachment of an N- terminal amino acid residue of the cyclic peptide or analog thereof to the macromolecular carrier, for use as a vaccine.

The Scripps Research Institute

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON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G V N A C* S
AIP-2
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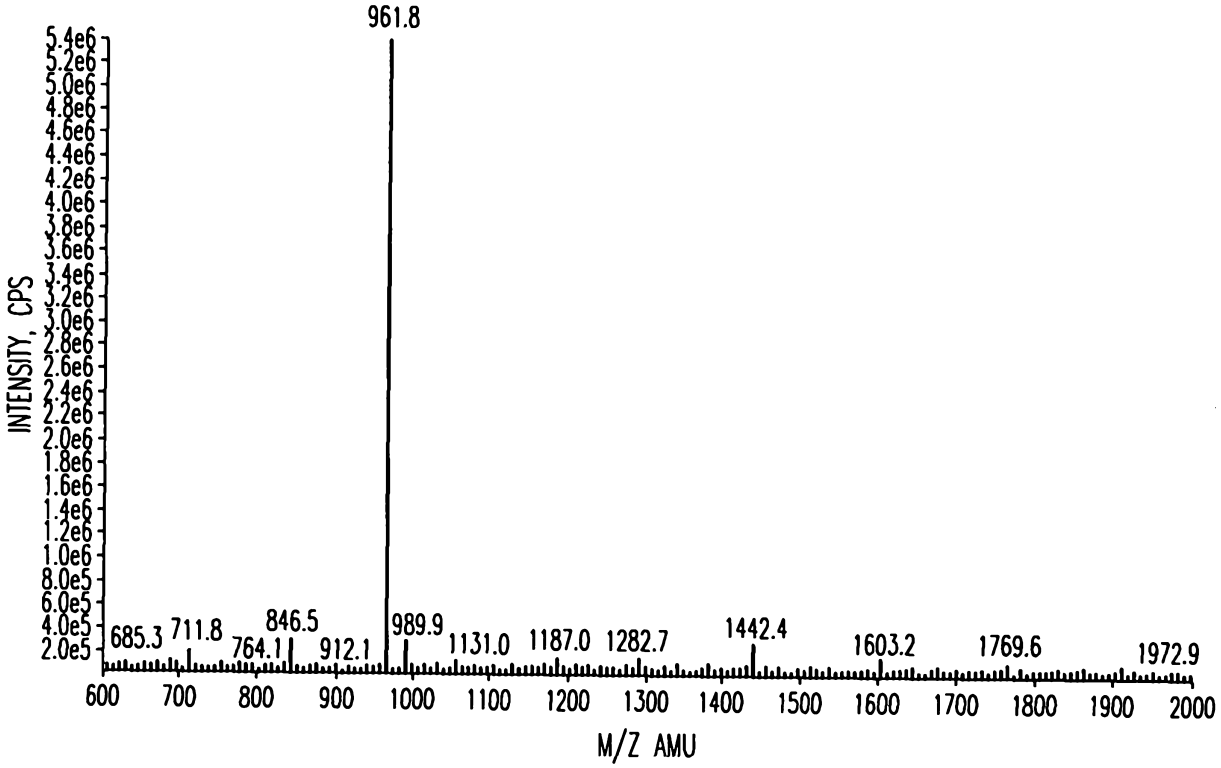
Y S T C* Y
AIP-4

FIG. 1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1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
685.3
711.8
764.1
846.5
912.1
989.9
1131.0
1187.0
1282.7
1442.4
1603.2
1769.6
1972.9
600
700
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900
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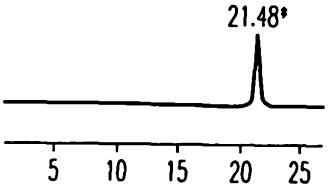
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
M/Z AMU

FIG. 2A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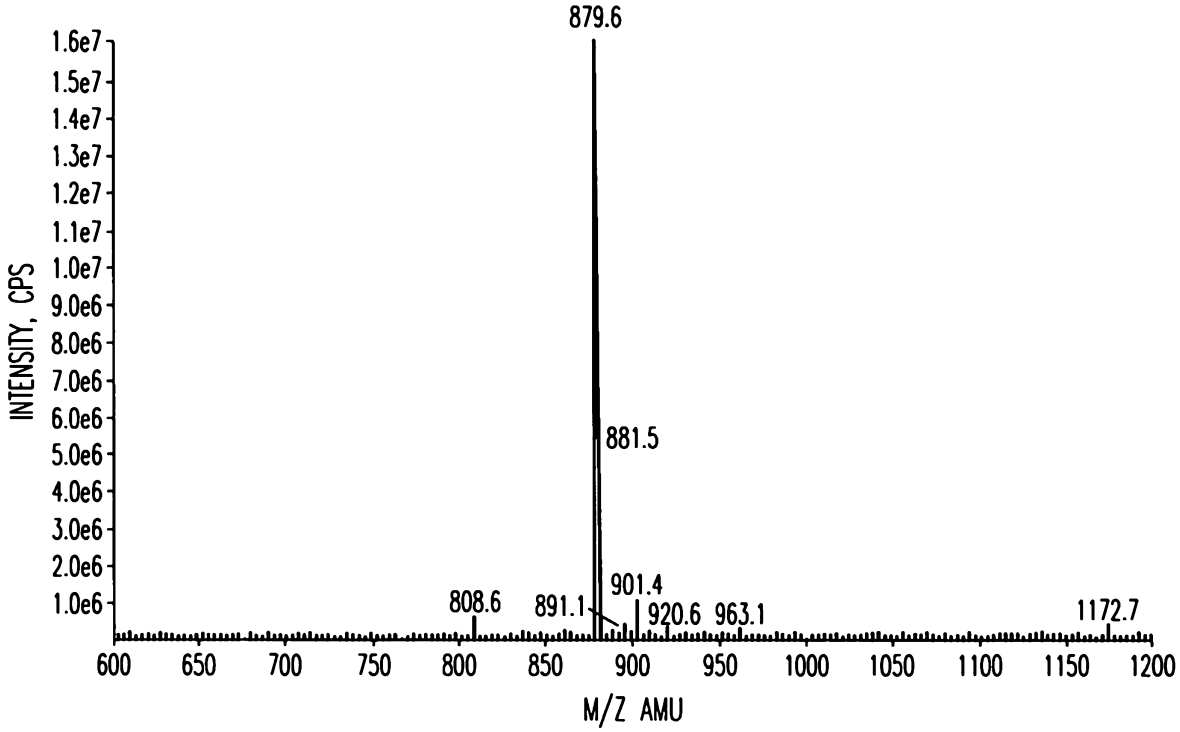
15
20
25

FIG. 2B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901.4
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920.6
963.1
1172.7
INTENSITY, CPS
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1.4e7
1.3e7
1.2e7
1.1e7
1.0e7
9.0e6
8.0e6
7.0e6
6.0e6
5.0e6
4.0e6
3.0e6
2.0e6
1.0e6
600
650
700
750
800
850
900
950
1000
1050
1100
1150
1200
M/Z AMU

FIG. 2C

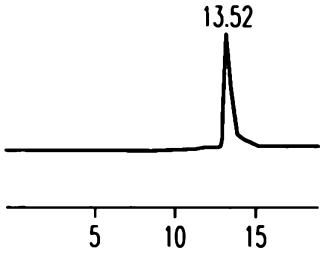
13.52
5
10
15

FIG. 2D

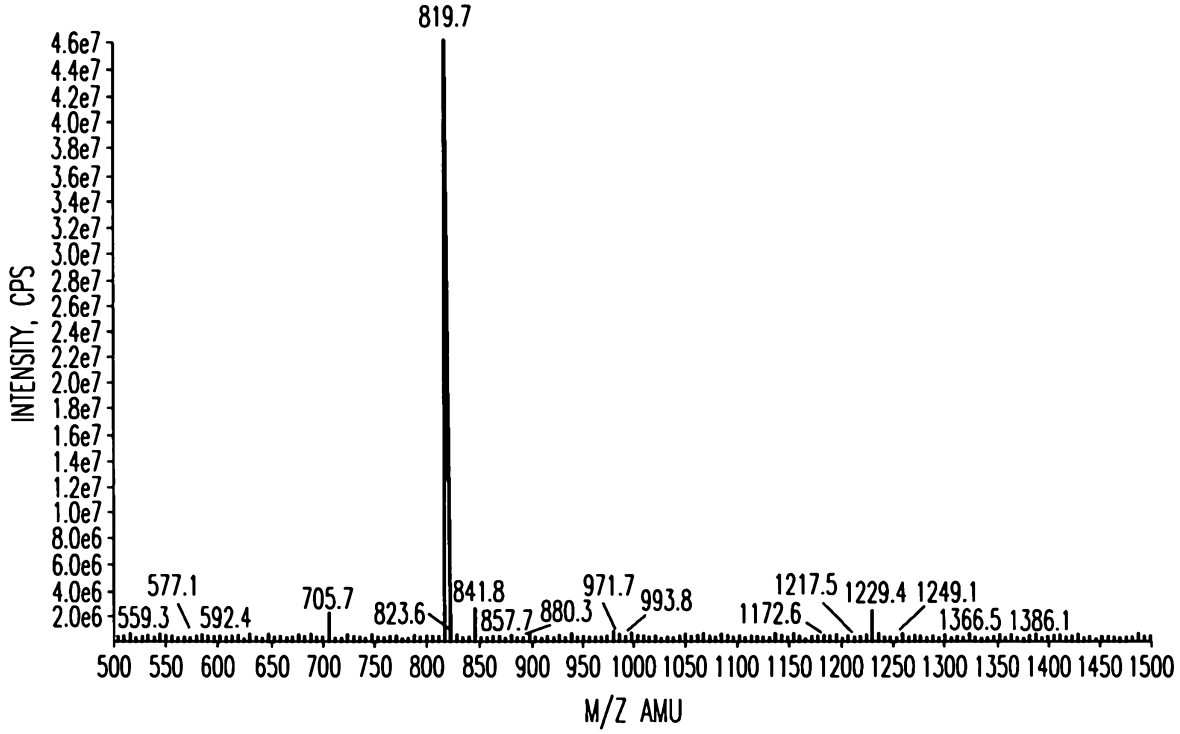
819.7
INTENSITY, CPS
4.6e7
4.4e7
4.2e7
4.0e7
3.8e7
3.6e7
3.4e7
3.2e7
3.0e7
2.8e7
2.6e7
2.4e7
2.2e7
2.0e7
1.8e7
1.6e7
1.4e7
1.2e7
1.0e7
8.0e6
6.0e6
4.0e6
2.0e6
577.1
559.3
592.4
705.7
823.6
841.8
857.7
880.3
971.7
993.8
1172.6
1217.5
1229.4
1249.1
1366.5
1386.1
500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500
M/Z AMU

FIG. 2E

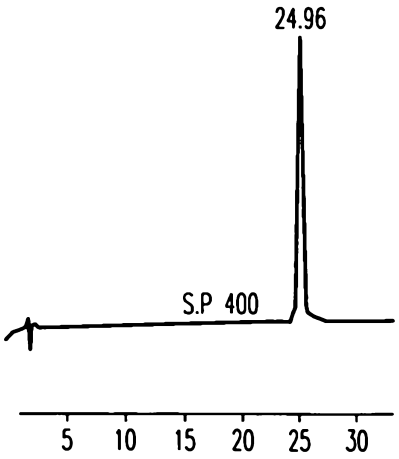
24.96
S.P 400
5 10 15 20 25 30

FIG. 2F

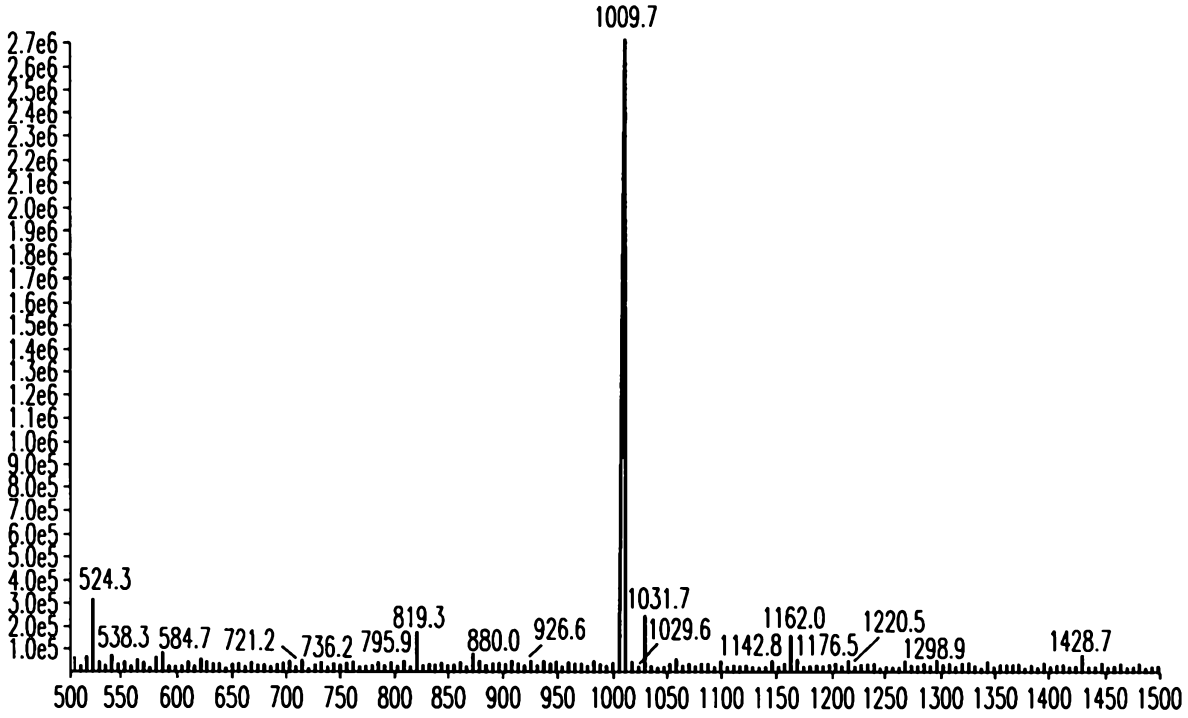
1009.7
524.3
538.3
584.7
721.2
736.2
795.9
819.3
880.0
926.6
1031.7
1029.6
1142.8
1162.0
1176.5
1220.5
1298.9
1428.7
2.7e6
2.6e6
2.5e6
2.4e6
2.3e6
2.2e6
2.1e6
2.0e6
1.9e6
1.8e6
1.7e6
1.6e6
1.5e6
1.4e6
1.3e6
1.2e6
1.1e6
1.0e6
9.0e5
8.0e5
7.0e5
6.0e5
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350 1400 1450 1500

FIG. 2G

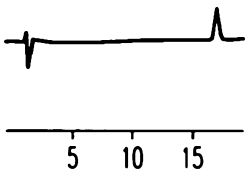
5
10
15

FIG. 2H

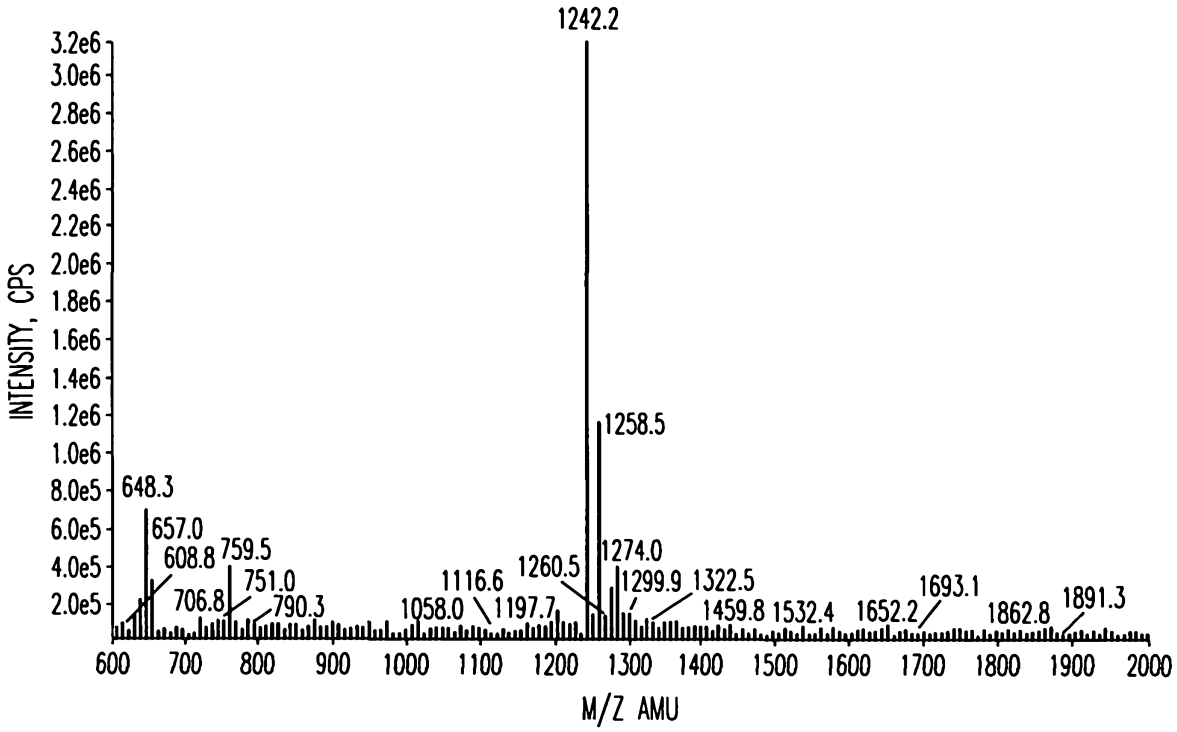
1242.2
1258.5
1274.0
1260.5
1299.9
1322.5
1116.6
1058.0
1197.7
1459.8
1532.4
1652.2
1693.1
1862.8
1891.3
648.3
657.0
608.8
759.5
751.0
706.8
790.3
INTENSITY, CPS
3.2e6
3.0e6
2.8e6
2.6e6
2.4e6
2.2e6
2.0e6
1.8e6
1.6e6
1.4e6
1.2e6
1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000
M/Z AMU

FIG. 2I

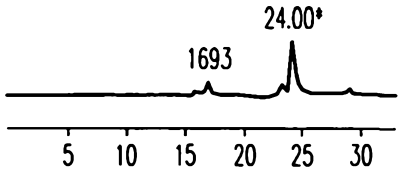
24.00*
1693
5 10 15 20 25 30

FIG. 2J

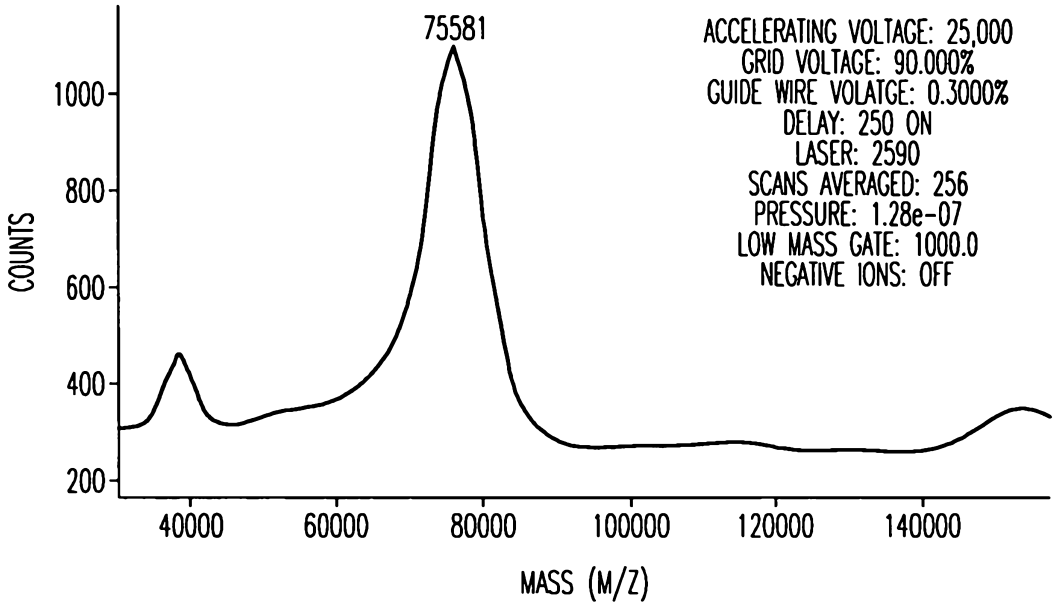
75581
ACCELERATING VOLTAGE: 25,000
GRID VOLTAGE: 90.000%
GUIDE WIRE VOLATGE: 0.3000%
DELAY: 250 ON
LASER: 2590
SCANS AVERAGED: 256
PRESSURE: 1.28e-07
LOW MASS GATE: 1000.0
NEGATIVE IONS: OFF
COUNTS
1000
800
600
400
200
40000
60000
80000
100000
120000
140000
MASS (M/Z)

FIG. 2K

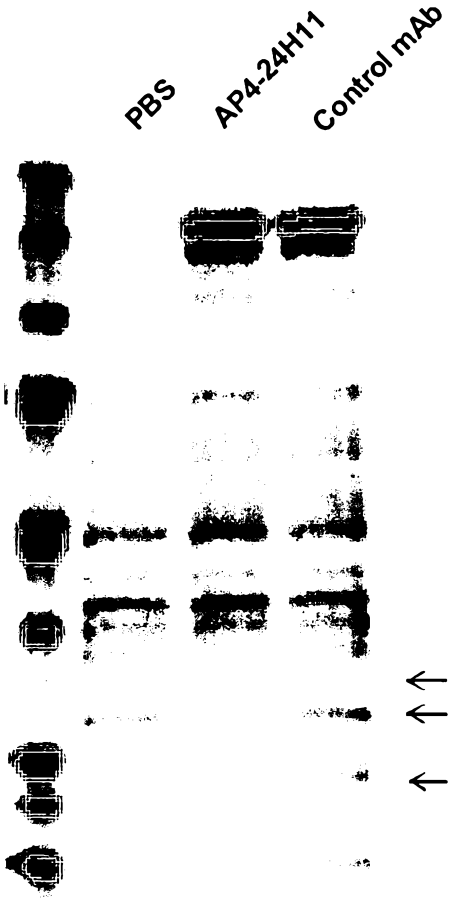

FIG. 3A

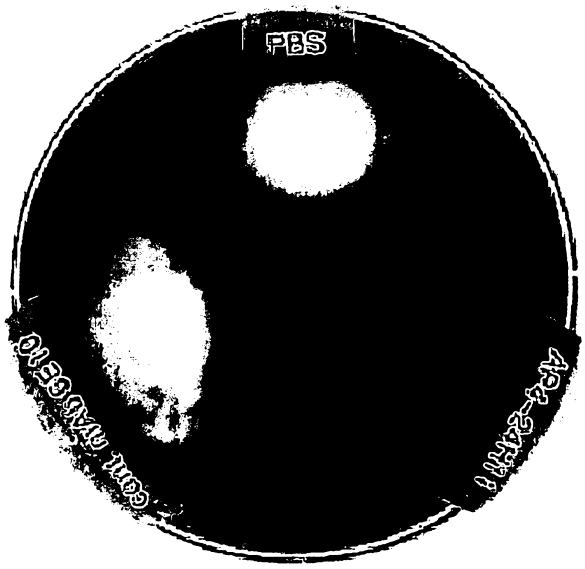

FIG. 3B

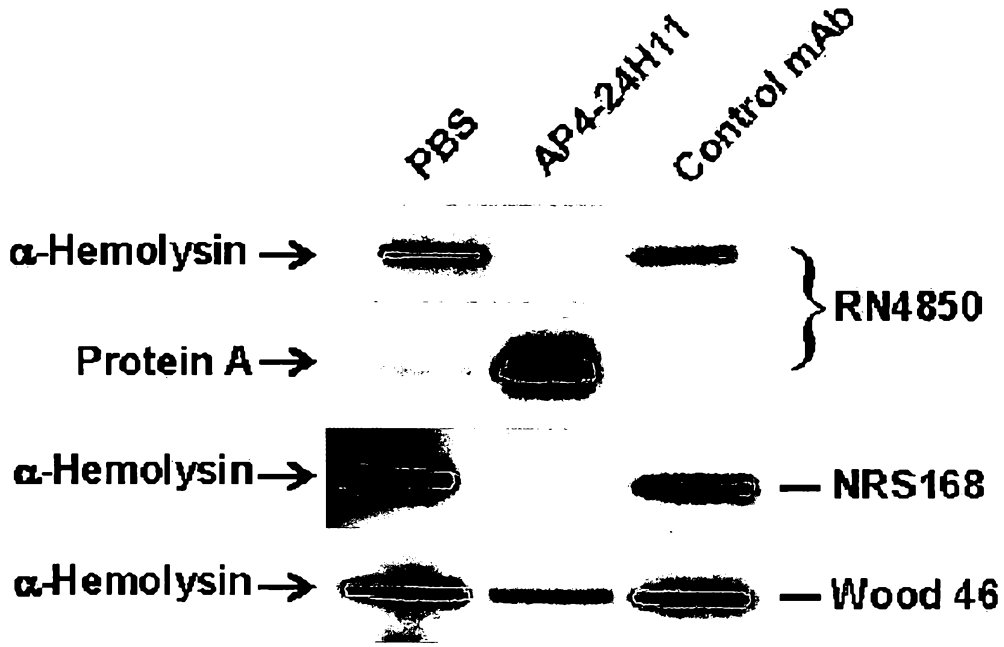

FIG. 4A

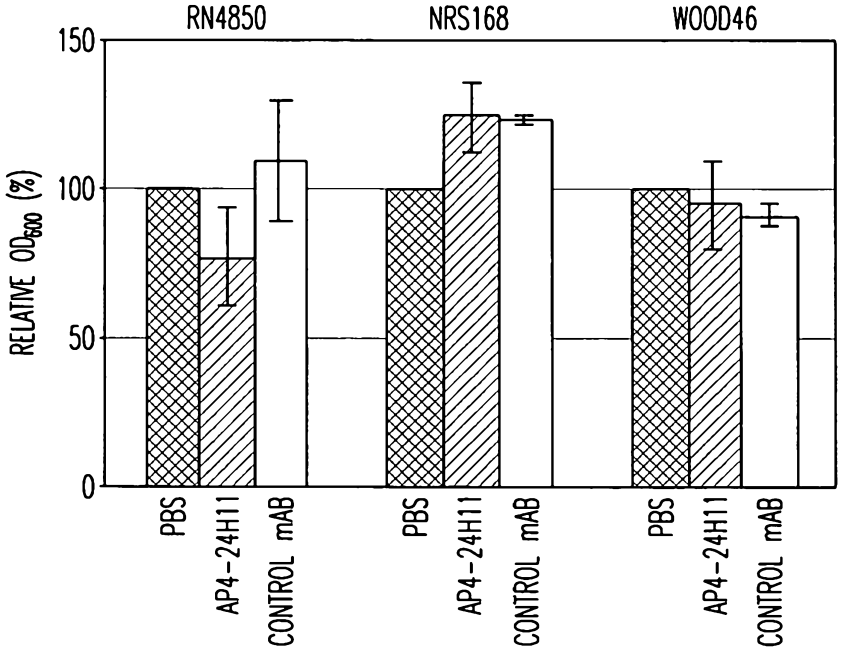

FIG. 4B

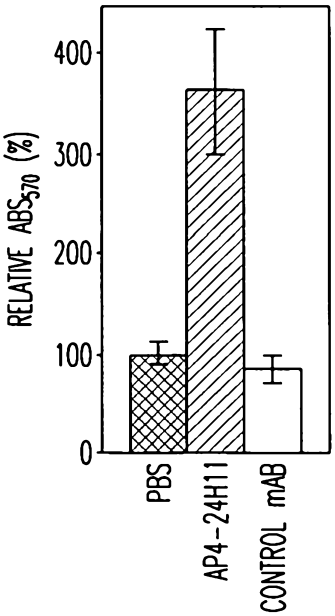

FIG. 4C

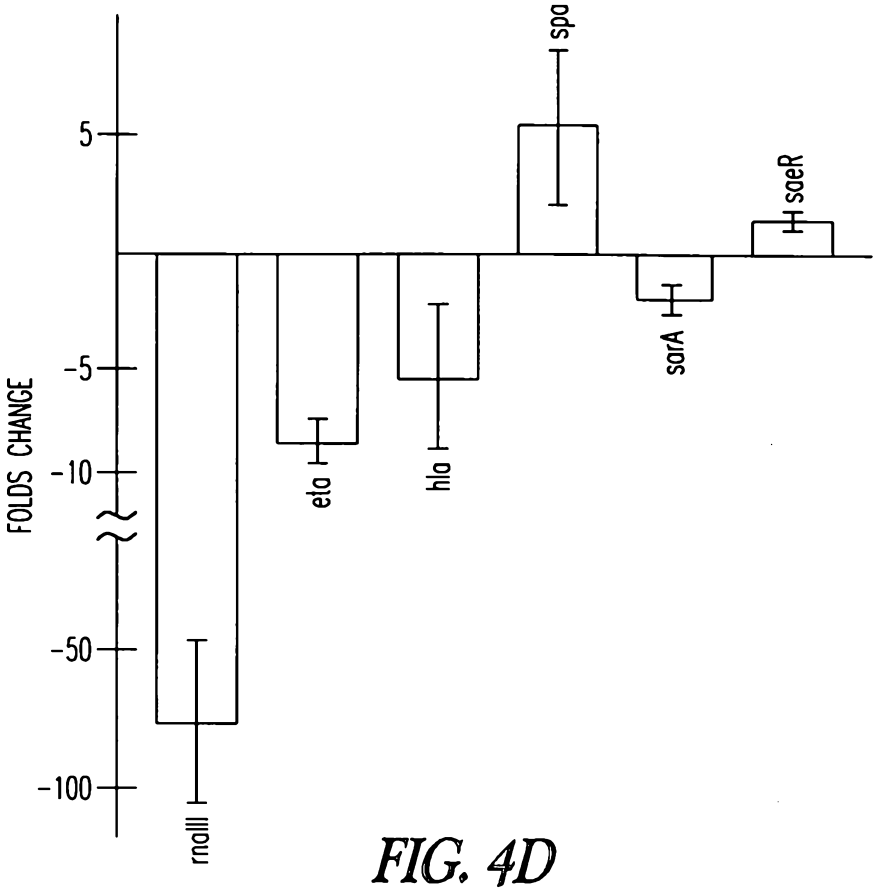
FOLDS CHANGE
5
-5
-10
-50
-100
rnaIII
eta
hla
spa
sarA
saeR

FIG. 4D

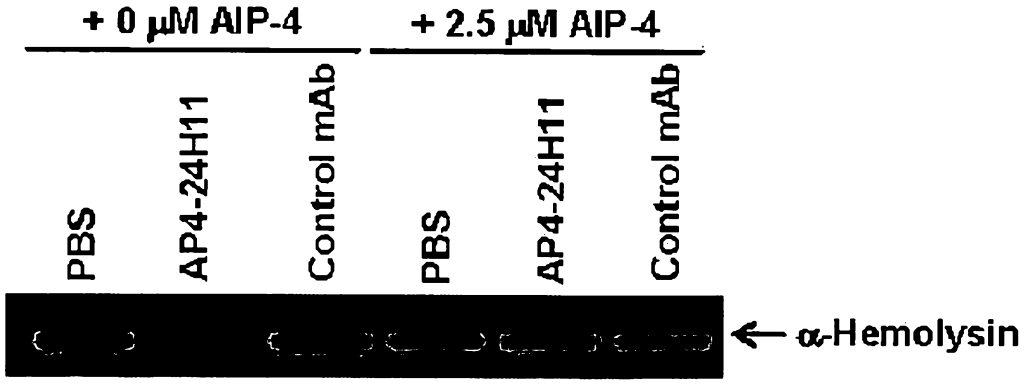
+ 0 μM AIP-4
+ 2.5 μM AIP-4
PBS
AP4-24H11
Control mAb
PBS
AP4-24H11
Control mAb
α-Hemolysin

FIG. 4E

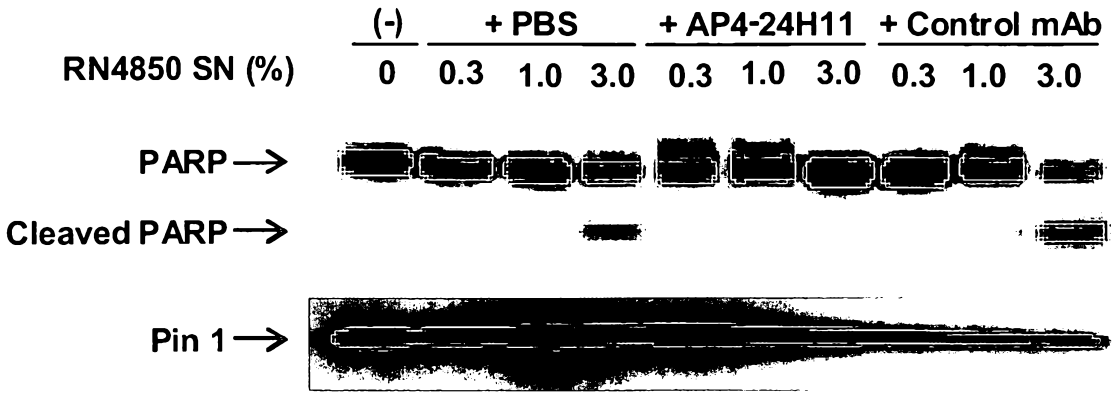
(-)
+ PBS
+ AP4-24H11
+ Control mAb
RN4850 SN (%)
0 0.3 1.0 3.0 0.3 1.0 3.0 0.3 1.0 3.0
PARP
Cleaved PARP
Pin 1

FIG. 5A

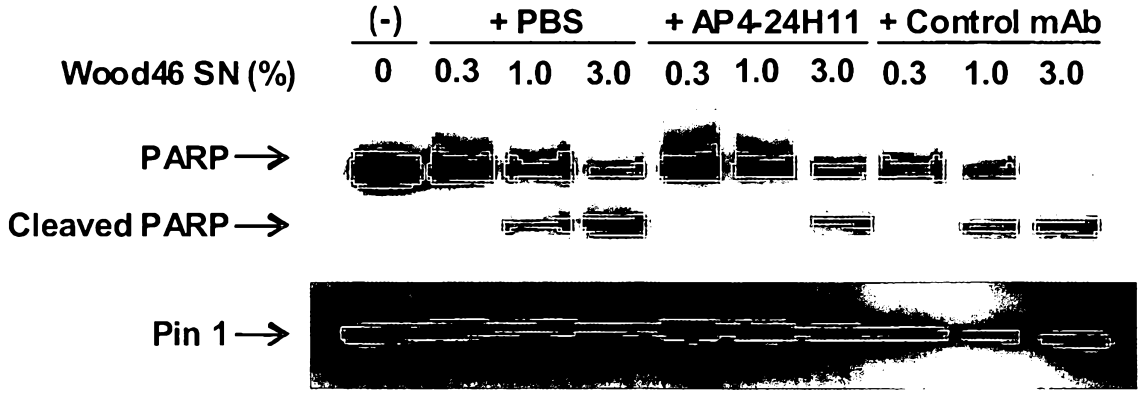
(-)
+ PBS
+ AP4-24H11
+ Control mAb
Wood46 SN (%)
0 0.3 1.0 3.0 0.3 1.0 3.0 0.3 1.0 3.0
PARP
Cleaved PARP
Pin 1

FIG. 5B

FIG. 6A

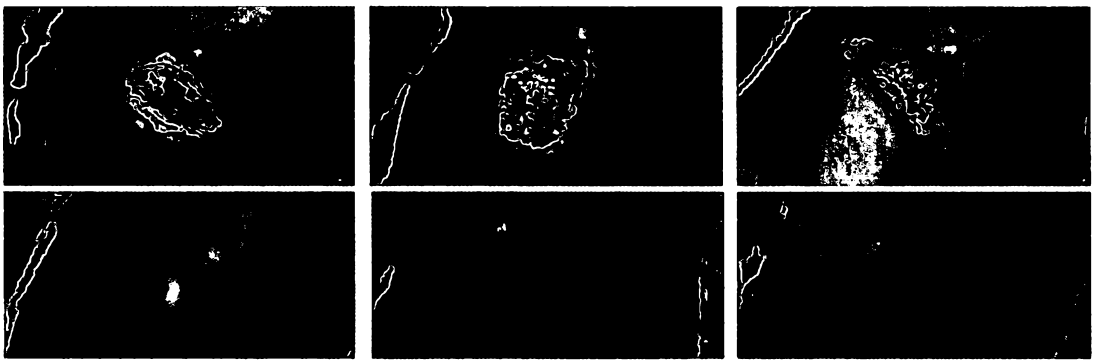

FIG. 6B

FIG. 7A

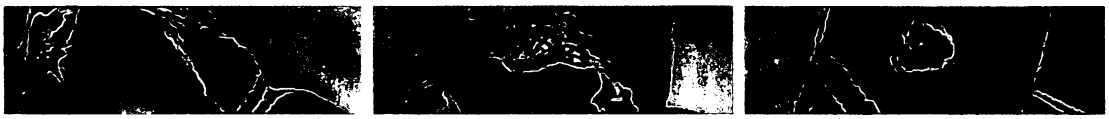

FIG. 7B

FIG. 7C

FIG. 7D

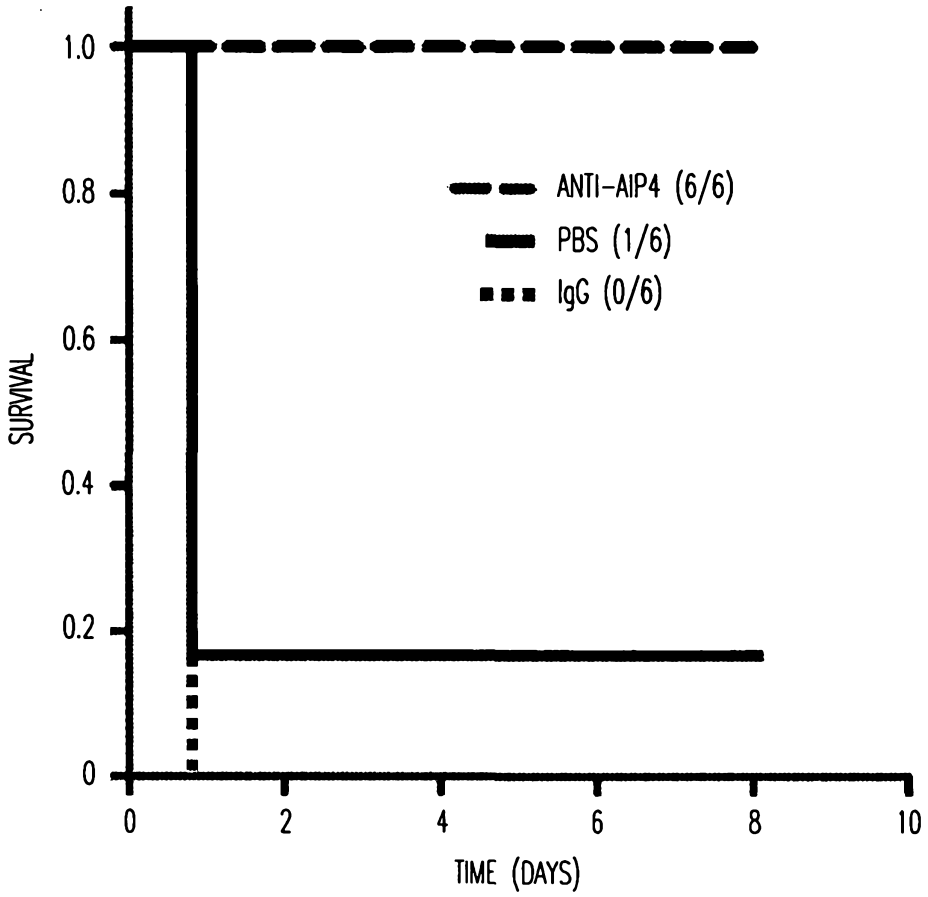
1.0
0.8
0.6
0.4
0.2
0
SURVIVAL
ANTI-AIP4 (6/6)
PBS (1/6)
IgG (0/6)
0
2
4
6
8
10
TIME (DAYS)

FIG. 8

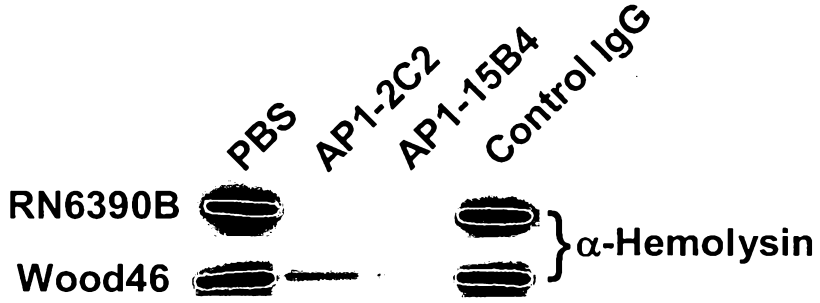
PBS
AP1-2C2
AP1-15B4
Control IgG
RN6390B
Wood46
α-Hemolysin

FIG. 9

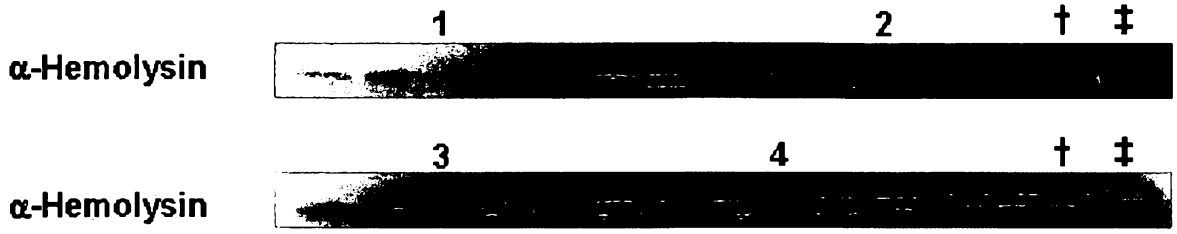

FIG. 10A

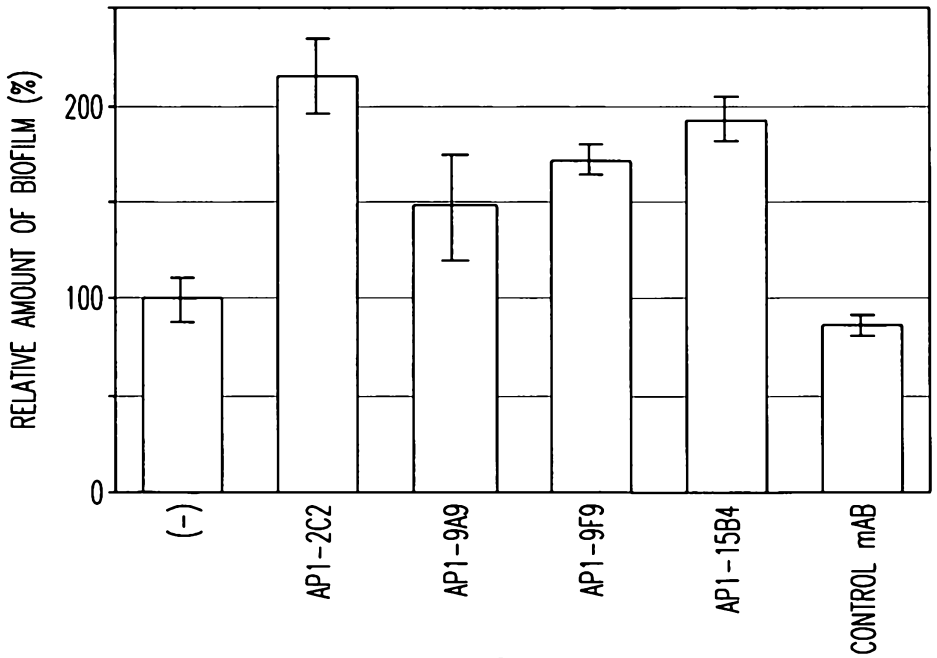

FIG. 10B

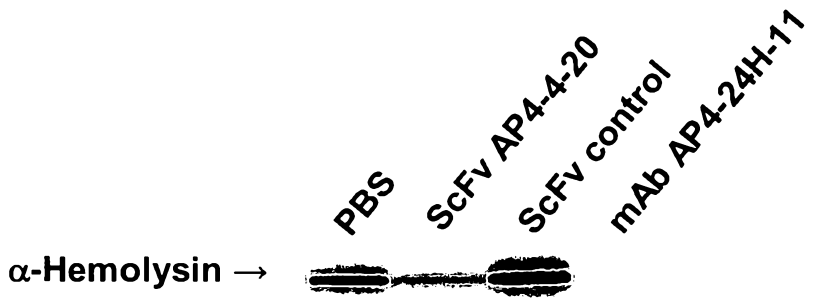
PBS
ScFv AP4-4-20
ScFv control
mAb AP4-24H-11
α-Hemolysin →

FIG. 11

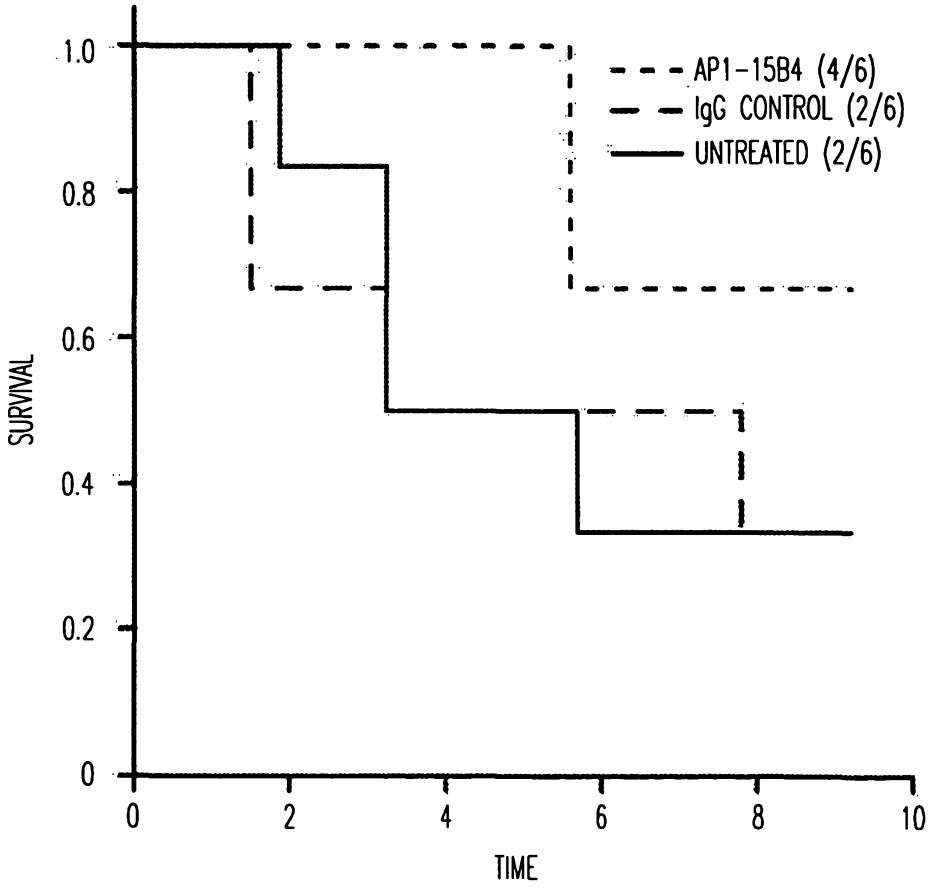
1.0
0.8
0.6
0.4
0.2
0
SURVIVAL
0
2
4
6
8
10
TIME
AP1-15B4 (4/6)
IgG CONTROL (2/6)
UNTREATED (2/6)

FIG. 12