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(54) **Title**: PEPTIDES AND METHODS FOR INDUCING CELL DEATH

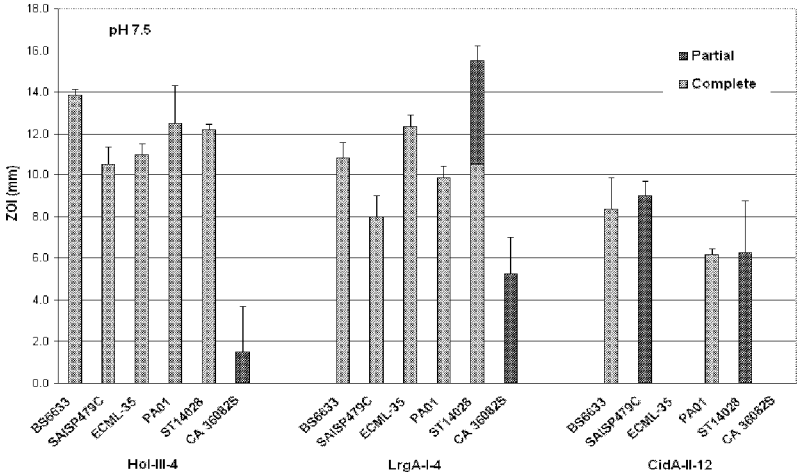


Figure 30

(57) **Abstract**: The invention provide isolated peptides, protides and conjugates having novel peptide sequences which are able to induce antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity. The invention also provides a method of inducing programmed cell death in a cell by contacting the cell with an isolated peptide, protide or conjugate described herein. In some aspects, the method can be used in the diagnosis, prevention, or treatment of a disease, such as an infection, cancer, autoimmune disease, or inflammatory disease.

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PEPTIDES AND METHODS FOR INDUCING CELL DEATH

This application claims the benefit of priority of United States Provisional application serial No. 61/338,747, filed February 22, 2010, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to compositions and methods for inducing cell death, and more specifically to peptides and compositions having antimicrobial, anti-cancer, anti-inflammatory and/or anti-proliferative activity and methods of using the peptides and compositions as therapeutics.

BACKGROUND OF THE INVENTION

Programmed cell death pathways are known to exist in most if not all organisms on Earth, ranging from microbes to man. Proteins that effect this function, also known as apoptosis, have been identified in human, other mammals, plants, protozoa, fungi, and bacteria, among other forms of life. In humans, these proteins target the mitochondria, causing permeabilization, dissipation of the membrane potential, activation of intracellular signaling pathways, and ultimate death of the cell. Eukaryotic pathogens also contain mitochondria, and mitochondria are now widely accepted by evolutionary biologists to be decedents of specialized symbiotic bacteria in eukaryotic cells.

Given these close parallels between mitochondria and bacteria, it is contemplated that specific human, eukaryotic or prokaryotic proteins have necessarily evolved to control prokaryotic symbionts (eg. mitochondria, chloroplasts) or competitors, and directly or indirectly prompt death of microbes or infected or abnormal cells. These types of proteins exhibit similarities in structures (eg., cationic helical domains) and mechanisms of action (eg., membrane interaction or perturbation that can lead to programmed cell death). Thus, such proteins may serve as excellent templates for novel therapeutic molecules, and reveal new insights into host-pathogen co-evolution, cancer biology, and other disease prevention, pathogenesis and treatment.

SUMMARY OF INVENTION

Embodiments of the invention provide isolated peptides, protides and conjugates having novel peptide sequences which are able to induce antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity. Peptides, protides and conjugates provided by the invention comprise, consist essentially of, or consist of amino acid sequences represented by SEQ ID NOS:1-263 and 288-289. In some aspects, peptides, protides and conjugates described herein have conservative amino acid substitutions or alternative residues at specific locations within a peptide sequence. Non-limiting examples of such substitutions or alternative residues include when the amino acid residue is represented by (x) a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue can be substituted, or alternatively, when the amino acid residue is represented by (b) a lysine, an arginine, an asparagine, a glutamine or a basic residue can be substituted, or alternatively, when the amino acid residue is represented by (j) a cysteine or a thiol residue can be substituted, or alternatively, when the amino acid residue is represented by (o) an anthrylalanine or other non-natural amino acid can be substituted.

Embodiments of the invention also provide methods of inducing programmed cell death in a cell by exposing the cell to an isolated peptide, protide or conjugate described herein. In some aspects, the methods can be used in the diagnosis, prevention, or treatment of a disease, such as an infection, cancer, autoimmune disease, or inflammatory disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence of exemplary programmed cell death / holin-like proteins (Dnm-1 (SEQ ID NO:280), Bax (SEQ IN NO:281) and Bcl-2 (SEQ ID NO:282)) identified in *Homo sapiens*,.

Figure 2 shows the amino acid sequence of exemplary programmed cell death proteins (CidA (SEQ ID NO:283) and LrgA (SEQ IN NO:284)) identified in *Staphylococcus aureus*,.

Figure 3 shows the amino acid sequence of exemplary candidate proteins (Perforin 1 from *Bos taurus* (SEQ ID NO:285), Bcl-2 from *Homo sapiens* (SEQ ID NO:286), and BCL-W from *Homo sapiens* (SEQ ID NO:287)) used for the iterative primary structure analysis of the

protein databases (Blastp and/or equivalent thereof) available from the National Center for Biotechnology Information utilizing the basic local alignment sequence tool (BLAST).

Figure 4 shows an exemplary data score table sorted by alignment score using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 5 shows exemplary multisequence alignments using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 6 shows a phylogram of candidate programmed cell death effector peptides using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 7 shows a similarity alignment of helical region 1 (amino acids - ~450-490) between candidate peptides identified in the phylogram of Figure 6 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 8 shows a similarity alignment of helical region 2 (amino acids - ~540-560) between candidate peptides identified in the phylogram of Figure 6 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 9 shows a similarity alignment of helical region 3 (amino acids - ~590-620) between candidate peptides identified in the phylogram of Figure 6 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 10 shows a cladogram of candidate programmed cell death effector molecule subset 1 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 11 shows a similarity alignment of helical region 1 / subset 1 (amino acids - ~180-225) between candidate programmed cell death effector molecules identified in the

cladogram of Figure 10 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 12 shows a similarity alignment of helical region 2 / subset 1 (amino acids - ~270-290) between candidate programmed cell death effector molecules identified in the cladogram of Figure 10 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 13 shows a similarity alignment of helical region 3 / subset 1 (amino acids - ~320-360) between candidate programmed cell death effector molecules identified in the cladogram of Figure 10 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 14 shows a similarity alignment of helical region 4 / subset 1 (amino acids - ~490-530) between candidate programmed cell death effector molecules identified in the cladogram of Figure 10 using the multisequence alignment tool ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI.

Figure 15 shows a secondary structure diagram of human Bcl-2, isoform 1 or 2. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence NREIVMKYIHYKLS (residues 1-14 of SEQ ID NO:48) of a peptide predicted to have antimicrobial activity is shown in grey.

Figure 16 shows a secondary structure diagram of human Bcl-2, isoform 1 or 2. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence HLALRQAGDDFSRRYR having a peptide predicted to have antimicrobial activity (SEQ ID NO:53) is shown in grey.

Figure 17 shows a secondary structure diagram of human Bcl-xL. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence SQSNRELVVDFLSYKLSQK (SEQ ID NO:288) of a peptide predicted to have antimicrobial activity is shown in grey. The amino acid sequence SQSNRELVVDFLSYKLSQK (SEQ ID NO:288) has also been identified as being conserved in multiple PCD-effector templates, including human Bcl-xL and Bcl-xβ, murine Bcl-xγ, and various related proteins.

Figure 18 shows a secondary structure diagram of human Bcl-W. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence TRALVADFVGYKLRQK (residues 1-16 of SEQ ID NO:14) of a peptide predicted to have antimicrobial activity is shown in grey.

Figure 19 shows a secondary structure diagram of human Bax. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence RVVALFYFASKLVLKALCTK (residues 1-20 of SEQ ID NO:7) of a peptide predicted to have antimicrobial activity is shown in grey.

Figure 20 shows a secondary structure diagram of human CTL Granulysin. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence RDYRTCLTIVQKLKKM having a peptide predicted of have antimicrobial activity (residues 3-17 of SEQ ID NO:224) is shown in grey.

Figure 21 shows secondary structurediagram of human CTL Granulysin. Cylinders represent alpha helices. The arrows on the helix cylinders point in the N-terminal to C-terminal direction. The amino acid sequence QKLKKMVDKPTQRSVSN (SEQ ID NO:289) of a peptide predicted to have antimicrobial activity is shown in grey.

Figure 22 shows a secondary structure ribbon diagram of human Bax (1F16) protein. Helix-1, residues 104-129 are represented in grey.

Figure 23 shows a secondary structure ribbon diagram of human Bax Helix-1, residues 104-129. Structure A shows the location of positive residues in dark grey. Structure B shows the most hydrophilic residues in dark grey and the most hydrophobic in medium grey.

Figure 24 shows a secondary structure ribbon diagram of human Bax (1F16) protein. Helix-2, residues 168-190 are represented in grey.

Figure 25 shows a secondary structure ribbon diagram of human Bax Helix-2, residues 168-190. Structure A shows the location of positive residues in dark grey. Structure B shows the most hydrophilic residues as represented in dark grey and the most hydrophobic residues as represented in medium grey.

Figure 26, panels A-D, show three dimensional alignments between human Bax Helix-1, residues 104-129 vs. IL-8 helix, residues 55-72. For the alignment analyses, comparative

sequences of X and Y length are entered, and the computation prioritizes which span of those length are most comparable. Panel A shows a sequence alignment based on the following structural alignment. Panel B shows a horizontal view of a ribbon diagram alignment between Bax, residues 104-129 (medium grey) and IL-8, residues 56-72 (dark grey), whereas panel C shows an axial view of the same alignment. Panel D shows the same ribbon alignment as panel B, wherein the most hydrophilic residues are represented in drak grey and the most hydrophobic residues are represented in medium grey. Also included in the figure are the root mean square deviation (RMSD) score and other results from the alignment.

Figure 27, panels A-D, show three dimensional alignments between human Bax helix, residues 168-190 vs. IL-8 helix, residues 55-72. For the alignment analyses, comparative sequences of X and Y length are entered, and the computation prioritizes which span of those length are most comparable. Panel A shows a sequence alignment based on the following structural alignment. Panel B shows a horizontal view of a ribbon diagram alignment between Bax, residues 168-190 (medium grey) and IL-8, residues 55-72 (dark grey), whereas panel C shows an axial view of the same alignment. Panel D shows the same ribbon alignment as panel B, wherein the most hydrophilic residues are represented in dark grey and the most hydrophobic residues are represented in medium grey. Also included in the figure are the root mean square deviation (RMSD) score and other results from the alignment.

Figure 28, panels A-D, show three dimensional alignments between human Bax helix, residues 104-129 vs. magainin residues 1-16. For the alignment analyses, comparative sequences of X and Y length are entered, and the computation prioritizes which span of those length are most comparable. Panel A shows a sequence alignment based on the following structural alignment. Panel B shows a horizontal view of a ribbon diagram alignment between Bax, residues 104-129 (medium grey) and magainin, residues 1-16 (dark grey), whereas panel C shows an axial view of the same alignment. Panel D shows the same ribbon alignment as panel B, wherein the most hydrophilic residues are represented in dark grey and the most hydrophobic residues are represented in light grey. Also included in the figure are the root mean square deviation (RMSD) score and other results from the alignment.

Figure 29, panels A-D, show three dimensional alignments between human Bax helix, residues 168-190 vs. magainin residues 1-16. For the alignment analyses, comparative sequences of X and Y length are entered, and the computation prioritizes which span of those length are most comparable. Panel A shows a sequence alignment based on the following

structural alignment. Panel B shows a horizontal view of a ribbon diagram alignment between Bax, residues 168-190 (medium grey) and magainin, residues 1-16 (dark grey), whereas panel C shows an axial view of the same alignment. Panel D shows the same ribbon alignment as panel B, wherein the most hydrophilic residues are represented in dark grey and the most hydrophobic residues are represented in medium grey. Also included in the figure are the root mean square deviation (RMSD) score and other results from the alignment.

Figure 30 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4, LrgA-I-4 and CidA-II-12 against pathogenic bacteria and fungi at pH 7.5. The size of the complete zone of inhibition (ZOI) is represented by a ligh grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar. See Tables 22 and 23 for peptide and microorganism designations.

Figure 31 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4, LrgA-I-4 and CidA-II-12 against pathogenic bacteria and fungi at pH 5.5. The size of the complete zone of inhibition (ZOI) is represented by a ligh grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar. See Tables 22 and 23 for peptide and microorganism designations.

Figure 32 shows a histogram of the antimicrobial spectra of exemplary peptides Dnm2-II-4, Dnm1-IV-2, Ncl-VIII-6 and Mfn1-II-2 against pathogenic bacteria and fungi at pH 7.5. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar. See Tables 22 and 23 for peptide and microorganism designations.

Figure 33 shows a histogram of the antimicrobial spectra of exemplary peptides Dnm2-II-4, Dnm1-IV-2, Ncl-VIII-6 and Mfn1-II-2 against pathogenic bacteria and fungi at pH 5.5. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar. See Tables 22 and 23 for peptide and microorganism designations.

Figure 34 shows a histogram of the antimicrobial spectra of exemplary peptides BclWP-I-4, Csp3-II-12, BclXb-I-2 and BaxP-I-18 against pathogenic bacteria and fungi at pH 7.5. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar. See Tables 22 and 23 for peptide and microorganism designations.

Figure 35 shows a histogram of the antimicrobial spectra of exemplary peptides BclWP-I-4, Csp3-II-12, BclXb-I-2 and BaxP-I-18 against pathogenic bacteria and fungi at pH 5.5. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar. See Tables 22 and 23 for peptide and microorganism designations.

Figure 36 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Pseudomonas aeruginosa* CRM27853 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 37 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Pseudomonas aeruginosa* PA 01 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 38 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Pseudomonas aeruginosa* XEN 5 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 39 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* 19606 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 40 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii*

17978 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 41 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter calcoaceticus* 23055 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 42 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter haemolyticus* 17906 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 43 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #ATCC at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 44 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #1 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 45 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #6 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the

negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 46 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #12 at pH 5.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 47 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Pseudomonas aeruginosa* CRM27853 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 48 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Pseudomonas aeruginosa* PA 01 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 49 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Pseudomonas aeruginosa* XEN 5 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 50 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* 19606 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the

complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 51 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* 17978 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 52 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter calcoaceticus* 23055 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 53 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter haemolyticus* 17906 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 54 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #ATCC at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 55 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #1 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the

complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 56 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #6 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

Figure 57 shows a histogram of the antimicrobial spectra of exemplary peptides Hol-III-4 (SEQ ID NO. 268) and NCl-VIII-6 (SEQ ID NO. 152) against *Acinetobacter baumannii* HUMC #12 at pH 7.5. Positive control peptides RP1, 6W-RP1, IK and PMP-2, whereas the negative control of double-distilled water (DDH2O) are also shown. The size of the complete zone of inhibition (ZOI) is represented by a light grey bar, whereas the size of the partial (ZOI) is represented by a dark grey bar.

DETAILED DESCRIPTION OF THE INVENTION

Over the last several years, unforeseen structural, functional, and evolutionary relationships among host defenses and other proteins across all kingdoms of life have been discovered. In the course of the studies described herein, unifying themes among polypeptides based on sequence formulae, functional mechanisms, and/or 3D structures have been identified. Embodiments provided by the invention are based in part on the observations that 1) programmed cell death and apoptosis pathway proteins contain archetype sequences that confer membrane interacting / modifying domains similar to those of antimicrobial or other host defense peptides; 2) such sequences encode helical or other sequence and/or 3D structural signatures; and 3) such peptides exert antimicrobial and anti-cancer cell activities. Without being bound by theory, such peptides induce or regulate programmed cell death or related responses in target cells (e.g. microbial pathogens, cancer cells, etc.) leading to death of these cells. It is also contemplated that the mechanisms of action of peptides based on programmed cell death may activate archetypal apoptosis pathways in target cells, thus killing the target cell and circumventing resistance to existing antimicrobial, anti-cancer, or other preventive or therapeutic agents.

Embodiments of the invention provide that novel antimicrobial, anti-cancer, anti-inflammatory and/or anti-proliferative activity peptides reside in peptide sequences of programmed cell death effector proteins by virtue of the evolutionary necessity for control of microbial and cancer cell survival by increasingly complex eukaryotic systems / symbionts. Thus mitochondrial, chloroplast, and/or nuclear-encoded proteins capable of activating and/or modulating programmed cell death pathways are contemplated to be evolutionary relatives / descendents of polypeptides that originally provided a survival advantage in the face of microbial or neoplastic challenge.

The peptides, protides and conjugates described herein have the potential to create, augment, or improve several existing therapeutic, prophylactic, diagnostic, and basic research problems. For example, therapeutically, these peptides, protides and conjugates can address the problem of antibiotic-resistant infections and antineoplastic-resistant cancers. Likewise, the peptides, protides and conjugates may serve as immunotherapeutic agents to enhance or restore efficacy of endogenous host defenses. As adjunctive agents, these peptides, protides and conjugates will increase efficacy of conventional agents (such as antibiotics or anti-neoplastic agents), enhance immune functions, and activate or inactivate apoptotic

mechanisms of cell regulation associated with aging or other degenerative conditions, and many other potential applications. The scope and diversity of other uses for these peptides, protides and conjugates are considerable. For example, the peptides, protides and conjugates described herein can be used as diagnostic probes in isotopic or non-isotopic forms to localize or characterize diseases or conditions containing signatures such as those characteristic of microbial, neoplastic, necrotic, apoptotic, or other tissues or cells. Additionally, extensions of the above concepts are applicable to the construction, design, delivery, and use of such peptides as research reagents.

As will be clear to those skilled in the art, the above novel concepts relating to structure-activity relationships in programmed cell death proteins enabled the design of novel antimicrobial, anti-cancer, anti-inflammatory and anti-proliferative peptides, protides and conjugates. These peptides and compositions are useful as diagnostic, prophylactic, and/or therapeutic agents that exploit programmed cell death pathways in pathogens, cancer cells, autoimmune cells, and other disease-caused cells and tissues. Specific examples of peptides, variants, congeners, and mimetics of these molecules are included herein. Embodiments of the invention provide conjugates in which one given molecule can represent or include one or more antimicrobial, anti-cancer, anti-inflammatory, immunomodulatory peptide and one or more non-peptide functional motifs or domains, or combinations of these. Embodiments of the invention also provide protides which are multifunctional and context-activated polypeptides that have two or more effectors with individually distinct biological functions and one or more corresponding activator sites that can each initiate or amplify the biological function of one or more effectors upon context-activation. Therefore, peptides, protides and conjugates exemplified herein are relevant to Antibiotide, Immodulotide, Antineotide, Apoptide, and/or Cascatide class peptides.

The novel concepts, peptide design strategies, and exemplifying peptides encompass conceptual as well as material inventions. Moreover, variations upon these fundamental themes are applicable to novel therapeutic agents and strategies in virtually any area of medicine, including, but not limited to diagnosis, prevention, and therapy of infectious diseases, cancer and cancer-like diseases, immune and autoimmune disorders, cardiology, aging, and/or other conditions or disease states. Furthermore, the novel peptides based on programmed cell death effectors described herein represent agents and strategies to treat human, animal, and agricultural diseases. Other applications include their use in diagnosis,

prevention, or research of diseases, or as research tools to investigate pathogenesis, apoptosis, or related biological phenomena.

Embodiments of the invention, herein provide an isolated peptide comprising one or more amino acid sequences selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263, wherein the amino acid residue represented by (x) is a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue, wherein the amino acid residue represented by (b) is a lysine, an arginine, an asparagine, a glutamine or a basic residue, wherein the amino acid residue represented by (j) is a cysteine or a thiol residue, wherein in the amino acid residue represented by (o) is an anthrylalanine or other non-natural amino acid and wherein the peptide induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bax protein, which are represented by amino acid sequences of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 264, 270 and 271. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bcl-W protein, which are represented by amino acid sequences of SEQ ID NOS: 17, 18, 19, 21-25, 269 and 272. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bcl-xβ protein, which are represented by amino acid sequences of SEQ ID NOS: 30, 31-36 and 273. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bak protein, which are represented by amino acid sequences of SEQ ID NOS: 39-47. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bcl-2 protein, which are represented by amino acid sequences of SEQ ID NOS: 49-52. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bcl-2 isoform 1 protein, which are represented by amino acid sequences of SEQ ID NOS: 54-57. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Mfn-1 protein, which are represented by amino acid sequences of SEQ ID NOS: 59-63 and 274. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Mfn-2 protein, which are represented by amino acid sequences of SEQ ID NOS: 66-75. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Dnm-1 protein, which are represented by amino acid

sequences of SEQ ID NOS: 84-93 and 275. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Dnm-2 protein, which are represented by amino acid sequences of SEQ ID NOS: 102-106, 108-121, 267, 276 and 277. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Ncl protein, which are represented by amino acid sequences of SEQ ID NOS: 132-175. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Csp3 protein, which are represented by amino acid sequences of SEQ ID NOS: 179-187, 266 and 278. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Bad protein, which are represented by amino acid sequences of SEQ ID NOS: 191-199. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Prf-1 protein, which are represented by amino acid sequences of SEQ ID NOS: 205-209 and 211-223. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Granulysin protein, which are represented by amino acid sequences of SEQ ID NOS: 227-235. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the CidA protein, which are represented by amino acid sequences of SEQ ID NOS: 238-243, 245-247, 265 and 279. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the LrgA protein, which are represented by amino acid sequences of SEQ ID NOS: 249-251. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Lambda S21 protein, which are represented by amino acid sequences of SEQ ID NOS: 253-256. In one aspect, the isolated peptide comprises one or more amino acid sequence, identified from the Holin protein, which are represented by amino acid sequences of SEQ ID NOS: 260-263 and 268.

Embodiments of the invention provide an isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9, 12, 14-16, 20, 26-29, 37, 38, 48, 53, 58, 64, 65, 72, 76-83, 94-101, 107, 114, 122-131, 170, 176-178, 188-190, 200-204, 210, 224-226, 236, 237, 244, 248, 252, 257-259 and 288-289, wherein the peptide induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Bax protein, which are represented by amino acid sequences of SEQ ID NOS: 1, 2, 5, 7, 9 and 12. In one aspect, the isolated peptide consists of one or more amino acid

sequence, identified from the Bcl-W protein, which are represented by amino acid sequences of SEQ ID NOS: 14-16 and 20. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Bcl-xβ protein, which are represented by amino acid sequences of SEQ ID NOS: 26-29. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Bak protein, which are represented by amino acid sequences of SEQ ID NOS: 37 or 38. In one aspect, the isolated peptide consists of the amino acid sequence, identified from the Bcl-2 protein, which is represented by amino acid sequence of SEQ ID NO: 48. In one aspect, the isolated peptide consists of the amino acid sequence, identified from the Bcl-2 isoform 1 protein, which is represented by amino acid sequence of SEQ ID NOS: 53. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Mfn-1 protein, which are represented by amino acid sequences of SEQ ID NOS: 58 or 64. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Mfn-2 protein, which are represented by amino acid sequences of SEQ ID NOS: 65 or 72. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Dnm-1 protein, which are represented by amino acid sequences of SEQ ID NOS: 76-83. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Dnm-2 protein, which are represented by amino acid sequences of SEQ ID NOS: 94-101, 107 and 114. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Ncl protein, which are represented by amino acid sequences of SEQ ID NOS: 122-131 and 170. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Csp3 protein, which are represented by amino acid sequences of SEQ ID NOS: 176-178. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Bad protein, which are represented by amino acid sequences of SEQ ID NOS: 188-190. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Prf-1 protein, which are represented by amino acid sequences of SEQ ID NOS: 200-204 and 210. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Granulysin protein, which are represented by amino acid sequences of SEQ ID NOS: 224-226. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the CidA protein, which are represented by amino acid sequences of SEQ ID NOS: 236, 237 and 244. In one aspect, the isolated peptide consists of the amino acid sequence, identified from the LrgA protein, which is represented by amino acid sequence of SEQ ID NOS: 248. In one aspect, the isolated peptide consists of the amino acid sequence, identified from the Lambda S21 protein,

which is represented by amino acid sequences of SEQ ID NOS: 252. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from the Holin protein, which are represented by amino acid sequences of SEQ ID NOS: 257-259. In one aspect, the isolated peptide consists of one or more amino acid sequence, identified from human Bcl-xL protein, which is represented by the amino acid sequence SEQ ID NO: 288 or human CTL Granulysin, which is represented by the amino acid sequence SEQ ID NO: 289. In one aspect, an isolated peptide as described herein has a C-terminus comprising a carboxamide.

Embodiments of the invention are intended to be used as in ways similar to antibiotic, anti-cancer, or similar medical administration either as local (e.g. topical, oral rinse, inhaled, nebulized, etc.) or systemic (oral ingestion, intravenous, intramuscular, etc) agents. Additionally, the peptides may be used as research tools for basic molecular biology, microbiology, biochemistry or other disciplines as they relate broadly to cellular or molecular biology, infection and immunity, cell regulation and apoptosis, gene expression, signal transduction, or any other area of investigation in which a concept, approach, or specific peptide or may be used.

In certain embodiments, the invention provides novel isolated peptides having one or more continuous amino acids sequences. As used herein, a "peptide" generally has from about 3 to about 100 amino acids, whereas a polypeptide or protein has about 100 or more amino acids, up to a full length sequence translated from a gene. Additionally, as used herein a peptide can be a subsequence or a portion of a polypeptide or protein. In certain embodiments the size of at least one peptide may comprise, but is not limited to, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 amino acid residues.

As used herein, an "amino acid residue" refers to any naturally or non-naturally occurring amino acid, any amino acid derivative or any amino acid mimic known in the art. In certain embodiments, the residues of the peptide are sequential, without any non-amino acid interrupting the sequence of amino acid residues. In other embodiments, the sequence may comprise one or more non-amino acid moieties. In particular embodiments, the sequence of residues of the peptide may be interrupted by one or more non-amino acid moieties.

Accordingly, the term peptide encompasses amino acid sequences comprising at least one of the 20 common amino acids found in naturally occurring proteins, or at least one modified or unusual or non-natural amino acid, including, but not limited to, Anthrylalanine, 2 Aminoadipic acid (Aad), N Ethylasparagine (EtAsn), 3 Aminoadipic acid (Baad), Hydroxylysine (Hyl), β alanine, β Amino propionic acid (Bala), allo Hydroxylysine (AHyl), 2 Aminobutyric acid (Abu), 3 Hydroxyproline (3Hyp), 4 Aminobutyric acid (4Abu), 4 Hydroxyproline (4Hyp), 6 Aminocaproic acid (Acp), Isodesmosine (Ide), 2 Aminoheptanoic acid (Ahe), allo Isoleucine (AIle), 2 Aminoisobutyric acid (Aib), N Methylglycine (MeGly), 3 Aminoisobutyric acid (Baib), N Methylisoleucine (MeIle), 2 Aminopimelic acid (Apm), 6 N Methyllysine (MeLys), 2,4 Diaminobutyric acid (Dbu), N Methylvaline (MeVal), Desmosine (Des), Norvaline (Nva), 2,2'Diaminopimelic acid (Dpm), Norleucine (Nle), 2,3 Diaminopropionic acid (Dpr), Ornithine (Orn), or N Ethylglycine (EtGly).

A peptide containing one or more mimetic structures having a similar charge and spatial or steric arrangements as the reference amino acid residues is included within the definition of the term so long as the peptide containing the mimetic portion exhibits a similar or enhanced activity as compared with the reference peptide. It is thus understood that a peptide described herein includes such mimetics as chemically modified peptides, peptide-like molecules containing non-naturally occurring amino acids, and peptoids, which are peptide-like molecules resulting from oligomeric assembly of N-substituted glycines, with similar or enhanced activity as compared with the reference protide upon which the mimetic is derived or having any other property desired by the user, for example, enhanced biostability (see, for example, Goodman and Ro, Peptidomimetics for Drug Design, in "Burger's Medicinal Chemistry and Drug Discovery" Vol. 1 (ed. M. E. Wolff; John Wiley & Sons 1995), pages 803 861), which is incorporated herein by reference in its entirety. Mimetics also include constrained-structures so as to maintain optimal spacing and charge interactions of the amino acid or of the amino acid functional groups. Those skilled in the art know or can determine what structures constitute functionally equivalent amino acid analogs and amino acid mimetics useful for preparation of a peptide described herein.

Specific examples of amino acid analogs and mimetics can be found described in, for example, Roberts and Vellaccio, The Peptides: Analysis, Synthesis, Biology, Eds. Gross and Meinhofer, Vol. 5, p. 341, Academic Press, Inc., New York, N.Y. (1983), the entire volume of which is incorporated herein by reference. Other examples include peralkylated amino

acids, particularly permethylated amino acids. See, for example, Combinatorial Chemistry, Eds. Wilson and Czarnik, Ch. 11, p. 235, John Wiley & Sons Inc., New York, N.Y. (1997), which is incorporated herein by reference in its entirety. Yet other examples include amino acids whose amide portion and, therefore, the amide backbone of the resulting peptide, has been replaced, for example, by a sugar ring, steroid, benzodiazepine or carbo cycle. See, for example, Burger's Medicinal Chemistry and Drug Discovery, supra, Ch. 15, pp. 619 620, which is incorporated herein by reference in its entirety. Methods for synthesizing peptides, polypeptides, peptidomimetics and proteins are well known in the art (see, for example, U.S. Patent 5,420,109; Bodanzsky, Principles of Peptide Synthesis (1st ed. & 2d rev. ed.), Springer-Verlag, New York, N.Y. (1984 & 1993), see Chapter 7; Stewart and Young, Solid Phase Peptide Synthesis, (2d ed.), Pierce Chemical Co., Rockford, Ill. (1984), each of which is incorporated-herein by reference in its entirety).

In one aspect, the peptide, protide or conjugate can comprise conservatively substituted sequences or alternative residues at specifically identified positions described herein, for example, residues identified in SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263. In general, a conservative substitution refers to replacement of a given amino acid residue with a residue having similar physiochemical characteristics. Examples of conservative substitutions include (1) non-polar amino acids (Gly, Ala, Val, Leu, and Ile); (2) polar neutral amino acids (Cys, Met, Ser, Thr, Asn, and Gln); (3) polar acidic amino acids (Asp and Glu); (4) polar basic amino acids (Lys, Arg and His); and (5) aromatic amino acids (Phe, Trp, Tyr, and His). Other such conservative substitutions, for example, include substitutions of entire regions having similar hydrophobicity characteristics or substitution of one H-bond donor/acceptor with another H-bond donor/acceptor. An alternative residue refers to a residue that may not be traditionally considered a conservative substitution, but when substituted at the designated position does not adversely effect the functional characteristics of the peptide.

Hydrogen bonding (H-bond) is a non-covalent type of bonding between molecules or within them, intermolecularly or intramolecularly, and in the context of the peptides described herein include H-bond between amino acids. The H-bond donor is the molecule that has a hydrogen atom bonded to a highly electronegative, small atom with available valence. For example, H-O, H-N, and H-F bonds are extremely polar and as a result, the electron density is

easily withdrawn from the hydrogen atom towards the electronegative atom. The partially positive hydrogen in one molecule attracts to partially negative lone pair of the electronegative atom on the other molecule, i.e. an H-bond acceptor, and thus a H-bond forms as a result of such an interaction.

A "basic" residue refer to an amino acid residue which has a second basic group, which can be, but is not limited to, an amino group (i.e. lysine), a guanidine group (i.e. arginine), or an imidazole ring (i.e. histidine).

A "thiol" residue refers to an amino acid residue which has a functional sulfur-hydrogen present in the side chain (i.e. cysteine or methionine). A thiol residue, such as cysteine, can also play an important role in the folding and stability of some peptides and proteins through the formation of disulfide bonds.

A pathological condition appropriate for treatment with a peptide, protide or conjugate described here can be a symptomatic disease or other abnormal condition or injury of a mammalian cell or tissue. Such pathological conditions include, for example, hyperproliferative and unregulated neoplastic cell growth, degenerative conditions, inflammatory diseases, autoimmune diseases and infectious diseases. Hyperplastic and cancer cells proliferate in an unregulated manner, causing destruction of tissues and organs. Specific examples of hyperplasias include benign prostatic hyperplasia and endometrial hyperplasia.

Abnormal cellular growth can also result from infectious diseases in which foreign organisms cause excessive growth. For example, human papilloma viruses can cause abnormal growth of tissues. The growth of cells infected by a pathogen is abnormal due to the alteration of the normal condition of a cell resulting from the presence of a foreign organism. Specific examples of infectious diseases include DNA and RNA viral diseases, bacterial diseases, fungal diseases, and protozoal or parasitic diseases. Similarly, the cells mediating autoimmune and inflammatory diseases are aberrantly regulated which results in, for example, the continued proliferation and activation of immune mechanisms with the destruction of tissues and organs. Accordingly, "anti-inflammatory activity" refers to a cellular response to a substance or treatment that reduces inflammation and "anti-proliferative activity" refers to a cellular response to a substance that prevents the proliferation or uncontrolled dividing of cells. Specific examples of autoimmune diseases include, for

example, rheumatoid arthritis and systemic lupus erythmatosis. Specific examples of degenerative disease include osteoarthritis and Alzheimer's disease. Similarly, the terms infectious diseases, degenerative diseases, autoimmune diseases and inflammatory diseases are intended to include all classes and types of these pathological conditions. Those skilled in the art will know the various classes and types of proliferative, neoplastic, infectious, autoimmune and inflammatory diseases.

By specific mention of the above categories of pathological conditions, those skilled in the art will understand that such terms include all classes and types of these pathological conditions. For example, the term cancer is intended to include all known cancers, whether characterized as malignant, benign, soft tissue or solid tumors, or hematologic tumors relating to cells in circulation, such as Anal Cancer, Basal Cell Carcinoma, Bladder Cancer, Bone Cancer (Osteosarcoma and Malignant Fibrous Histiocytoma), Brain Tumor, Breast Cancer, Bronchial Tumors, Burkitt Lymphoma, Central Nervous System Lymphoma, Cervical Cancer, Childhood Cancers, Colon Cancer, Colorectal Cancer, Eye Cancer (Intraocular Melanoma or Retinoblastoma), Gallbladder Cancer, Gastric (Stomach) Cancer, Germ Cell Tumor, Head and Neck Cancer, Kidney (Renal Cell) Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer (Non-Small Cell), Lung Cancer (Small Cell), Neuroblastoma, Oral Cancer (Oropharyngeal Cancer), Ovarian Epithelial Cancer, Pancreatic Cancer, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Prostate Cancer, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoma, Skin Cancer (Nonmelanoma), Skin Cancer (Melanoma), Skin Carcinoma (Merkel Cell), Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Urethral Cancer and Vaginal Cancer. Accordingly, "anti-cancer activity" refers to a cellular response to a substance that kills or inhibits the growth of a cancer cell. Cancer cells typically display uncontrolled growth (division beyond the normal limits), invasion (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). Peptides, protides and conjugates described herein which have anti-cancer activity can kill the cancer cell or prevent the invasion or metastasis of the cancer cell into other tissues. It is contemplated that the mechanism of action through which this activity occurs is through the programmed cell death or related responses in the cells.

"Antimicrobial activity" refers to a cellular response to a substance that kills or inhibits the growth of a microorganism, such as bacteria, fungi or protozoans. Peptides described herein

which have antimicrobial activity can either kill the microorganism (microbicidal) or prevent the growth of the microorganism (microbistatic). In some aspects, the peptides, protides and conjugates described herein show antimicrobial activity again pathogenic microorganisms. A "pathogenic microorganism" refers to a microorganism that causes a disease, disorder or condition, which is commonly referred to as an infection. Pathogenic microorganisms are well known to one of skill in the art and include pathogenic bacteria such as *Acinotobacter baumannii, Acinotobacter calcoaceticus, Acinotobacter haemolyticus, Bordetella pertussis, Borrelia burgdorferi, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatis, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Enterococcus faecalis, Enterococcus faecum, Escherichia coli, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Leptospira interrogans, Listeria monocytogenes, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitides, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Vibrio cholerae* and *Yersinia pestis,* pathogenic fungi such as *Cryptococcus neoformans*, A*spergillus fumigatus, Aspergillus flavus, Aspergillus clavatus, Cryptococcus neoformans, Cryptococcus laurentii, Crytococcus albidus, Histoplasma capsulatum, Pneumocystis jirovecii, Stachybotrys chartarum* and several members of the Canidida species, such as *C. albicans, C. glabrata, C. tropicalis, C. stellatoidea, C. glabrata, C. Krusei, C. parapsilosis, C. guilliermondii, C. viswanathii* and *C. lusitaniae*. Additional examples of pathogenic microorganisms are described by Jorgensen and Pfaller in "A Clinician's Dictionary of Pathogenic Microorganisms" ASM Press (2004), which is herein incorporated by reference in its entirety.

Human infections due to antibiotic-resistant bacteria and fungi are increasing in frequency and severity. Microbial pathogens exhibiting resistance to one or more antibiotics can now commonly be found in community and nosocomial settings. Antibiotic resistant pathogens currently of the greatest concern are methicillin (multiple) resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* (VRE), multi-drug-resistant *Streptococcus pneumoniae* (MDRSPn) or *Streptococcus pyogenes* (MDRBRSPy), *Pseudomonas aeruginosa* (MDRA), and *Candida albicans* (MDRCA).

"Programmed cell death" or "PCD" is death of a cell in any form, mediated by an intracellular program. In contrast to other types of cell death, such as necrosis, PCD is carried out as a regulated process which generally confers advantages during an organism's life-cycle. A strategic advantage of apoptosis is that it typically does not induce broader inflammatory responses, which can be injurious and/or delay wound healing, whereas necrosis often iduces considerable inflammation. PCD is commonly categorized by two types, apoptosisin (type I cell-death) or autophagic (type II cell death). Apoptosisin, also known as apoptosis, is a series of biochemical events leading to a characteristic cell morphology including blebbing, loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation known as laddering. Autophagic PCD is a catabolic process involving the degradation of a cell's own components and organelles through the lysosomal machinery prior to the nucleus being destroyed. Additionally, in some aspects, PCD refers to other pathways that have been described including non-apoptotic (i.e. caspase-independent) programmed cell-death, necrosis-like programmed cell death, anoikis, excitotoxicity and Wallerian degeneration.

Embodiments of the invention provide a context-activated protide having at least one activator site and two or more effectors, wherein at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263, wherein the amino acid residue represented by (x) is a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue, wherein the amino acid residue represented by (b) is a lysine, an arginine, an asparagine, a glutamine or a basic residue, wherein the amino acid residue represented by (j) is a cysteine or a thiol residue, wherein the amino acid residue represented by (o) is an anthrylalanine or other non-natural amino acid and wherein the at least one effector induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity. Embodiments of the invention also provide a context-activated protide comprising at least one activator site and two or more effectors, wherein at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9, 12, 14-16, 20, 26-29, 37, 38, 48, 53, 58, 64, 65, 72, 76-83, 94-101, 107, 114, 122-131, 170, 176-178, 188-190, 200-204, 210, 224-226, 236, 237, 244, 248, 252, 257-259 and 288-289, wherein the at least one

effector induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity

In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bax protein, referenced by the amino acid sequence of SEQ ID NOS: 1-13, 264, 270 and 271. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bcl-W protein, referenced by the amino acid sequence of SEQ ID NOS: 14-25, 269 and 272. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bcl-xβ protein, referenced by the amino acid sequence of SEQ ID NOS: 26-36 and 273. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bak protein, referenced by the amino acid sequence of SEQ ID NOS: 37-47. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bcl-2 protein, referenced by the amino acid sequence of SEQ ID NOS: 48-52. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bcl-2 isoform 1 protein, referenced by the amino acid sequence of SEQ ID NOS: 53-57. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Mfn-1 protein, referenced by the amino acid sequence of SEQ ID NOS: 58-64 and 274. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Mfn-2 protein, referenced by the amino acid sequence of SEQ ID NOS: 65-75. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Dnm-1 protein, referenced by the amino acid sequence of SEQ ID NOS: 76-93 and 275. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Dnm-2 protein, referenced by the amino acid sequence of SEQ ID NOS: 94-121, 267, 276 and 277. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Ncl protein, referenced by the amino acid sequence of SEQ ID NOS: 122-175. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Csp3 protein, referenced by the amino acid sequence of SEQ ID NOS: 176-187, 266 and 278. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Bad protein, referenced by the amino acid sequence of SEQ ID NOS: 188-199. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence,

identified from the Prf-1 protein, referenced by the amino acid sequence of SEQ ID NOS: 200-223. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Granulysin protein, referenced by the amino acid sequence of SEQ ID NOS: 224-235. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the CidA protein, referenced by the amino acid sequence of SEQ ID NOS: 236-247, 265 and 279. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the LrgA protein, referenced by the amino acid sequence of SEQ ID NOS: 248-251. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Lambda S21 protein, referenced by the amino acid sequence of SEQ ID NOS: 252-256. In one aspect of the invention, the protide has at least one effector comprising an amino acid sequence, identified from the Holin protein, referenced by the amino acid sequence of SEQ ID NOS: 257-263 and 268. In one aspect, the protide has at least one effector comprising an amino acid sequence, identified from human Bcl-xL protein, which is represented by the amino acid sequence SEQ ID NO: 288 or human CTL Granulysin, which is represented by the amino acid sequence SEQ ID NO: 289.

In one aspect, the activator site is context-activated. The invention also provides that upon context-activation, the protide inititate programmed cell death of the target cells. In some aspects, the context-activation results from a physiologal condition, such as, but not limited to acidity, alkalinity, ionic strength or osmotic strength. In some aspects, the context-activation results from association with an activator molecule. The activator molecules can modify the activator site upon association. In some aspects, modification of the activator site includes cleavage of the activator site. In some aspects, the activoator molecule is an enzyme, such as a protease, esterase or lipase. In one aspect, the activator is expressed by a pathogenic microorganism as described herein. In another aspect, the activator is present in the context of a tumor cell, such as a tumor-specific protease. An example of a tumor-specific protease is a matrix bound protein such as matrix metalloproteinase. In another aspect, the activator is present in the context of an inflammatory response, wherein activators such as thrombin, bradykinin, elastase and metalloproteinase are expressed.

The term "protide," as used herein, refers to a mosaic molecule composed of two or more peptide or non-peptide functional domains, referred to as effectors, and one or more corresponding activator sites. A protide can consist of an indefinite number of effector and

activator domains that can vary in function, activation, position, continuity, or sequence. Additional examples of protide compositions and designs are described in U.S. Patent Application Publication 2006-0074016, 2006-0135416 and U.S. Patent 7,067,621, which are herein incorporated by reference.

The protides described herein have two or more distinct biological functions and are designed to be activated within a defined or characteristic context. The protides described herein have at least one activator site and two or more effectors, wherein at least one of the effectors has and amino acid sequence of a peptide described herein. Protides have the advantage of designs that can be customized, engineered, chosen, or combined to allow for highly selective correspondence to or association with or unique to a specific pathological condition or etiology. The distinct biological functions can further be associated with distinct functional aims, for example, therapy, prevention, amplification and detoxification. As described herein, a multifunctional, context-activated protide can be designed to be activated in any context desired by the user, a feature which makes the protides useful to applications in many areas of medicine and biomedical research, including, for example, diagnosis, imaging, detection, speciation or other specification, prevention/prophylaxis, and therapy of a wide range of pathological conditions such as infectious diseases, neoplastic diseases, immune and autoimmune disorders, cardiovascular conditions, disorders in metabolism or physiology, diseases of inheritance or genetic abnormality, a variety of pathological conditions associated with gene expression, mitochondrial dysfunction or regulation, as well as cell death and/or cellular senescence.

As described herein, in addition to their direct antimicrobial efficacies, the peptides, protides and conjugate described herein are useful based on their ability to circumvent or minimize conventional resistance mechanisms by pathogens or tumor cells. For example, this can be the result of activation by activators that are present outside of the target cell such that the peptides, protides or conjugate need not necessarily enter the target cell to be activated and to achieve subsequent efficacy, thus minimizing the likelihood for resistance due to reduced target access or increased efflux of the peptides, protides or conjugate. Furthermore, in many conventional resistance mechanisms, resistance can be induced by the presence of the anti-infective agent itself. In particular, protides can be designed to be activated by such microbial counter-responses or virulence factors. Thus, the more of the activator that is made by the organism, the more protide activation results, yielding an expected amplification of the

anti-pathogenic efficacy of the protide. Conversely, decreased production of the activators can translate in turn to decreased presence or function of these same activators such as virulence factors or mediators of pathogenesis, in essence turning off the pathogenic potential of the target cell, or reducing its ability to protect itself from otherwise normal host defenses. Similarly, protides can be beneficial by reconstituting tumor cell or microbial pathogen susceptibility to conventional therapeutic agents, to which these pathogenic cells would otherwise be resistant. Thus, the protides can either be activated from upregulation of resistance- or virulence factor expression, or can impact efficacy by effecting the downregulation of virulence factor expression by pathogenic cells or organisms.

In applications of the methods described herein, involving an established infection or a host response to infection, activators can be present or generated. An activator useful for activation of a protide of the invention can be advantageously selected based on a high concentration in the immediate proximity of the infection locus so as to allow for activation of the majority of protides in the desired context. One skilled in the art will be able to select an activator that represents the desired activation context. For applications of the invention methods in the arena of microbial infection, context-activation can be designed to specifically occur in the local context of infection so as to effect optimal relative protide effector concentrations in specific contexts of infection. In addition to context activation that maximizes efficacy, the protides and methods of the invention also minimize the potential for inadvertent host cytotoxicity in areas that do not represent the context. Therefore, in the absence of infection, the protide activators are either absent or are present at concentrations insufficient for effective protide activation, thereby minimizing inadvertent or indiscriminant acute toxicity.

In addition to specific pathogen or host molecules that can serve as activators as described above, protides can also be designed to become activated to diagnose, prevent, or treat infection in unique and/or specific biochemical or physiological contexts associated with microbial pathogens. Examples of such biochemical or environmental contexts include ionic, osmotic, pH, oxidation/reduction, or other conditions that are unique to, characteristic of, or present in the context of infection or disease processes that occur upon infection, or host responses to these events. For example, a protide can be designed to require the influence of protonation, conformation change, or other modification that occurs uniquely or disproportionately in the context of acidic pH, to activate the protide or its ensuing effectors

by altering their structure-activity relationship(s) from inactive to active. As one example, genitourinary tissues, such as renal-tissues or genitourinary mucosa, can exhibit pH values that are decreased normally, or in the setting of infection. A protide designed to be activated only under such acidic conditions could be designed to either be vulnerable to activation in these conditions, or directly activated by these conditions, and thus would be predicted to be active only in such contexts. Alternatively, protides can be designed to be inactive in particular contexts or conditions, such as conditions of relatively high osmotic strength or relatively high pH, so as to minimize or prevent untoward or toxic effects such as nephro- or hepatotoxicity. By way of a further example, activation as well as leukocyte accumulation are conditions associated with infection. Moreover, a fundamental strategy of host defense phagocytes is to phagocytize the microbial pathogen, subjecting it to the harsh environment of the acidic phagolysosome. The compartment so created can become acidified to pH values of 5.5 or lower as the leukocyte responds to the pathogen. Therefore, a protide can be designed that is activated or has amplified or antimicrobial activities, for example, by pH, phagolysosomal enzymes or reactants, or a combination of these conditions, or can amplify or potentiate the antimicrobial mechanisms of leukocytes or other host cells within such settings, so as to inhibit or kill pathogens that enter such cells.

Protide activation also can include conformational, oxidiation or reduction-mediated changes in disulfide array, assembly into multimers of two or more homomeric (identical) or heteromeric (non-identical) effectors, or other modifications of the protide and/or its subsequent effectors. In a particular embodiment, protide activation is triggered as a result of protide accumulation, or its resulting effector components, so as to achieve or surpass threshold concentrations required to optimize or catalyze activation or activity through multimerization or other modification in structure or function of the protide or its effectors.

It is understood, that activation can involve combinations of the protide activation strategies described above. For example, a protide can be designed that is not responsive to an activator unless both the protide and the activator are present within a context associated with or resulting from infection or other disease.

The term "context-activated," as used herein in reference to a protide of the invention, refers to the initiation, activation or amplification of a biological or other desired, for example, diagnostic or prophylactic function of one or more protide effectors in a particular temporal, spatial, pathological and/or biochemical context. Context-activation can be initiated by direct

or indirect interaction between a protide activator site and a corresponding activator that is selectively associated with the particular context. As used herein, context-activation encompasses activation in a wide variety of contexts that can include, for example, local, regional, systemic, and/or temporal proximity; as well as the presence or absence of an etiological agent, pathologic condition, or characteristic components thereof.

Thus, context need not be limited to a place, time or quality, but also can be the presence or absence of an activator, for example, an enzyme elaborated by an organism such as, for example, a specific strain of bacteria. The context for activation can consequently be of any breadth desired by the user, for example, can target a class of organisms or cell types, for example, by using an activator that is ubiquitous to the targeted class, or can alternatively have a more narrow focus by using an activator that represents a more narrowly defined target, for example, a particular genus, organism, species, subspecies, strain, or cell or tissue type. The context can be associated with a pathological condition, but also can be selected to represent a non-pathological environment, for example, in prophylactic applications of the invention practiced to preserve a normal or homeostatic condition.

As used herein, the term "effector" refers to the peptide or non-peptide functional domains of a protide provide herein that have specific individual functions, which are initiated or amplified upon activation and achieve specific functions relating to the diagnosis, prevention, or treatment of a disease. As described herein, a protide has at least two effector domains with distinct, complementary and/or synergistic biological functions. An effector is inactive or exhibits relatively reduced or attenuated biological activity unless an activator, by virtue of either its presence or absence, alters or facilitates or allows the altering of its corresponding activator site and, as a result, initiates or amplifies the diagnostic, prophylactic, therapeutic, or other biological function(s) of the effector(s). Multiple effectors can be induced by the same activator site. Peptide and non-peptide effectors can be present in the same protide, which can be referred to as a hybrid protide. Similarly, a protide can consist exclusively of peptide effectors, also referred to as a peptide protide. Similarly, a protide of the invention can consist exclusively of non-peptidic effectors. The biological function(s) of an effector that corresponds to a protide described herein can be, for example, antimicrobial, immunomodulatory, pro- or anti-inflammatory, tumoricidal, pro- or anti-apoptotic, pro- and anti-angiogenic and/or hemolytic.

As described herein, a protide of the invention can be bifunctional or multifunctional, with two or more unique complementary effectors, and one or more activators as determined by specific effector and activator site domains engineered into the mosaic protide, which can be activated by specific molecules or conditions present in unique or strategic contexts of interest. Examples of such effectors can include one or more antimicrobial, anti-neoplastic, anti-inflammatory, immunomodulatory, or other peptide or non-peptide functional domains, or combinations thereof.

As used herein, the term "activator site" when used in reference to a protide of the invention, refers to a domain of the protide that, in the presence of an activator, initiates, promotes, amplifies or modulates the specific biological function of one or more effectors. As described herein, an activator site can be modified, cleaved, processed or otherwise altered in the presence of an activator. In addition, an activator site can be sensitive either to the absolute presence or absence of an activator as well as can be sensitive to a threshold concentration of an activator rather than its mere presence.

An activator site useful in the invention can include one or more sites for cleavage, modification, processing or other triggering by strategic activators, which can be, for example, proteases, esterases, lipases, or other endogenous enzymatic activators or cascades generated by or associated with a specific condition such as, for example, the presence of pathogenic microorganisms, damaged or inflamed tissues, or hematologic or solid neoplastic or pre-neoplastic cells or tumors. Such an activator site also can be selected to exploit contexts associated with biochemical or physical conditions such as requisite acidity or alkalinity, for example, acidic phagolysomes containing intracellular bacteria or fungi; or ionic or osmotic strength, for example, in a renal context, that represent a specific pathologic or non-pathologic context. Furthermore, an activator site can be selected to exploit normal rather than a pathologic context.

An activator site can be subject to proteolytic as well as non-proteolytic activation. For example, the activator site can be located within the peptide moiety, and require a protease activator. In other embodiments, the non-proteolytic activator can target a non-proteinaceous substrate component of the protide. For example, a protide of the invention can include an esterase activator and can link peptide and/or non-peptide moieties (eg. a protide consisting of peptide and conventional antibiotic effectors) by means of an ester bond. Other biochemically relevant bonds or linkages that can serve as activation sites in an invention

protide can include, for example, lipase- (lipid cleaving), nuclease- (nucleic acid cleaving), and kinase or phosphatase- (phosphate addition or removal) sensitive activators that target substrates other than peptides. For example, certain microbial pathogens or tumor cells can express, or abnormally express restriction enzymes that can provide a suitable basis for design of a protide that could be activated only-within the target cell, further reducing indiscriminant host cytotoxicity.

As used herein, the term "activator" refers to a molecule or condition that, by altering the activator site, causes the liberation or onset of a specific diagnostic or biological function of effector(s). As described herein, an activator can be a normal or abnormal exogenous or endogenous cell, structure or molecule, a condition or milieu (normal or abnormal), or a combination thereof that is associated with a specific context in which activation of the protide is desired. Thus, an activator can be selected based on its presence in a temporal, spatial, or physiological context, which can be normal or abnormal, that is associated with the desired context for protide activation. An activator can consequently include physiological conditions including, for example, acidity, alkalinity, conditions of oxidation or reduction, and/or ionic and/or osmotic strength, that are associated with a particular context, and modulate protide activation. Alternatively, an activator can be a structure or molecule, for example, an enzyme, that is present in a particular spatial, temporal or pathological context. The activator molecule can modify the activator site upon association, for example, by cleavage or other modification that results in activation in the particular context, or can facilitate interaction between protide and activator(s). The activator molecule can be an enzyme including, for example, protease, esterase, lipase, nucleases or peptidase.

In one embodiment of the invention, an activator site can encompass one or more domains for cleavage, modification, processing or any other type of liberation by an activator, for example, a protease, esterase, lipase or other endogenous or exogenous enzymatic activator or cascade. The choice of one or more activator sites that correspond to specific activators depends directly on the desired context for activation. Thus, an activator can be a particular pathologic setting or condition that is chosen based on its association with a particular etiological agent or host response. In the presence of the activator, one or more effectors are liberated so as to achieve a specific function relating to, for example, the treatment, prevention, or diagnosis of a targeted disease. An activator site can thus be strategically designed to become activated in temporal and spatial proximity to activator expression,

thereby allowing the activation of a protide to be targeted to a particular context and over time so as to maximize the desired therapeutic or prophylactic effect, while minimizing untoward or undesirable toxicities or other side effects.

As described herein, an activator site is selected based on its correspondence and/or association with the context in which the two or more protide effectors are to be liberated so as to initiate or potentiate their functions. Therefore, as long as an activator is associated with the context, the invention can be practiced with any context desired. Those skilled in the art will appreciate that, given the versatility of activators useful for practicing the invention as described herein, a protide can be designed based on virtually any context desired, inluding, for example, vascular injury, presence of a neoplasm or cancer, infection, and inflammation.

In one embodiment, the protide is an antimicrobial protide, which also can be referred to as an antimicrotide. Cleavage sites for strategic proteases can be engineered into multifunctional antimicrobial protides so as to represent the activator site of the protide. Upon activation of the protease in the localized or generalized context of tissue injury or infection, as selected by the user, the inactive protide is cleaved, liberating independent and active molecules to effect their respective biological functions. Prior to and beyond the setting of activation of the strategic protease representing the activator, the mosaic protide construct is relatively inactive both with respect to antimicrobial function and host cell toxicity. A mosaic protide construct can consist of an indefinite number (1 through n) of effector and activator domains that can vary in function, activation, position, continuity, or sequence. Effectors corresponding to one or more protides activated by the same or distinct activators also can function synergistically, and/or can recombine in a manner facilitating their complementary functions. As an example, in the context of vascular injury, a protide activator can be selected that specifically represents this particular context, for example, a clotting cascade protease such as thrombin, or a complement fixing protease such as a C3 convertase, for example, C4B2A or C3bBb. Similarly, as another example, a protide activator can be selected that represents a broader constellation of symptoms or conditions, such as sepsis, in which corresponding activators can include serine proteases associated with systemic inflammation, sepsis, or injury, such as activated protein C.

A further embodiment of the invention encompasses anti-neoplastic protides, which also are referred to as antineotides. Many tumor cells produce or overexpress characteristic activators, such as matrix metalloproteinases (MMP) or other enzymes that are not expressed

by, or at levels much higher than normal cells. Consequently, the activator can be a tumor-specific protease, for example, a matrix metalloproteinase or thymidylate synthase (TS), which is overexpressed in the majority of cancers. A tumor-specific protease also can be associated with a more narrow neoplastic context, such as a serine protease that is specifically expressed in prostate cells, for example, PSA, human kallikrein-2 (hK2), human kallikrein-11 (hK11) and TMPRSS2.

Embodiment of the invention provide a conjugate having one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263 and a moiety, wherein the amino acid residue represented by (x) is a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue, wherein the amino acid residue represented by (b) is a lysine, an arginine, an asparagine, a glutamine or a basic residue, wherein the amino acid residue represented by (j) is a cysteine or a thiol residue, wherein in the amino acid residue represented by (o) is an anthrylalanine or other non-natural amino acid and wherein the conjugate induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity. Embodiments of the invention also provide a conjugate having one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9, 12, 14-16, 20, 26-29, 37, 38, 48, 53, 58, 64, 65, 72, 76-83, 94-101, 107, 114, 122-131, 170, 176-178, 188-190, 200-204, 210, 224-226, 236, 237, 244, 248, 252, 257-259 and 288-289 and a moiety, wherein said conjugate induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Bax protein, referenced by the amino acid sequence of SEQ ID NOS: 1-13, 264, 270 and 271. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Bcl-W protein, referenced by the amino acid sequence of SEQ ID NOS: 14-25, 269 and 272. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Bcl-xβ protein, referenced by the amino acid sequence of SEQ ID NOS: 26-36 and 273. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Bak protein, referenced by the amino acid sequence of SEQ ID NOS: 37-47. In one aspect of the

invention, the conjugate has at least one or more amino acid sequence, identified from the Bcl-2 protein, referenced by the amino acid sequence of SEQ ID NOS: 48-52. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Bcl-2 isoform 1 protein, referenced by the amino acid sequence of SEQ ID NOS: 53-57. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Mfn-1 protein, referenced by the amino acid sequence of SEQ ID NOS: 58-64 and 274. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Mfn-2 protein, referenced by the amino acid sequence of SEQ ID NOS: 65-75. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Dnm-1 protein, referenced by the amino acid sequence of SEQ ID NOS: 76-93 and 275. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Dnm-2 protein, referenced by the amino acid sequence of SEQ ID NOS: 94-121, 267, 276 and 277. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Ncl protein, referenced by the amino acid sequence of SEQ ID NOS: 122-175. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Csp3 protein, referenced by the amino acid sequence of SEQ ID NOS: 176-187, 266 and 278. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Bad protein, referenced by the amino acid sequence of SEQ ID NOS: 188-199. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Prf-1 protein, referenced by the amino acid sequence of SEQ ID NOS: 200-223. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Granulysin protein, referenced by the amino acid sequence of SEQ ID NOS: 224-235. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the CidA protein, referenced by the amino acid sequence of SEQ ID NOS: 236-247, 265 and 279. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the LrgA protein, referenced by the amino acid sequence of SEQ ID NOS: 248-251. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Lambda S21 protein, referenced by the amino acid sequence of SEQ ID NOS: 252-256. In one aspect of the invention, the conjugate has at least one or more amino acid sequence, identified from the Holin protein, referenced by the amino acid sequence of SEQ ID NOS: 257-263 and 268. In one aspect, the conjugate has at least one or more amino acid sequence, identified from human Bcl-xL protein, which is represented by the amino acid sequence SEQ

ID NO: 288 or human CTL Granulysin, which is represented by the amino acid sequence SEQ ID NO: 289.

In one aspect, the moiety comprises a therapeutic agent, a targeting peptide or a label. In one aspect, therapeutic agent is a cytotoxic agent, such as an antibiotic or a chemotherapeutic agent. In one aspect, the targeting peptide selectively homes a conjugate described herein to a microorganism, a tumor tissue, tumor cell or tumor vasculature. In one aspect, the targeting peptide selectively homes the conjugate to an immune regulatory cell or an immune effector cell. In one aspect, the conjugate described herein has a targeting peptide, such as, but not limited to, an antibody or a fragment thereof. In another aspect, the moiety of the conjugate described herein is a label, such as a radioisotope or a dye.

As used herein, the term “conjugate” refers to a peptide having an amino acid sequence as described herein linked to a moiety. A "moiety" is used broadly to mean a physical, chemical, or biological material that is linked to a peptide for the purpose of targeting the peptide to a select organ, tissue or cell type or providing an additional functional group to the peptide. In particular, a moiety is a biologically useful moiety such as therapeutic moiety, a diagnostic moiety or a drug delivery vehicle. Thus, a moiety can be a therapeutic agent, for example, a cancer chemotherapeutic agent. Cancer chemotherapeutic agents are well known to one of skill in the art and include, without limitation, alkylating agents such as cyclophosphamide, mechlorethamine, chlorambucil and melphalan, anthracyclines such as daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone and valrubicin, cytoskeletal disruptors such as paclitaxel and docetaxel, epothilones such as epothilones A through F, inhibitors of topoisomerase II such as etoposide, teniposide and tafluposide, nucleotide analogs and precursor analogs such as azacitidine, azathioprine, capecitabine, cytarabine, doxifluridine, fluorouracil, gemcitabine, mercaptopurine, methotrexate, and tioguanine, peptide antibiotics, such as bleomycin, platinum-based agents such as carboplatin, cisplatin and oxaliplatin, retinoids such as tretinoin, and vinca alkaloids or their derivatives such as vinblastine, vincristine, vindesine and vinorelbine. Such a moiety when linked to a peptide, provides a conjugate useful for treating a cancer in a subject. In addition, a moiety can be a drug delivery vehicle such as a chambered microdevice, a cell, a liposome or a virus, which can contain an agent such as a drug or a nucleic acid.

A moiety also can be a targeting peptide or nucleic acid, to which a peptide as described herein is grafted for the purpose of directing the peptide to a selected organ, tissue, tumor or

cell (Smith et al., *J. Biol. Chem.* 269:32788-32795 (1994); Goldman et al., *Cancer Res.* 15:1447-1451 (1997) and U.S. Patent 6,576,239, each of which is incorporated herein by reference). For example, a targeting peptide or nucleic acid can be expressed as a fusion protein with a desired peptide such that the peptide or nucleic acid targets the grafted peptide to a selected tumor tissue, tumor cell or tumor vasculature. Such a desired peptide, which is grafted to the tumor homing peptide, can be a polypeptide involved in initiating a programmed cell death pathway as described herein or inducing any other cellular response resulting in anti-cancer activity. Additionally, targeting peptides, which can be grafted to a peptide as described herein having antimicrobial activity, include peptides that selectively home to a microorganism. For example, peptide sequences have been identified that selectively bind to surface molecules of fugal pathogens such as invasive Aspergillus species as described in U.S. Patent Application 2005-0187161. Still further, the invention provides a conjugate wherein the targeting peptide selectively homes the desired peptide to a cell involved in the immune response, including immune regulatory cells such as lymphocytes or immune effector cells such as macrophages or granulocytes. Conjugates provided herein include these and other exemplary peptide or nucleic acid sequences grafted to a peptide described herein. tumor tissue, tumor cell, tumor vasculature, immune regulatory cell or immune effector cell.

A "targeting peptide" is a peptide comprising a contiguous sequence of amino acids, which is characterized by selective localization to an organ, tissue, or cell type. Selective localization may be determined, for example, by methods disclosed below, wherein the putative targeting peptide sequence is incorporated into a protein that is displayed on the outer surface of a phage. Administration to a subject of a library of such phage that have been genetically engineered to express a multitude of such targeting peptides of different amino acid sequence is followed by collection of one or more organs, tissues, or cell types from the subject and identification of phage found in that organ, tissue, or cell type. A phage expressing a targeting peptide sequence is considered to be selectively localized to a tissue or organ if it exhibits greater binding in that tissue or organ compared to a control tissue or organ. Preferably, selective localization of a targeting peptide should result in a two-fold or higher enrichment of the phage in the target organ, tissue, or cell type, compared to a control organ, tissue, or cell type. Selective localization resulting in at least a three-fold, four-fold, five-fold, six-fold, seven-fold, eight-fold, nine-fold, ten-fold or higher enrichment in the target organ compared to a control organ, tissue or cell type is more preferred. Alternatively, a phage expressing a

targeting peptide sequence that exhibits selective localization preferably shows an increased enrichment in the target organ compared to a control organ when phage recovered from the target organ are reinjected into a second host for another round of screening. Further enrichment may be exhibited following a third round of screening. Another alternative means to determine selective localization is that phage expressing the putative target peptide preferably exhibit a two-fold, more preferably a three-fold or higher enrichment in the target organ or tissue compared to control phage that express a non-specific peptide or that have not been genetically engineered to express any putative target peptides. Another means to determine selective localization is that localization to the target organ or tissue of phage expressing the target peptide is at least partially blocked by the co-administration of a synthetic peptide containing the target peptide sequence. "Targeting peptide" and "homing peptide" are used synonymously herein.

A targeting peptide is useful, for example, for targeting a desired peptide to the selected tumor as discussed above. In addition, a targeting peptide in conjunction with a detectable label can be used to identify the delivery of a desired peptide to a sample. As used herein, the term "sample" is used in its broadest sense to mean a cell, tissue, organ or portion thereof, including a tumor, that is isolated from the body. A sample can be, for example, a histologic section or a specimen obtained by biopsy or cells that are placed in or adapted to tissue culture.

The term "antibody" is well-known in the art and refers to a protein functionally defined as a binding protein and structurally defined as comprising an amino acid sequence that is recognized by one of skill in the art as having variable and constant regions. A typical antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” and one “heavy” chain. The N-terminal portion of each chain defines the variable region of about 100 to about 110 amino acids, which are primarily responsible for antigen recognition and binding. The terms variable heavy chain (V_H) and variable light chain (V_L) regions refer to these light and heavy chains, respectively. The variable region includes the segments of Framework 1 (FR1), CDR1, Framework 2 (FR2), CDR2, Framework 3, CDR3 and Framework 4 (FR4). Antibodies are typically divided into five major classes, IgM, IgG, IgA, IgD, and IgE, based on their constant region structure and immune function. The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. Heavy chains γ,

α and δ have a constant region composed of three tandem (in a line) Ig domains, and a hinge region for added flexibility; heavy chains μ and ε have a constant region composed of four immunoglobulin domains. Antibody classes can also be divided into subclasses, for example, there are four IgG subclasses IgG1, IgG2, IgG3 and IgG4. The structural characteristics that distinguish these subclasses from each other are known to those of skill in the art and can include the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains. The constant region also determines the mechanism used to destroy the bound antigen. A light chain has two successive regions: one constant region, which are designated as κ and λ, and one variable region.

As used herein, the term "functional fragment" when used in reference to the antibodies described herein is intended to refer to a portion of the antibody including heavy or light chain polypeptides which still retains some or all or the binding activity of the antibody. Such functional fragments can include, for example, antibody functional fragments such as Fab, $F(ab)_2$ Fv, and single chain Fv (scFv). Other functional fragments can include, for example, heavy or light chain polypeptides, variable region polypeptides or CDR polypeptides or portions thereof so long as such functional fragments retain binding activity, specificity, inhibitory and activation activity. The term is also intended to include polypeptides encompassing, for example, modified forms of naturally occurring amino acids such as D-stereoisomers, non-naturally occurring amino acids, amino acid analogues and mimetics so long as such polypeptides retain functional activity as defined above.

A moiety can be a detectable label such a radiolabel or can be a cytotoxic agent, including a toxin such as ricin or a drug such as a chemotherapeutic agent or can be a physical, chemical or biological material such as a liposome, microcapsule, micropump or other chambered microdevice, which can be used, for example, as a drug delivery system. Generally, such microdevices, should be nontoxic and, if desired, biodegradable. Various moieties, including microcapsules, which can contain an agent, and methods for linking a moiety, including a chambered microdevice, to a molecule of the invention are well known in the art and commercially available (see, for example, "Remington's Pharmaceutical Sciences" 18th ed. (Mack Publishing Co. 1990), chapters 89-91; Harlow and Lane, Antibodies: A laboratory manual (Cold Spring Harbor Laboratory Press 1988), each of which is incorporated herein by reference; see, also, Hermanson, Bioconjugate Techniques (Academic Press 1996)).

A "label" refers a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into a HIPK1 nucleic acids, proteins and antibodies at any position. For example, the label should be capable of producing, either directly or indirectly, a detectable signal. The detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the label may be employed, including those methods described by Hunter et al., *Nature*, 144:945 (1962); David et al., *Biochemistry*, 13:1014 (1974); Pain et al., *J. Immunol. Meth.*, 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.*, 30:407 (1982).

Peptides, protides and conjugates, which are identified herein, can be synthesized in required quantities using routine methods of solid state peptide synthesis or can be purchased from commercial sources (for example, Anaspec; San Jose Calif.) and a desired moiety can be linked to the peptide. Several methods useful for linking a moiety to a peptide are known in the art, depending on the particular chemical characteristics of the molecule. For example, methods of linking haptens to carrier proteins as used routinely in the field of applied immunology (see, for example, Harlow and Lane, *supra*, 1988; Hermanson, *supra*, 1996).

A moiety such as a therapeutic or diagnostic agent can be conjugated to a peptide using, for example, carbodiimide conjugation (Bauminger and Wilchek, *Meth. Enzymol.* 70:151-159 (1980), which is incorporated herein by reference). Carbodiimides comprise a group of compounds that have the general formula R-N=C=N-R', where R and R' can be aliphatic or aromatic, and are used for synthesis of peptide bonds. The preparative procedure is simple, relatively fast, and is carried out under mild conditions. Carbodiimide compounds attack carboxylic groups to change them into reactive sites for free amino groups. Carbodiimide conjugation has been used to conjugate a variety of compounds to carriers for the production of antibodies.

The water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)ca- rbodiimide (EDC) is particularly useful for conjugating a moiety to a peptide and was used to conjugate doxorubicin to tumor homing peptides (U.S. Patent Application Publication 2004-0131623).

The conjugation of doxorubicin and a tumor homing peptide requires the presence of an amino group, which is provided by doxorubicin, and a carboxyl group, which is provided by the peptide.

In addition to using carbodlimides for the direct formation of peptide bonds, EDC also can be used to prepare active esters such as N-hydroxysuccinimide (NHS) ester. The NHS ester, which binds only to amino groups, then can be used to induce the formation of an amide bond with the single amino group of a moiety. The use of EDC and NHS in combination is commonly used for conjugation in order to increase yield of conjugate formation (Bauminger and Wilchek, *supra*, 1980).

Other methods for conjugating a moiety to a peptide can also be used. For example, sodium periodate oxidation followed by reductive alkylation of appropriate reactants can be used, as can glutaraldehyde crosslinking. However, it is recognized that, regardless of which method of producing a conjugate of the invention is selected, a determination may be needed to confirm that the peptide described herein maintains its antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity and that the moiety maintains its relevant function. Methods for determining the activity of the conjugates are well know to one of skill in the art.

An agar radial diffusion assay has been used as described herein to determine antimicrobial activities of proteins against microbial pathogens *in vitro*. One million CFU will be mixed into 10 ml (i.e., 1 x 10^5 CFU/ml) of melted 1% agarose (in 10 mM $NaHPO_4$ and cooled to 42°C) containing minimal nutrient and adjusted to either pH 5.5 or pH 7.2. The agar is solidified in culture dishes, and sample wells are formed. Peptides at various concentrations are dissolved in 10 µl of 0.01% acetic acid buffer (pH 5.5 or 7.2), loaded into individual wells, and incubated at 37°C for three hours. The plate is then overlayed with 1% agarose containing nutrients and incubated (37°C, for at least 24 hours). Peptides purified by RP-HPLC lacking antimicrobial activity are tested in parallel as controls. Zones of inhibition are measured to quantify antimicrobial activity. This assay will not distinguish between microbicidal and microbiostatic actions, but is highly sensitive to peptides with one or both functions.

Minimum inhibitory (MIC) and microbiocidal concentration (MMC) assays can also be performed, and may include a microvolume assay which is used to quantitatively screen

peptides for antimicrobial activities. In this assay, suspensions of bacteria or fungi in appropriate media are placed in 100-200 µl final volumes in microtiter plates. Poly-L-lysine coated or otherwise positively charged plates are used for these assays, since cationic peptides may bind to anionic surfaces. Purified peptides are then serially diluted, descending from 100 µg/ml. Organisms are inoculated into wells to a concentration of 1 x 10^5 CFU/ml, and plates incubated (37°C, for at least 24 hours). Well turbidities are then assessed visually and by spectrophotometry to quantify growth inhibition versus wells containing no peptide. MMCs are then determined by quantitative culture of MIC wells exhibiting no visible growth.

Microbicidal kinetics of purified peptides are assessed by resuspending the peptides in 0.01% acetic acid buffer (pH 5.5 or 7.2), and organisms are resuspended to a concentration of 1 x 10^5 CFU/ml in 50-250 µl of sterile buffer containing peptide concentrations from 0 to 40 µg/ml. Controls contain buffer alone or non-antimicrobial proteins and organism as above. Mixtures are incubated at 37°C for up to 48 hours, after which aliquots are quantitatively cultured and incubated for 24 to 48 hours. Killing is expressed as decrease in logarithm_{10} surviving CFU/ml. The limit of sensitivity in microbicidal assays is considered to be a 1 log reduction in viable cells.

Flow cytometry can also be used to examine kinetics and mechanisms of the action of the peptides on bacterial membrane integrity and energetics. Peptides which differ in activity or specificity for their ability to depolarize and/or permeabilize microbial membranes can also be compared by analysis of membrane depolarization, and permeabilization. $DiOC_5$ is a charged lipophilic dye which partitions into the cytoplasm, and is dependent on intact transmembrane potential ($\Delta\Psi$) for intracellular retention. Organisms prepared as above are labeled in darkness for 30 minutes at about 20°C in PBS containing 0.05 µM $DiOC_5$ Organisms are resuspended to a concentration of 5 x 10^8 CFU/ml in K^+ MEM containing an individual peptide, and incubated at 37°C. For flow cytometry, organisms are washed, sonicated, counted, and resuspended in K^+ MEM buffer. Reductions in mean $DiOC_5$ fluorescence relative to controls are interpreted to represent loss of $DiOC_5$, indicating membrane depolarization. Positive control cells exposed to valinomycin, as well as control cells not exposed to any peptides, are analyzed for $DiOC_5$ fluorescence in parallel.

Propidium iodide is excluded from cells with normal membrane integrity, but enters cells permealized to molecules ≥2 nm in diameter, and can be stimulated to emit fluorescence at

>620 nm. Organisms prepared as above are resuspended to a concentration of 5 x 10^8 CFU/ml in K^+ MEM containing a selected peptide, and incubated for pre-selected times (ranging from zero up to about 120 minutes) at 37°C. Cells are washed in fresh K^+ MEM, sonicated, counted, and resuspended in K^+ MEM buffer containing 20 μM propidium iodide. Control cells exposed to ethanol (positive control for permeabilization) are assessed for propidium iodide uptake in parallel. Increases in mean propidium iodide fluorescence relative to control cells are interpreted to indicate increases in permeability.

Erythrocyte permeabilizing and hemolytic activities of peptides exhibiting potent microbicidal activity are also studied as indicators of potential *in vivo* toxicity. Four-percent (vol/vol) of washed human erythrocytes (in PBS alone, or in PBS plus 10% heat-inactivated PNHS are incubated with selected peptides ranging in concentration up to 100 times greater than geometric mean MICs. After 24 hours of incubation at 37°C, erythrocyte permeabilization and hemolysis are determined spectrophotometrically. Permeabilization and hemolysis will be compared to buffers alone, and with a triton X-100 control (100% hemolysis).

Endothelial cell injury due to peptides can also be measured using a standard ^{51}Cr release assay, described in Filler et al., *J Infect Dis.*, 164:928-935 (1991); Filler, et al., *Infect Immun.* 62:1064-1069 (1994); Filler et al., *Infect Immun.* 63:976-983 (1995). Briefly, endothelial cells in 96 well tissue culture plates are incubated with $Na^51\ CrO_4$ overnight. The following day, the unincorporated isotope tracer is removed by rinsing, and peptides in 0.01% acetic acid buffer are added to the endothelial cells. Control wells are exposed to buffer alone. After a predetermined incubation period, the medium is aspirated and the amount of ^{51}Cr released into the medium is measured by scintillation. This approach facilitates toxicity screening of multiple peptides simultaneously, and minimizes the amount of peptide necessary for assessment.

Each antimicrobial and toxicity assay described above is performed independently a minimum of two times, and means ± standard error is calculated for each peptide under varying exposure conditions (concentration or pH) as compared with control samples. Statistical analyses of microbicidal data are performed using Student t test or Kruskall-Wallis rank sum analysis for non-parametric data, and corrected for multiple comparisons as appropriate.

The yield of moiety/peptide conjugate formed is determined using routine methods. For example, HPLC or capillary electrophoresis or other qualitative or quantitative method can be used (see, for example, Liu et al., *J. Chromatogr.* 735:357-366 (1996); Rose et al., *J. Chromatogr.* 425:419-412 (1988), each of which is incorporated herein by reference). In particular, the skilled artisan will recognize that the choice of a method for determining yield of a conjugation reaction depends, in part, on the physical and chemical characteristics of the specific moiety and peptide. Following conjugation, the reaction products can be desalted to remove any free peptide and free drug.

Embodiments of the invention provide a method of inducing programmed cell death in a cell, including contacting the cell with an isolated peptide, protide or conjugate described herein. In one aspect, the cell is a microorganism, or in some aspects a pathogenic microorganism. In some aspects, the pathogenic microorganism is *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus subtilis, Acinotobacter baumannii, Acinotobacter calcoaceticus, Acinotobacter haemolyticus, Pseudomonas aeruginosa, Candida albicans* or any of the pathogenic microorganisms described herein. In another aspect, the cell is a tumor cell, including both a malignant and non-malignant tumor cell, or in a preferred aspect a malignant cell. In yet another aspect, the cell is an immune regulatory cell or an immune effector cell.

A peptide, protide or conjugate of the invention useful for practicing the methods of the invention can be formulated and administered by those skilled in the art in a manner and in an amount appropriate for the pathological condition to be treated, for example, an infection, neoplastic disorder, inflammation; the rate or amount of inflammation; the weight, gender, age and health of the individual; the biochemical nature, bioactivity, bioavailability and side effects of the particular compound; and in a manner compatible with concurrent treatment regimens. An appropriate amount and formulation for decreasing the severity of a pathological condition in humans can be extrapolated from credible animal models known in the art of the particular disorder. It is understood, that the dosage of a therapeutic substance has to be adjusted based on the binding affinity of the substance, such that a lower dose of a substance exhibiting significantly higher binding affinity can be administered compared to the dosage necessary for a substance with lower binding affinity. For a peptide, protide or conjugate described herein several factors can be taken into account when determining the proper dosage. For example, for a protide, the nature of the protide effectors and their

bioactivity upon activation, the anticipated concentration of activator and the responsiveness of the activator site to presence of the activator, may be taken into account.

The total amount of peptide, protide or conjugate can be administered as a single dose or by infusion over a relatively short period of time, or can be administered in multiple doses administered over a more prolonged period of time. Such considerations will depend on a variety of case-specific factors such as, for example, whether the disease category is characterized by acute episodes or gradual or chronic deterioration. For an individual affected with an acute infection or inflammatory response, for example, as associated with a bacterial infection, the substance can be administered as a single dose or by infusion of several large doses in a relatively short period of time. For an individual affected with chronic deterioration, for example, as associated with a neuroinflammatory disorder, the substance can be administered in a slow-release matrice, which can be implanted for systemic delivery or at the site of the target tissue, which means an area proximal to the desired context. Contemplated matrices useful for controlled release of therapeutic compounds are well known in the art, and include materials such as DepoFoam™, biopolymers, micropumps, and the like.

The peptides, protides and conjugates administered in the methods of the invention can be administered to the individual by any number of routes known in the art including, for example, systemically, such as intravenously or intraarterially. A therapeutic peptide, protide or conjugate can be provided in the form of isolated and substantially purified polypetides in pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes, including for example, topical, transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, oral, rectal or parenteral such as intravenous, intraspinal, intrathecal, subcutaneous or intramuscular routes. I ntrathecal administration of a therapeutic peptide, protide or conjugate into the intradural or subarachnoid space can be an appropriate route for decreasing the severity of a neuroinflammatory condition. Intravenous administration of a terhapeutic substance containing a peptide, protide or conjugate also is a preferred route for practicing the invention. In addition, a therapeutic substance administered in the methods of the invention can be incorporated into biodegradable polymers allowing for sustained release of the substance useful for prophylactic and reconstitutive applications described above.

Biodegradable polymers and their use are described, for example, in Brem et al., *J. Neurosurg.* 74:441 446 (1991), which is incorporated herein by reference.

The methods for treating a particular pathological condition additionally can be practiced in conjunction with other therapies. For example, for treating cancer, the methods of the invention can be practiced prior to, during, or subsequent to conventional cancer treatments such as surgery, chemotherapy, including administration of cytokines and growth factors, radiation or other methods known in the art. Similarly, for treating pathological conditions which include infectious disease, the methods of the invention can be practiced prior to, during, or subsequent to conventional treatments, such as antibiotic administration, against infectious agents or other methods known in the art. Treatment of pathological conditions of autoimmune disorders also can be accomplished by combining the methods of the invention for inducing an immune response with conventional treatments for the particular autoimmune diseases. Conventional treatments include, for example, chemotherapy, steroid therapy, insulin and other growth factor and cytokine therapy, passive immunity and inhibitors of T cell receptor binding. The peptides, protides and conjugates of the invention can be administered in conjunction with these or other methods known in the art and at various times prior, during or subsequent to initiation of conventional treatments. For a description of treatments for pathological conditions characterized by aberrant cell growth see, for example, The Merck Manual, Sixteenth Ed, (Berkow, R., Editor) Rahway, N.J., 1992.

As described above, administration of a peptide, protide or conjugate can be, for example, simultaneous with or delivered in alternative administrations with the conventional therapy, including multiple administrations. Simultaneous administration can be, for example, together in the same formulation or in different formulations delivered at about the same time or immediately in sequence. Alternating administrations can be, for example, delivering a peptide, protide or conjugate formulation and a conventional therapeutic treatment in temporally separate administrations. Temporally separate administrations of a peptide, protide or conjugate and conventional therapy can use different modes of delivery and routes.

A therapeutic peptide, protide or conjugate containing substance administered in the methods of the invention also can be administered as a solution or suspension together with a pharmaceutically acceptable medium. Such a pharmaceutically acceptable medium can include, for example, sterile aqueous solvents such as sodium phosphate buffer, phosphate buffered saline, normal saline or Ringer's solution or other physiologically buffered saline, or

other solvent or vehicle such as a glycol, glycerol, an oil such as olive oil or an injectable organic ester. A pharmaceutically acceptable medium can additionally contain physiologically acceptable compounds that act, for example, stabilize the neutralizing agent, increase its solubility, or increase its absorption. Such physiologically acceptable compounds include, for example, carbohydrates such as glucose, sucrose or dextrans; antioxidants such as ascorbic acid or glutathione; receptor mediated permeabilizers, which can be used to increase permeability of the blood-brain barrier; chelating agents such as EDTA, which disrupts microbial membranes; divalent metal ions such as calcium or magnesium; low molecular weight proteins; lipids or liposomes; or other stabilizers or excipients. Those skilled in the art understand that the choice of a pharmaceutically acceptable carrier depends on the route of administration of the compound containing the protides and on its particular physical and chemical characteristics.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions such as the pharmaceutically acceptable mediums described above. The solutions can additionally contain, for example, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Other formulations include, for example, aqueous and non-aqueous sterile suspensions which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and can be stored in a lyophilized condition requiring, for example, the addition of the sterile liquid carrier, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules and tablets of the kind previously described.

For applications that require the peptide, protide, or conjugate containing compounds to cross the blood-brain barrier, formulations that increase the lipophilicity of the compound are particularly desirable. For example, the neutralizing agent can be incorporated into liposomes (Gregoriadis, Liposome Technology, Vols. I to III, 2nd ed. (CRC Press, Boca Raton Fla. (1993)). Liposomes, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

A therapeutic peptide, protide or conjugate containing substance administered in the methods of the invention can also be prepared as nanoparticles. Adsorbing peptide compounds onto the surface of nanoparticles has proven effective in delivering peptide drugs to the brain (see

Kreuter et al., *Brain Research* 674:171 174 (1995)). Exemplary nanoparticles are colloidal polymer particles of poly-butylcyanoacrylate with a therapeutic protide-containing substance to be administered in the methods of the invention adsorbed onto the surface and then coated with polysorbate 80.

Image-guided ultrasound delivery of a therapeutic peptide, protide or conjugate containing substance administered in the methods of the invention through the blood-brain barrier to selected locations in the brain can be utilized as described in U.S. Patent 5,752,515. Briefly, to deliver a therapeutic substance past the blood-brain barrier a selected location in the brain is targeted and ultrasound used to induce a change detectable by imaging in the central nervous system (CNS) tissues and/or fluids at that location. At least a portion of the brain in the vicinity of the selected location is imaged, for example, via magnetic resonance imaging (MRI), to confirm the location of the change. An therapeutic substance administered in the methods of the invention into the patient's bloodstream can be delivered to the confirmed location by applying ultrasound to effect opening of the blood-brain barrier at that location and, thereby, to induce uptake of the substance.

In addition, polypeptides called receptor mediated permeabilizers (RMP) can be used to increase the permeability of the blood-brain barrier to molecules such as therapeutic, prophylactic or diagnostic substances as described in U.S. Patents 5,268,164; 5,506,206; and 5,686,416. These receptor mediated permeabilizers can be intravenously co-administered to a host with molecules whose desired destination is the cerebrospinal fluid compartment of the brain, for example, in the treatment of a neuroinflammatory condition. The permeabilizer polypeptides or conformational analogues thereof allow therapeutic substances to penetrate the blood-brain barrier and arrive at their target destination which can be selected based on its proximity to the desired activation context. Such polypeptides can be designed as part of strategic invention protides.

In current treatment regimes for most diseases, more than one compound is often administered to an individual for management of the same or different aspects of the disease. Similarly, in the methods of the invention for treating neoplastic condition, microbial infection, a condition associated with decreased cell death or inflammatory condition, a therapeutic peptide, protide or conjugate containing substance can advantageously be formulated with a second therapeutic compound such as an anti-inflammatory compound, antimicrobail compound, chemotherapeutic compound, immunosuppressive compound or

any other compound that manages the same or different aspects of the particular disease. As an example, for treatment of an infectious disease a therapeutic substance can advantageously be formulated with a second therapeutic compound such as an antibiotic. Contemplated methods of treating a pathological condition by administering to a subject a therapeutically effective amount of a peptide, protide or conjugate therefore include administering a therapeutic substance useful in the methods of the invention alone, in combination with, or in sequence with, such other compounds. Alternatively, combination therapies can consist of fusion proteins, where a therapeutic substance useful for treating a particular pathological condition is linked to a heterologous protein, such as an invention protide.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also provided within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Antimicrobial, Anti-cancer, and other Therapeutic Applications of Peptides Designed from Programmed Cell Death Effector Proteins

Candidate proteins representing a variety of nuclear- or mitochondrial-encoded programmed cell death effector and/or modulatory molecules from eukaryotic sources were identified for comparison. When candidate proteins were compared, similarities in sequences and helical domains that have amphipathic and cationic characteristics were identified. Analogues were also identified where possible from prokaryotic sources.

Template proteins were initially chosen based on the following: 1) mammalian or other eukaryotic cell nuclear-encoded peptides with structure-activity relationships to antimicrobial peptide sequences that afford interaction / control / inhibition of mitochondria or chloroplast symbionts, or host cell apoptosis (examples include Bax, Bcl-W, dynamin, mitofusin, nucleolin, and other programmed cell death effector proteins); and 2) structural and/or functional homologues of such proteins in prokaryotes (examples include CidA/B, LrgA/B, and other holin-like or programmed cell death effector proteins). The identification of candidate peptide sequences was achieved through an iterative process, which included a search of protein databases for additional candidate proteins, multiseqeunce alignments of the identified candidates, followed by an integration and analysis of candidate sequences.

Upon identification of an initial group of template proteins having the above-identified characteristics, additional candidate proteins were identified using primary sequence similarity searches of protein databases using the basic local alignment search tool (BLAST) available from the National Center for Biotechnology Information. The searches utilized the amino acid sequences of candidate template proteins having the desired characteristics as query sequences. Figure 3 shows exemplary amino acid sequence used as the query sequences.

Once all additional candidate template proteins were identified, a compiled set of query sequences were submitted for analysis to the modeling server ClustalW (Larkin et al., *Bioinformatics* 23(21): 2947-2948 (2007)) available online from EMBL-EBI. Utilizing the modeling server, multisequence alignments were performed to identify regions of sequence homology within the candidate sequences or with known host defense or antimicrobial peptide sequences. For example, candidate proteins were ranked based on their alignment

score (Figure 4) and/or analyzed for conserved residues through multisequence alignments (Figure 5). Additionally, phylogenetic and cladogenetic analyses were conducted between the candidate proteins, followed by multiseqeunce alignments of identified putative helical domains (Figures 6-14).

The results from the above processes were integrated to analyze and prioritize candidate sequences. The criteria for their prioritization included: 1) conservation of sequence homology or motif(s); 2) homology to known antimicrobial or anti-cancer peptides; 3) similarity to known or recognized antimicrobial peptide structure-activity relationships (SAR; including presence, periodicity, and distribution of cationic, hydrophobic, and aromatic residues); and 4) visual inspection. Candidate sequences that were identified included mitochondria-targeting proteins, such as Bcl-2, Bcl-W, Bax, and Mitofusin; NK/Tcyto cell effectors, such as Granulysin, Granzyme H, Perforin-1 and Azurocidin (CAP37); apoptosis/cell signaling proteins, such as Fas ligand, Caspase 7 and Dynamin 1; and other related proteins, such as Serpin B9 (CytoPro3), Fractalkine (CX3CL1), CXCL3 and Atrophin 1.

Following the above interactive primary structure analysis, a secondary (2°) structure analysis was conducted. This analysis included visualization, qualitative, and/or quantitative analyses of candidate sequences identified above. This analysis, including 3D visualization of target sequences, conformation 3D homology, qualitative 3D analysis of target sequences, quantitative 3D analysis of target sequences and comparative 3D refinement of target sequences.

3D visualization of target sequences was achieved using Cn3D software available through PubMed. Exemplary 3D visualization of identified candidate peptides within the native total protein are shown in Figure 15-21. These results identified specific sequences as novel targets for further analysis / design. The conformational 3D homology of target sequences to known antimicrobial or anti-cancer template polypeptides was assessed using the threading and 3D homology fold recognition server Protein Homology/analogy Recognition Engine (PHYRE) "Protein structure prediction on the web: a case study using the Phyre server" (Kelley and Sternberg *Nature Protocols* 4:363-371 (2009). Statistical e values were used to guide prioritization of molecules for further analysis.

Priority target sequences identified above were visualized for qualitative analysis of distribution of 3D physicochemical attributes using the public domain UCSF software package Chimera. Next, priority target sequences were quantitatively evaluated for structural homology and/or structure-activity relationships to known antimicrobial and/or anti-cancer peptides / proteins using a combinatorial extension method of Shindyalov and Bourne, *Protein Engineering* 11:739-747 (1998). The results from these analyses provided quantitative alignment of compositional elements, including charged and hydrophobic residues (Figures 22-29). The results included root mean square deviation (RMSD) scores as quantitative data that allowed further prioritization of target sequences. The priority target sequences emanating from the process described above were then used as novel templates for 3D analyses using VAST and/or 3Dpssm software to identify homologous sequences and discover other novel target sequences.

As a final step, a computation simulation of the antimicrobial activity of the selected sequences was conducted. Selected target sequences emerging from the above process were subjected to a computational assessment tool which integrates multiple physical and biochemical attributes of polypeptide sequences to generate a predicted minimal inhibitory concentration (MIC) based on the inverse of the target sequence calculated hydrophobic moment (1/MH; see U.S. Patent 6,743,769).

Based on the above process, specific peptide sequences were identified, which have the desired primary and secondary structure within the candidate proteins (Tables 1-20). These identified peptides are predicted to have antimicrobial and anti-cancer activity. Exemplifying predictive accuracy, candidate peptides have been synthesized and antimicrobial efficacy has been demonstranted *in vitro* against a panel of Gram-positive and Gram-negative bacteria and fungi (see Example II). Furthermore, specific amino acid substitutions were identified for several peptide sequences (Tables 1-19). The identified peptide residues were prioritized/ranked utilizing to above process (Table 21).

Table 1: Engineered peptides based on Bax protein (pro-apoptotic protein / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
1.	^{104}nfnwgrvvalfyfasklvlkalctkv^{129}
2.	^{168}twqtvtifvagvltasltiwkk^{190}
Synthetic Peptides	
1.	H_2N–nfnwgrvvalfyfasklvlkalctkv–COOH
1.	H_2N–nfnwgrvvalfyfasklvlkalctkv–$CONH_2$
3.	H_2N–nfnwgrvvalfyfasklvlkal<u>x</u>tkv–COOH
3.	H_2N–nfnwgrvvalfyfasklvlkal<u>x</u>tkv–$CONH_2$
4.	H_2N–nfnwgrvvalfyfasklvlkal<u>b</u>t<u>j</u>v–COOH
4.	H_2N–nfnwgrvvalfyfasklvlkal<u>b</u>t<u>j</u>v–$CONH_2$
5.	H_2N–wgrvvalfyfasklvlkalctkv–COOH
5.	H_2N–wgrvvalfyfasklvlkalctkv–$CONH_2$
6.	H_2N–wgrvvalfyfasklvlkal<u>x</u>tkv–COOH
6.	H_2N–wgrvvalfyfasklvlkal<u>x</u>tkv–$CONH_2$
7.	H_2N–rvvalfyfasklvlkalctkv–COOH
7.	H_2N–rvvalfyfasklvlkalctkv–$CONH_2$
8.	H_2N–rvvalfyfasklvlkal<u>x</u>tkv–COOH
8.	H_2N–rvvalfyfasklvlkal<u>x</u>tkv–$CONH_2$
9.	H_2N–alfyfasklvlkalctkv–COOH
9.	H_2N–alfyfasklvlkalctkv–$CONH_2$
10.	H_2N–alfyfasklvlkal<u>x</u>tkv–COOH
10.	H_2N–alfyfasklvlkal<u>x</u>tkv–$CONH_2$
2.	H_2N–twqtvtifvagvltasltiwkk–COOH
2.	H_2N–twqtvtifvagvltasltiwkk–$CONH_2$
11.	H_2N–twqtvtifva<u>b</u>vltasltiwkk–COOH
11.	H_2N–twqtvtifva<u>b</u>vltasltiwkk–$CONH_2$
12.	H_2N–tvtifvagvltasltiwkk–COOH
12.	H_2N–tvtifvagvltasltiwkk–$CONH_2$
13.	H_2N–tvtifva<u>b</u>vltasltiwkk–COOH
13.	H_2N–tvtifva<u>b</u>vltasltiwkk–$CONH_2$

Underlined residues indicate substitutions; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue; b = k, r, n, q, other natural / non-natural basic residue; j = cysteine or other natural / non-natural thiol residue.

Table 2: Engineered peptides based on Bcl-W protein (pro-apoptotic protein / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
14.	^{10}tralvadfvgyklrqkgyv^{28}
15.	^{55}trfrrtfsdlaaqlhvt^{71}
16.	^{159}arrlregnwasvrtvltgavalgalvtvgaffask^{193}
Synthetic Peptides	
14.	H_2N–tralvadfvgyklrqkgyv–COOH
14.	H_2N–tralvadfvgyklrqkgyv–$CONH_2$
17.	H_2N–tralva<u>b</u>fvgyklrqkgyv–COOH
17.	H_2N–tralva<u>b</u>fvgyklrqkgyv–$CONH_2$
15.	H_2N–trfrrtfsdlaaqlhvt–COOH
15.	H_2N–trfrrtfsdlaaqlhvt–$CONH_2$
18.	H_2N–trfr<u>b</u>tfsdlaaqlhvt–COOH
18.	H_2N–trfr<u>b</u>tfsdlaaqlhvt–$CONH_2$
19.	H_2N–trfrrtfs<u>b</u>laaqlhvt–COOH
19.	H_2N–trfrrtfs<u>b</u>laaqlhvt–$CONH_2$
20.	H_2N–rrlregnwasvrtvltgavalgalvtvgaffask–COOH
20.	H_2N–rrlregnwasvrtvltgavalgalvtvgaffask–$CONH_2$
21.	H_2N–rrlr<u>b</u>gnwasvrtvltgavalgalvtvgaffask–COOH
21.	H_2N–rrlr<u>b</u>gnwasvrtvltgavalgalvtvgaffask–$CONH_2$
22.	H_2N–rrlregnwasvrtvlt<u>b</u>avalgalvtvgaffask–COOH
22.	H_2N–rrlregnwasvrtvlt<u>b</u>avalgalvtvgaffask–$CONH_2$
23.	H_2N–rrlregnwasvrtvltgaval<u>b</u>alvtvgaffask–COOH
23.	H_2N–rrlregnwasvrtvltgaval<u>b</u>alvtvgaffask–$CONH_2$
24.	H_2N–rrlregnwasvrtvltgavalgalvtv<u>b</u>affask–COOH
24.	H_2N–rrlregnwasvrtvltgavalgalvtv<u>b</u>affask–$CONH_2$
25.	H_2N–rrlr<u>b</u>gnwasvrtvlt<u>b</u>aval<u>b</u>alvtv<u>b</u>affask–COOH
25.	H_2N–rrlr<u>b</u>gnwasvrtvlt<u>b</u>aval<u>b</u>alvtv<u>b</u>affask–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue.

Table 3: Engineered peptides based on Bcl-xβ protein (apoptotic protein / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
26.	^{12}flsyklsqkgyswsqfs^{28}
27.	^{99}lryrrafsdltsqlhitpgtayqsf^{122}
28.	^{187}gwvrtkplvcpfslasgqrs^{196}
29.	^{203}gqrsptalllylfllcwvivg^{223}
Synthetic Peptides	

SEQ ID NO:	Amino Acid Sequence
26.	H_2N–flsyklsqkgyswsqfs–COOH
26.	H_2N–flsyklsqkgyswsqfs–$CONH_2$
27.	H_2N–lryrrafsdltsqlhitpgtayqsf–COOH
27.	H_2N–lryrrafsdltsqlhitpgtayqsf–$CONH_2$
30.	H_2N–lryrrafsbltsqlhitpgtayqsf–COOH
30.	H_2N–lryrrafsbltsqlhitpgtayqsf–$CONH_2$
28.	H_2N–gwvrtkplvcpfslasgqrs–COOH
28.	H_2N–gwvrtkplvcpfslasgqrs–$CONH_2$
31.	H_2N–gwvrtkplvxpfslasgqrs–COOH
31.	H_2N–gwvrtkplvxpfslasgqrs–$CONH_2$
32.	H_2N–gwvrtkplvxpfslasbqrs–COOH
32.	H_2N–gwvrtkplvxpfslasbqrs–$CONH_2$
29.	H_2N–gqrsptalllylfllcwvivg–COOH
29.	H_2N–gqrsptalllylfllcwvivg–$CONH_2$
33.	H_2N–gqrsptalxlylfllcwvivg–COOH
33.	H_2N–gqrsptalxlylfllcwvivg–$CONH_2$
34.	H_2N–gqrsptalllylfllxwvivg–COOH
34.	H_2N–gqrsptalllylfllxwvivg–$CONH_2$
35.	H_2N–gqrsptalllylfllcwvivb–COOH
35.	H_2N–gqrsptalllylfllcwvivb–$CONH_2$
36.	H_2N–gqrsptalxlylfllxwvivb–COOH
36.	H_2N–gqrsptalxlylfllxwvivb–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 4: Engineered peptides based on Bak protein (apoptotic protein / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
37.	^{127}rvvallgfgyrlalhvyq^{144}
38.	^{187}ilnvlvvlgvvllgqfvvrrffks^{211}
Synthetic Peptides	
37.	H_2N–rvvallgfgyrlalhvyq–COOH
37.	H_2N–rvvallgfgyrlalhvyq–$CONH_2$
39.	H_2N–rvvallbfgyrlalhvyq–COOH
39	H_2N–rvvallbfgyrlalhvyq–$CONH_2$
40.	H_2N–rvvallgfbyrlalhvyq–COOH
40.	H_2N–rvvallgfbyrlalhvyq–$CONH_2$
41.	H_2N–rvvallbfbyrlalhvyq–COOH
41.	H_2N–rvvallbfbyrlalhvyq–$CONH_2$

SEQ ID NO:	Amino Acid Sequence
42.	H_2N–rvvalygfgyrlalhvyq–COOH
42.	H_2N–rvvalygfgyrlalhvyq–$CONH_2$
43.	H_2N–rvvalwgfgyrlalhvyq–COOH
43.	H_2N–rvvalwgfgyrlalhvyq–$CONH_2$
44.	H_2N–rvvalybfbyrlalhvyq–COOH
44.	H_2N–rvvalybfbyrlalhvyq–$CONH_2$
45.	H_2N–rvvalwbfbyrlalhvyq–COOH
45.	H_2N–rvvalwbfbyrlalhvyq–$CONH_2$
38.	H_2N–ilnvlvvlgvvllgqfvvrrffks–COOH
38.	H_2N–ilnvlvvlgvvllgqfvvrrffks–$CONH_2$
46.	H_2N–ilnvlvxlgvvllgqfvvrrffks–COOH
46.	H_2N–ilnvlvxlgvvllgqfvvrrffks–$CONH_2$
47.	H_2N–ilnvlvblvlgqfvrfks–COOH
47.	H_2N–ilnvlvblvlgqfvrfks–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 5: Engineered peptides based on Bcl-2 protein (apoptotic regulator / human).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domain	
48.	^{11}nreivmkyihyklsqrgy^{28}
Synthetic Peptides	
48.	H_2N–nreivmkyihyklsqrgy–COOH
48.	H_2N–nreivmkyihyklsqrgy–$CONH_2$
49.	H_2N–nrbivmkyihyklsqrgy–COOH
49.	H_2N–nrbivmkyihyklsqrgy–$CONH_2$
50.	H_2N–nreivxkyihyklsqrgy–COOH
50.	H_2N–nreivxkyihyklsqrgy–$CONH_2$
51.	H_2N–nreivmkyibyklsqrgy–COOH
51.	H_2N–nreivmkyibyklsqrgy–$CONH_2$
52.	H_2N–nrbivxkyibyklsqrgy–COOH
52.	H_2N–nrbivxkyibyklsqrgy–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor residue.

Table 6: Engineered peptides based on Bcl-2 isoform 1 protein (apoptotic regulator / human).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domain	
53.	^{54}lalrqagddfsrryrg^{69}
Synthetic Peptides	
53.	H_2N–lalrqagddfsrryrg–COOH
53.	H_2N–lalrqagddfsrryrg–$CONH_2$
54.	H_2N–lalrqagbdfsrryrg–COOH
54.	H_2N–lalrqagbdfsrryrg–$CONH_2$
55.	H_2N–lalrqagdbfsrryrg–COOH
55.	H_2N–lalrqagdbfsrryrg–$CONH_2$
56.	H_2N–lalrqagbxfsrryrg–COOH
56.	H_2N–lalrqagbxfsrryrg–$CONH_2$
57.	H_2N–lalrqaobxfsrryrg–COOH
57.	H_2N–lalrqaobxfsrryrg–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue; o = anthrylalanine or other non-natural amino acid.

Table 7: Engineered peptides based on Mfn-1 protein (mitofusin-1; human mitochondrial regulator).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domain	
58.	^{699}keidqlekiqnnskllrnkavqlenelenftkqfl^{734}
Synthetic Peptides	
58.	H_2N–keidqlekiqnnskllrnkavqlenelenftkqfl–COOH
58.	H_2N–keidqlekiqnnskllrnkavqlenelenftkqfl–$CONH_2$
59.	H_2N–keibqlekiqnnskllrnkavqlenelenftkqfl–COOH
59	H_2N–keibqlekiqnnskllrnkavqlenelenftkqfl–$CONH_2$
60.	H_2N–keibqlbkiqnnskllrnkavqlbnelenftkqfl–COOH
60.	H_2N–keibqlbkiqnnskllrnkavqlbnelenftkqfl–$CONH_2$
61.	H_2N–keibqlbkiqnnskllrnkavqlbnelenftkqfl–COOH
61.	H_2N–keibqlbkiqnnskllrnkavqlbnelenftkqfl–$CONH_2$
62.	H_2N–keibqlbkiqnnskllrnkavqlbnblenftkqfl–COOH
62.	H_2N–keibqlbkiqnnskllrnkavqlbnblenftkqfl–$CONH_2$
63.	H_2N–keibqlbkiqnnskllrnkavqlbnblbnftkqfl–COOH
63.	H_2N–keibqlbkiqnnskllrnkavqlbnblbnftkqfl–$CONH_2$
64.	H_2N–kiqnnskllrnkavql–COOH
64.	H_2N–kiqnnskllrnkavql–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue.

Table 8: Engineered peptides based on Mfn-2 protein (mitofusin-1; human mitochondrial regulator).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domain	
65.	^{718}nkkievldslqskakllrnkagwldselnmfthqylqpsr^{757}
Synthetic Peptides	
65.	H_2N–nkkievldslqskakllrnkagwldselnmfthqylqpsr–COOH
65.	H_2N–nkkievldslqskakllrnkagwldselnmfthqylqpsr–$CONH_2$
66.	H_2N–nkkibvldslqskakllrnkagwldselnmfthqylqpsr–COOH
66.	H_2N–nkkibvldslqskakllrnkagwldselnmfthqylqpsr–$CONH_2$
67.	H_2N–nkkievlbslqskakllrnkagwldselnmfthqylqpsr–COOH
67.	H_2N–nkkievlbslqskakllrnkagwldselnmfthqylqpsr–$CONH_2$
68.	H_2N–nkkievldslqskakllrnkagwlbselnmfthqylqpsr–COOH
68.	H_2N–nkkievldslqskakllrnkagwlbselnmfthqylqpsr–$CONH_2$
69.	H_2N–nkkievldslqskakllrnkagwldsblnmfthqylqpsr–COOH
69.	H_2N–nkkievldslqskakllrnkagwldsblnmfthqylqpsr–$CONH_2$
70.	H_2N–nkkievldslqskakllrnkagwldselnxfthqylqpsr–COOH
70.	H_2N–nkkievldslqskakllrnkagwldselnxfthqylqpsr–$CONH_2$
71.	H_2N–nkkibvlbslqskakllrnkagwlbsblnxfthqylqpsr–COOH
71.	H_2N–nkkibvlbslqskakllrnkagwlbsblnxfthqylqpsr–$CONH_2$
72.	H_2N–kkievldslqskakllrnkagwl–COOH
72.	H_2N–kkievldslqskakllrnkagwl–$CONH_2$
73.	H_2N–kkibvldslqskakllrnkagwl–COOH
73.	H_2N–kkibvldslqskakllrnkagwl–$CONH_2$
74.	H_2N–kkievlbslqskakllrnkagwl–COOH
74.	H_2N–kkievlbslqskakllrnkagwl–$CONH_2$
75.	H_2N–kkibvlbslqskakllrnkagwl–COOH
75.	H_2N–kkibvlbslqskakllrnkagwl–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 9: Engineered peptides based on Dnm-1 protein (dynamin-1; human mitochondrial regulator).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
76.	^{223}kllplrrgyigvvnrsqk^{240}
77.	^{256}rkfflshpsyrhla^{269}
78.	^{294}glrnklqsqllsiek^{309}
79.	^{323}arktkallqmvqqfavdf^{340}
80.	^{411}atvkkqvqklk^{501}
81.	^{519}virkgwltinnigimkggsk^{539}

SEQ ID NO:	Amino Acid Sequence
82.	^{569}nlklrdvekgfmsskhifalfnteqrnvyk^{598}
83.	^{617}kasflragvypervgdk^{633}
Synthetic Peptides	
76.	H_2N–kllplrrgyigvvnrsqk–COOH
76.	H_2N–kllplrrgyigvvnrsqk–$CONH_2$
77.	H_2N–rkfflshpsyrhla–COOH
77.	H_2N–rkfflshpsyrhla–$CONH_2$
78.	H_2N–glrnklqsqllsiek–COOH
78.	H_2N–glrnklqsqllsiek–$CONH_2$
84.	H_2N–glrnklqsqllsi<u>b</u>k–COOH
84.	H_2N–glrnklqsqllsi<u>b</u>k–$CONH_2$
79.	H_2N–arktkallqmvqqfavdf–COOH
79.	H_2N–arktkallqmvqqfavdf–$CONH_2$
85.	H_2N–arktkallqmvqqfav<u>b</u>f–COOH
85.	H_2N–arktkallqmvqqfav<u>b</u>f–$CONH_2$
80.	H_2N–atvkkqvqklk–COOH
80.	H_2N–atvkkqvqklk–$CONH_2$
81.	H_2N–virkgwltinnigimkggsk–COOH
81.	H_2N–virkgwltinnigimkggsk–$CONH_2$
82.	H_2N–nlklrdvekgfmsskhifalfnteqrnvyk–COOH
82.	H_2N–nlklrdvekgfmsskhifalfnteqrnvyk–$CONH_2$
86.	H_2N–nlklr<u>b</u>vekgfmsskhifalfnteqrnvyk–COOH
86.	H_2N–nlklr<u>b</u>vekgfmsskhifalfnteqrnvyk–$CONH_2$
87.	H_2N–nlklrdv<u>b</u>kgfmsskhifalfnteqrnvyk–COOH
87.	H_2N–nlklrdv<u>b</u>kgfmsskhifalfnteqrnvyk–$CONH_2$
88.	H_2N–nlklrdvekgfmsskhifalfnt<u>b</u>qrnvyk–COOH
88.	H_2N–nlklrdvekgfmsskhifalfnt<u>b</u>qrnvyk–$CONH_2$
89.	H_2N–nlklr<u>b</u>v<u>b</u>kgfmsskhifalfnteqrnvyk–COOH
89.	H_2N–nlklr<u>b</u>v<u>b</u>kgfmsskhifalfnteqrnvyk–$CONH_2$
90.	H_2N–nlklr<u>b</u>v<u>b</u>kgfmsskhifalfnt<u>b</u>qrnvyk–COOH
90.	H_2N–nlklr<u>b</u>v<u>b</u>kgfmsskhifalfnt<u>b</u>qrnvyk–$CONH_2$
83.	H_2N–kasflragvypervgdk–COOH
83.	H_2N–kasflragvypervgdk–$CONH_2$
91.	H_2N–kasflragvyp<u>b</u>rvgdk–COOH
91.	H_2N–kasflragvyp<u>b</u>rvgdk–$CONH_2$
92.	H_2N–kasflragvypervg<u>b</u>k–COOH
92.	H_2N–kasflragvypervg<u>b</u>k–$CONH_2$
93.	H_2N–kasflragvyp<u>b</u>rvg<u>b</u>k–COOH
93.	H_2N–kasflragvyp<u>b</u>rvg<u>b</u>k–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue.

Table 10: Engineered peptides based on Dnm-2 protein (dynamin-2; human mitochondrial regulator).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
94.	^{187}klakevdpqglrtigvitkl^{206}
95.	^{295}alrsklqsqllslek^{309}
96.	^{323}trktkallqmvqqfgv^{338}
97.	^{420}aivkkqvvklk^{421}
98.	^{499}aqqrstqlnkkraipnqg^{516}
99.	^{559}kekkymlpldnlkirdvekgfmsnkhvfaifnteqrnvyk^{598}
100.	^{569}nlkirdvekgfmsnkhvfaifnteqrnvyk^{598}
101.	^{615}swkasflragvypekdqa^{632}
Synthetic Peptides	
94.	H_2N–klakevdpqglrtigvitkl–COOH
94.	H_2N–klakevdpqglrtigvitkl–$CONH_2$
102.	H_2N–klakbvdpqglrtigvitkl–COOH
102.	H_2N–klakbvdpqglrtigvitkl–$CONH_2$
103.	H_2N–klakevbpqglrtigvitkl–COOH
103.	H_2N–klakevbpqglrtigvitkl–$CONH_2$
104.	H_2N–klakbvbpqglrtigvitkl–COOH
104.	H_2N–klakbvbpqglrtigvitkl–$CONH_2$
95.	H_2N–alrsklqsqllslek–COOH
95.	H_2N–alrsklqsqllslek–$CONH_2$
105.	H_2N–alrsklqsqllslbk–COOH
105	H_2N–alrsklqsqllslbk–$CONH_2$
96.	H_2N–trktkallqmvqqfgv–COOH
96.	H_2N–trktkallqmvqqfgv–$CONH_2$
97.	H_2N–aivkkqvvklk –COOH
97.	H_2N–aivkkqvvklk –$CONH_2$
106.	H_2N–oivkkqvvklk –COOH
106.	H_2N–oivkkqvvklk –$CONH_2$
98.	H_2N–aqqrstqlnkkraipnqg–COOH
98.	H_2N–aqqrstqlnkkraipnqg–$CONH_2$
99.	H_2N–kekkymlpldnlkirdvekgfmsnkhvfaifnteqrnvyk–COOH
99.	H_2N–kekkymlpldnlkirdvekgfmsnkhvfaifnteqrnvyk–$CONH_2$
107.	H_2N–kekkymlpldnlkir–COOH
107.	H_2N–kekkymlpldnlkir–$CONH_2$
108.	H_2N–kxkkymlpldnlkir–COOH
108.	H_2N–kxkkymlpldnlkir–$CONH_2$
109.	H_2N–kekkyxlpldnlkir–COOH
109.	H_2N–kekkyxlpldnlkir—$CONH_2$

SEQ ID NO:	Amino Acid Sequence
110.	H_2N–kekkymlplbnlkir–COOH
110.	H_2N–kekkymlplbnlkir—$CONH_2$
111.	H_2N–kekkymlplxnlkir–COOH
111.	H_2N–kekkymlplxnlkir—$CONH_2$
112.	H_2N–kxkkymlplbnlkir–COOH
112.	H_2N–kxkkymlplbnlkir—$CONH_2$
113.	H_2N–kxkkyxlplbnlkir–COOH
113.	H_2N–kxkkyxlplbnlkir—$CONH_2$
114.	H_2N–kgfmsnkhvfaifnteqrnvyk–COOH
114.	H_2N–kgfmsnkhvfaifnteqrnvyk—$CONH_2$
115.	H_2N–kxkkymlpldnlkirdvekgfmsnkhvfaifnteqrnvyk–COOH
115.	H_2N–kxkkymlpldnlkirdvekgfmsnkhvfaifnteqrnvyk—$CONH_2$
116.	H_2N–kekkyxlpldnlkirdvekgfmsnkhvfaifnteqrnvyk–COOH
116.	H_2N–kekkyxlpldnlkirdvekgfmsnkhvfaifnteqrnvyk—$CONH_2$
117.	H_2N–kekkymlpldnlkirdvekgfxsnkhvfaifnteqrnvyk–COOH
117.	H_2N–kekkymlpldnlkirdvekgfxsnkhvfaifnteqrnvyk—$CONH_2$
100.	H_2N–nlkirdvekgfmsnkhvfaifnteqrnvyk–COOH
100.	H_2N–nlkirdvekgfmsnkhvfaifnteqrnvyk–$CONH_2$
118.	H_2N–nlkirbvekgfmsnkhvfaifnteqrnvyk–COOH
118.	H_2N–nlkirbvekgfmsnkhvfaifnteqrnvyk–$CONH_2$
119.	H_2N–nlkirdvbkgfmsnkhvfaifnteqrnvyk–COOH
119.	H_2N–nlkirdvbkgfmsnkhvfaifnteqrnvyk–$CONH_2$
120.	H_2N–nlkirbvbkgfmsnkhvfaifntbqrnvyk–COOH
120.	H_2N–nlkirbvbkgfmsnkhvfaifntbqrnvyk–$CONH_2$
101.	H_2N–swkasflragvypekdqa–COOH
101.	H_2N–swkasflragvypekdqa–$CONH_2$
121.	H_2N–swkasflragvypbkdqa–COOH
121.	H_2N–swkasflragvypbkdqa–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; o = anthrylalanine or other non-natural amino acid; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 11: Engineered peptides based on Ncl protein (nucleolin; human mitochondrial regulator).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
122.	^{50}qkkgkkaaatsakkvvvs^{67}
123.	^{69}tkkvavatpakkaavt^{84}
124.	^{124}kkgaaipakgakngknakk^{142}
125.	^{216}akgkkaakvvpvkaknva^{233}
126.	^{273}vkeapgkrkkemakqkaa^{290}

SEQ ID NO:	Amino Acid Sequence
127.	362 kaleltglkvfgneiklek 380
128.	382 kgkdskkerdartllaknlpykvtq 406
129.	419 irlvskdgkskgiayi 434
130.	467 kgqnqdyrggknstwsgesktlvlsnlsysat 498
131.	508 katfikvpqnqngkskgyafi 528
Synthetic Peptides	
122.	H_2N–qkkgkkaaatsakkvvvs–COOH
122.	H_2N–qkkgkkaaatsakkvvvs–$CONH_2$
132.	H_2N–qkkgkkaxatsakkvyvs–COOH
132.	H_2N–qkkgkkaxatsakkvyvs–$CONH_2$
133.	H_2N–qkkgkkaaatsakkvyvs–COOH
133.	H_2N–qkkgkkaaatsakkvyvs–$CONH_2$
134.	H_2N–qkkgkkaaatsakkvwvs–COOH
134.	H_2N–qkkgkkaaatsakkvwvs–$CONH_2$
135.	H_2N–qkkgkkaxatsakkvyvs–COOH
135.	H_2N–qkkgkkaxatsakkvyvs–$CONH_2$
136.	H_2N–qkkgkkaxatsakkvwvs–COOH
136.	H_2N–qkkgkkaxatsakkvwvs–$CONH_2$
123.	H_2N–tkkvavatpakkaavt–COOH
123.	H_2N–tkkvavatpakkaavt–$CONH_2$
124.	H_2N–kkgaaipakgakngknakk–COOH
124.	H_2N–kkgaaipakgakngknakk–$CONH_2$
137.	H_2N–kkgaxipakgakngknakk–COOH
137.	H_2N–kkgaxipakgakngknakk–$CONH_2$
125.	H_2N–akgkkaakvvpvkaknva–COOH
125.	H_2N–akgkkaakvvpvkaknva–$CONH_2$
138.	H_2N–akgkkaakvvxvkaknva–COOH
138.	H_2N–akgkkaakvvxvkaknva–$CONH_2$
126.	H_2N–vkeapgkrkkemakqkaa–COOH
126.	H_2N–vkeapgkrkkemakqkaa–$CONH_2$
139.	H_2N–vkbapgkrkkemakqkaa–COOH
139	H_2N–vkbapgkrkkemakqkaa–$CONH_2$
140.	H_2N–vkeapgkrkkbmakqkaa–COOH
140.	H_2N–vkeapgkrkkbmakqkaa–$CONH_2$
141.	H_2N–vkeapgkrkkexakqkaa–COOH
141.	H_2N–vkeapgkrkkexakqkaa–$CONH_2$
142.	H_2N–vkbapgkrkkbxakqkaa–COOH
142.	H_2N–vkbapgkrkkbxakqkaa–$CONH_2$
127.	H_2N–kaleltglkvfgneiklek–COOH
127.	H_2N–kaleltglkvfgneiklek–$CONH_2$

SEQ ID NO:	Amino Acid Sequence
143.	H_2N–kalbltglkvfgneiklek–COOH
143.	H_2N–kalbltglkvfgneiklek–$CONH_2$
144.	H_2N–kaleltglkvfgnbiklek–COOH
144.	H_2N–kaleltglkvfgnbiklek–$CONH_2$
145.	H_2N–kaleltglkvfgneiklbk–COOH
145.	H_2N–kaleltglkvfgneiklbk–$CONH_2$
146.	H_2N–kalbltglkvfgnbiklbk–COOH
146.	H_2N–kalbltglkvfgnbiklbk–$CONH_2$
128.	H_2N–kgkdskkerdartllaknlpykvtq–COOH
128.	H_2N–kgkdskkerdartllaknlpykvtq–$CONH_2$
147.	H_2N–kgkxskkerdartllaknlpykvtq–COOH
147.	H_2N–kgkxskkerdartllaknlpykvtq–$CONH_2$
148.	H_2N–kgkdskkxrdartllaknlpykvtq–COOH
148.	H_2N–kgkdskkxrdartllaknlpykvtq–$CONH_2$
149.	H_2N–kgkdskkerbartllaknlpykvtq–COOH
149.	H_2N–kgkdskkerbartllaknlpykvtq–$CONH_2$
150.	H_2N–kgkxskkbrbartllaknlpykvtq–COOH
150.	H_2N–kgkxskkbrbartllaknlpykvtq–$CONH_2$
129.	H_2N–irlvskdgkskgiayi–COOH
129.	H_2N–irlvskdgkskgiayi–$CONH_2$
151.	H_2N–irlvskfgkskgiayi–COOH
151.	H_2N–irlvskfgkskgiayi–$CONH_2$
152.	H_2N–irlvskygkskgiayi–COOH
152.	H_2N–irlvskygkskgiayi–$CONH_2$
153.	H_2N–irlvskwgkskgiayi–COOH
153	H_2N–irlvskwgkskgiayi–$CONH_2$
154.	H_2N–irlvsklwgkskgiayi–COOH
154	H_2N–irlvsklwgkskgiayi–$CONH_2$
155.	H_2N–irlvskdgkskg–COOH
155.	H_2N–irlvskdgkskg–$CONH_2$
156.	H_2N–irlvskfgkskgi–COOH
156.	H_2N–irlvskfgkskg–$CONH_2$
157.	H_2N–irlvskygkskg–COOH
157.	H_2N–irlvskygkskg–$CONH_2$
158.	H_2N–irlvskwgkskg–COOH
158.	H_2N–irlvskwgkskg–$CONH_2$
159.	H_2N–irlvsklwgkskg–COOH
159.	H_2N–irlvsklwgkskgi–$CONH_2$
130.	H_2N–kgqnqdyrggknstwsgesktlvlsnlsysat–COOH
130.	H_2N–kgqnqdyrggknstwsgesktlvlsnlsysat–$CONH_2$
160.	H_2N–kgqnqbyrggknstwsgesktlvlsnlsysat–COOH

SEQ ID NO:	Amino Acid Sequence
160.	H_2N–kgqnqbyrggknstwsgesktlvlsnlsysat–$CONH_2$
161.	H_2N–kgqnqdyrggknstwsgbsktlvlsnlsysat–COOH
161.	H_2N–kgqnqdyrggknstwsgbsktlvlsnlsysat–$CONH_2$
162.	H_2N–kgqnqbyrggknstwsgbsktlvlsnlsysat–COOH
162	H_2N–kgqnqbyrggknstwsgbsktlvlsnlsysat–$CONH_2$
163.	H_2N–kgbnqdyrlgknstwsgbsktlvlsnlsysat–COOH
163.	H_2N–kgbnqdyrlgknstwsgbsktlvlsnlsysat–$CONH_2$
164.	H_2N–kgbnqdyrlgknstwsgbskt–COOH
164.	H_2N–kgbnqdyrlgknstwsgbskt–$CONH_2$
131.	H_2N–katfikvpqnqngkskgyafi–COOH
131.	H_2N–katfikvpqnqngkskgyafi–$CONH_2$
165.	H_2N–katfikvpqnqnxkskgyafi–COOH
165.	H_2N–katfikvpqnqnxkskgyafi–$CONH_2$
166.	H_2N–katfikvpqnqnlkskgyafi–COOH
166.	H_2N–katfikvpqnqnlkskgyafi–$CONH_2$
167.	H_2N–katfikvpqnqnykskgyafi–COOH
167.	H_2N–katfikvpqnqnykskgyafi–$CONH_2$
168.	H_2N–katfikvpqnqnfkskgyafi–COOH
168.	H_2N–katfikvpqnqnfkskgyafi–$CONH_2$
169.	H_2N–katfikvpqnqnwkskgyafi–COOH
169.	H_2N–katfikvpqnqnwkskgyafi–$CONH_2$
170.	H_2N–katfikvpqnqngkskgy–COOH
170.	H_2N–katfikvpqnqngkskgy–$CONH_2$
171.	H_2N–katfikvpqnqnxkskgy–COOH
171.	H_2N–katfikvpqnqnxkskgy–$CONH_2$
172.	H_2N–katfikvpqnqnlkskgy–COOH
172.	H_2N–katfikvpqnqnlkskgy–$CONH_2$
173.	H_2N–katfikvpqnqnykskgy–COOH
173.	H_2N–katfikvpqnqnykskgy–$CONH_2$
174.	H_2N–katfikvpqnqnfkskgy–COOH
174.	H_2N–katfikvpqnqnfkskgy–$CONH_2$
175.	H_2N–katfikvpqnqnwkskgy–COOH
175.	H_2N–katfikvpqnqnwkskgy–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 12: Engineered peptides based on Csp3 protein (caspase 3; apoptosis effector / nuclear encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
176.	^{10}sksiknlepkiihgs^{24}
177.	^{136}lkkitnffrgdrcrsltgkpklfiiqacrgt^{166}

SEQ ID NO:	Amino Acid Sequence
178.	[215]fiqslcamlkqyadklefmhiltrvnrkvat[245]
Synthetic Peptides	
176.	H_2N–sksiknlepkiihgs–COOH
176.	H_2N–sksiknlepkiihgs–$CONH_2$
179.	H_2N–sksiknlbpkiihgs–COOH
179.	H_2N–sksiknlbpkiihgs–$CONH_2$
180.	H_2N–sksiknlepkiiygs–COOH
180.	H_2N–sksiknlepkiiygs–$CONH_2$
181.	H_2N–sksiknlepkiiybs–COOH
181.	H_2N–sksiknlepkiiybs–$CONH_2$
182.	H_2N–sksiknlbpkiiybs–COOH
182.	H_2N–sksiknlbpkiiybs–$CONH_2$
177.	H_2N–lkkitnffrgdrcrsltgkpklfiiqacrgt–COOH
177.	H_2N–lkkitnffrgdrcrsltgkpklfiiqacrgt–$CONH_2$
183.	H_2N–lkkitnffrgbrcrsltgkpklfiiqacrgt–COOH
183.	H_2N–lkkitnffrgbrcrsltgkpklfiiqacrgt–$CONH_2$
184.	H_2N–lkkitnffrgdrxrsltgkpklfiiqacrgt–COOH
184.	H_2N–lkkitnffrgdrxrsltgkpklfiiqacrgt–$CONH_2$
185.	H_2N–lkkitnffrgdrcrsltgkpklfiiqaxrgt–COOH
185.	H_2N–lkkitnffrgdrcrsltgkpklfiiqaxrgt–$CONH_2$
186.	H_2N–lkkitnfrgbrxrsltgkpklfiiqaxrgt–COOH
186.	H_2N–lkkitnfrgbrxrsltgkpklfiiqaxrgt–$CONH_2$
187.	H_2N–lkkitnfrgbrxrsltgk–COOH
187.	H_2N–lkkitnfrgbrxrsltgk–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 13: Engineered peptides based on Bad protein (apoptotic protein / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
188.	[93]frgrsrsappnlwaaqrygrelrr[116]
189.	[115]rrmsdefvdsfkkglprpksagtatq[140]
190.	[121]fvdsfkkglprpksagtatq[140]
Synthetic Peptides	
188.	H_2N–frgrsrsappnlwaaqrygrelrr–COOH
188.	H_2N–frgrsrsappnlwaaqrygrelrr–$CONH_2$
191.	H_2N–frgrsrsappnlwaaqrygrblrr–COOH
191.	H_2N–frgrsrsappnlwaaqrygrblrr–$CONH_2$
189.	H_2N–rrmsdefvdsfkkglprpksagtatq–COOH
189.	H_2N–rrmsdefvdsfkkglprpksagtatq–$CONH_2$
192.	H_2N–rrmsbefvdsfkkglprpksagtatq–COOH

SEQ ID NO:	Amino Acid Sequence
192.	H_2N–rrmsbefvdsfkkglprpksagtatq–$CONH_2$
193.	H_2N–rrmsdbfvdsfkkglprpksagtatq–COOH
193.	H_2N–rrmsdbfvdsfkkglprpksagtatq–$CONH_2$
194.	H_2N–rrmsdefvbsfkkglprpksagtatq–COOH
194.	H_2N–rrmsdefvbsfkkglprpksagtatq–$CONH_2$
195.	H_2N–rrmsbbfvbsfkkglprpksagtatq–COOH
195.	H_2N–rrmsbbfvbsfkkglprpksagtatq–$CONH_2$
196.	H_2N–rrxsbbfvbsfkkglprpksagtatq–COOH
196.	H_2N–rrxsbbfvbsfkkglprpksagtatq–$CONH_2$
190.	H_2N–fvdsfkkglprpksagtatq–COOH
190.	H_2N–fvdsfkkglprpksagtatq–$CONH_2$
197.	H_2N–fvbsfkkglprpksagtatq–COOH
197.	H_2N–fvbsfkkglprpksagtatq–$CONH_2$
198.	H_2N–fvbsfkkglxrpksag–COOH
198.	H_2N–fvbsfkkglxrpksag–$CONH_2$
199.	H_2N–fvbsfkkglyrpksag–COOH
199.	H_2N–fvbsfkkglyrpksag–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 14: Engineered peptides based on Prf-1 protein (perforin-1 / apoptotic / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
200.	^{32}krshkfvpgawlag^{45}
201.	^{49}vtslrrsgsfpvdtqrflr^{68}
202.	^{123}rsirndwkvgldvtpk^{138}
203.	^{356}rrealrralsqyltdrarwr^{375}
204.	^{520}nlnhghlkfryhar^{534}
Synthetic Peptides	
200.	H_2N–krshkfvpgawlag–COOH
200.	H_2N–krshkfvpgawlag–$CONH_2$
205.	H_2N–krshkfvxgawlag–COOH
205.	H_2N–krshkfvxgawlag–$CONH_2$
201.	H_2N–vtslrrsgsfpvdtqrflr–COOH
201.	H_2N–vtslrrsgsfpvdtqrflr–$CONH_2$
206.	H_2N–vtslrrsgsfxvdtqrflr–COOH
206.	H_2N–vtslrrsgsfxvdtqrflr–$CONH_2$
207.	H_2N–vtslrrsgsfpvbtqrflr–COOH
207.	H_2N–vtslrrsgsfpvbtqrflr–$CONH_2$
208.	H_2N–vtslrrsgsfxvbtqrflr–COOH
208.	H_2N–vtslrrsgsfxvbdtqrflr–$CONH_2$

SEQ ID NO:	Amino Acid Sequence
202.	H_2N–rsirndwkvgldvtpk–COOH
202.	H_2N–rsirndwkvgldvtpk–$CONH_2$
209.	H_2N–rsirnbwkvgldvtpk–COOH
209.	H_2N–rsirnbwkvgldvtpk–$CONH_2$
210.	H_2N–rsirndwkvgldvt–COOH
210.	H_2N–rsirndwkvgldvt–$CONH_2$
211.	H_2N–rsirnbwkvgldvt–COOH
211.	H_2N–rsirnbwkvgldvt–$CONH_2$
203.	H_2N–rrealrralsqyltdrarwr–COOH
203.	H_2N–rrealrralsqyltdrarwr–$CONH_2$
212.	H_2N–rrbalrralsqyltdrarwr–COOH
212.	H_2N–rrbalrralsqyltdrarwr–$CONH_2$
213.	H_2N–rrealrralsqyltbrarwr–COOH
213.	H_2N–rrealrralsqyltbrarwr–$CONH_2$
214.	H_2N–rrbalrralsqyltbrarwr–COOH
214.	H_2N–rrbalrralsqyltbrarwr–$CONH_2$
215.	H_2N–rxbalrralsqyltbrarwr–COOH
215.	H_2N–rxbalrralsqyltbrarwr–$CONH_2$
204	H_2N–nlnhghlkfryhar–COOH
204.	H_2N–nlnhghlkfryhar–$CONH_2$
216.	H_2N–nlnbghlkfryhar–COOH
216.	H_2N–nlnbghlkfryhar–$CONH_2$
217.	H_2N–nlnbgblkfryhar–COOH
217.	H_2N–nlnbgblkfryhar–$CONH_2$
218.	H_2N–nlnbgblkfrybar–COOH
218.	H_2N–nlnbgblkfrybar–$CONH_2$
219.	H_2N–nlnxghlkfryhar–COOH
219.	H_2N–nlnxghlkfryhar–$CONH_2$
220.	H_2N–nlnbgxlkfryhar–COOH
220.	H_2N–nlnbgxlkfryhar–$CONH_2$
221.	H_2N–nlnbgblkfryxar–COOH
221.	H_2N–nlnbgblkfryxar–$CONH_2$
222.	H_2N–olnbgblkfrybar–COOH
222.	H_2N–olnbgblkfrybar–$CONH_2$
223.	H_2N–olnbgblkfryxar–COOH
223.	H_2N–olnbgblkfryxar–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue; o = anthrylalanine or other non-natural amino acid.

Table 15: Engineered peptides based on Granulysin protein (granulysin-1 / apoptotic / human / nuclear-encoded).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
224.	^{62}lgrdyrtcltivqklkk^{78}
225.	^{82}kptqrsvsnaatrvcrtgrsrwr^{104}
226.	^{101}srwrrryqsrvtqglvag^{125}
Synthetic Peptides	
224.	H_2N–lgrdyrtcltivqklkk–COOH
224.	H_2N–lgrdyrtcltivqklkk–$CONH_2$
227.	H_2N–lgrbyrtcltivqklkk–COOH
227.	H_2N–lgrbyrtcltivqklkk–$CONH_2$
228.	H_2N–lgrdyrtxltivqklkk–COOH
228.	H_2N–lgrdyrtxltivqklkk–$CONH_2$
229.	H_2N–lgrbyrtxltivqklkk–COOH
229.	H_2N–lgrbyrtxltivqklkk–$CONH_2$
225.	H_2N–kptqrsvsnaatrvcrtgrsrwr–COOH
225.	H_2N–kptqrsvsnaatrvcrtgrsrwr–$CONH_2$
230.	H_2N–kptqrsvsnaatrvxrtgrsrwr–COOH
230.	H_2N–kptqrsvsnaatrvxrtgrsrwr–$CONH_2$
231.	H_2N–kptqrsvsnaatrvxrtg–COOH
231.	H_2N–kptqrsvsnaatrvxrtg–$CONH_2$
232.	H_2N–kptqrsvsnyatrvxrtg–COOH
232.	H_2N–kptqrsvsnyatrvxrtg–$CONH_2$
233.	H_2N–kptqrsvsnfatrvxrtg–COOH
233.	H_2N–kptqrsvsnfatrvxrtg–$CONH_2$
226.	H_2N–srwrrryqsrvtqglvag–COOH
226.	H_2N–srwrrryqsrvtqglvag–$CONH_2$
234.	H_2N–srwrryqsrvtqylvag–COOH
234.	H_2N–srwrryqsrvtqylvag–$CONH_2$
235.	H_2N–orwrryqsrvtqylvag–COOH
235.	H_2N–orwrryqsrvtqylvag–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 16: Engineered peptides based on CidA protein (pro-programmed cell death protein / *S. aureus*).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
236.	^{28}qkifhlplagsivglflfylllqfkiv^{54}
237.	^{88}eitlnyilffaviiigtcivalssgyiaekmsvkhkqrkgi^{127}
Synthetic Peptides	
236.	H_2N–qkifhlplagsivglflfylllqfkiv–COOH
236.	H_2N–qkifhlplagsivglflfylllqfkiv–$CONH_2$
238.	H_2N–qkifhlplabsivglflfylllqfkiv–COOH
238.	H_2N–qkifhlplabsivglflfylllqfkiv–$CONH_2$

SEQ ID NO:	Amino Acid Sequence
239.	H_2N–qkifhlplagsivglflfylglqfkiv–COOH
239.	H_2N–qkifhlplagsivglflfylglqfkiv–$CONH_2$
240.	H_2N–qkifhlplabsivglflfylglqfkiv–COOH
240.	H_2N–qkifhlplabsivglflfylglqfkiv–$CONH_2$
241.	H_2N–labsivglflfylglqfkiv–COOH
241.	H_2N–labsivglflfylglqfkiv–$CONH_2$
242.	H_2N–labsivblflfylglqfkiv–COOH
242.	H_2N–labsivblflfylglqfkiv–$CONH_2$
237.	H_2N–eitlnyilffaviiigtcivalssgyiaekmsvkhkqrkgi–COOH
237.	H_2N–eitlnyilffaviiigtcivalssgyiaekmsvkhkqrkgi–$CONH_2$
243.	H_2N–eitlnyilffaviiigtxivalssgyiaekxsvkhkqrkgi–COOH
243.	H_2N–eitlnyilffaviiigtxivalssgyiaekxsvkhkqrkgi–$CONH_2$
244.	H_2N–aekmsvkhkqrkgi–COOH
244.	H_2N–aekmsvkhkqrkgi–$CONH_2$
245.	H_2N–abkmsvkhkqrkgi–COOH
245.	H_2N–abkmsvkhkqrkgi–$CONH_2$
246.	H_2N–alkmsvkhkqrkgi–COOH
246.	H_2N–alkmsvkhkqrkgi–$CONH_2$
247.	H_2N–alkxsvkhkqrkgi–COOH
247.	H_2N–alkxsvkhkqrkgi–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 17: Engineered peptides based on LrgA protein (anti-programmed cell death protein / *S. aureus*).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
248.	^{123}kvtsrskgdkvtkkiki^{139}
Synthetic Peptides	
248.	H_2N–kvtsrskgdkvtkkiki–COOH
248.	H_2N–kvtsrskgdkvtkkiki–$CONH_2$
249.	H_2N–kvtsrskgdkvtkwiki–COOH
249.	H_2N–kvtsrskgdkvtkwiki–$CONH_2$
250.	H_2N–kvtsrskgdkvtkziki–COOH
250.	H_2N–kvtsrskgdkvtkziki–$CONH_2$
251.	H_2N–kvtsrskgdkvtkxiki–COOH
251.	H_2N–kvtsrskgdkvtkxiki–$CONH_2$

Underlined residues indicate substitutions; z = d, e, other anionic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 18: Engineered peptides based on Lambda S21 protein (lytic regulator protein / λ21 phage).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
252.	^{44}slvlgfltyltnlyfkiredrrkaarge^{71}
Synthetic Peptides	
252.	H_2N–slvlgfltyltnlyfkiredrrkaarge–COOH
252.	H_2N–slvlgfltyltnlyfkiredrrkaarge–$CONH_2$
253.	H_2N–slvlgfltyltnlyfkirbdrrkaarge–COOH
253.	H_2N–slvlgfltyltnlyfkirbdrrkaarge–$CONH_2$
254.	H_2N–slvlgfltyltnlyfkirebrrkaarge–COOH
254.	H_2N–slvlgfltyltnlyfkirebrrkaarge–$CONH_2$
255.	H_2N–slvlgfltyltnlyfkirxxrrkaarge–COOH
255.	H_2N–slvlgfltyltnlyfkirxxrrkaarge–$CONH_2$
256.	H_2N–lyfkirxxrrkaarg–COOH
256.	H_2N–lyfkirxxrrkaarg–$CONH_2$

Underlined residues indicate substitutions; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 19: Engineered peptides based on Holin protein (lytic regulatory protein / Enterobacteria λ phage).

SEQ ID NO:	Amino Acid Sequence
Native Sequence Domains	
257.	^{27}aylrgrynggaftktvi^{44}
258.	^{84}sigslikrfaakkagvedgrnq^{105}
259.	^{84}sigslikrfaakkagv^{100}
Synthetic Peptides	
257.	H_2N–aylrgrynggaftktvi–COOH
257.	H_2N–aylrgrynggaftktvi–$CONH_2$
260.	H_2N–oylrgrynggaftktvi–COOH
260.	H_2N–oylrgrynggaftktvi–$CONH_2$
258.	H_2N–sigslikrfaakkagvedgrnq–COOH
258.	H_2N–sigslikrfaakkagvedgrnq–$CONH_2$
261.	H_2N–sigslikrfaakkagvbdgrnq–COOH
261.	H_2N–sigslikrfaakkagvbdgrnq–$CONH_2$
262.	H_2N–sigslikrfaakkagvebgrnq–COOH
262.	H_2N–sigslikrfaakkagvebgrnq–$CONH_2$
259	H_2N–sigslikrfaakkagv–COOH
259.	H_2N–sigslikrfaakkagv–$CONH_2$
263.	H_2N–sigslikrfaxkkagv–COOH
263.	H_2N–sigslikrfaxkkagv–$CONH_2$

Underlined residues indicate substitutions; o = anthrylalanine or other non-natural amino acid; b = k, r, n, q, other natural / non-natural basic residue; x = s, t, y, other natural / non-natural H-bond donor/acceptor residue.

Table 20: Additional engineered peptides based on programmed cell death effector proteins.

Native Protein	SEQ ID NO:	Amino Acid Sequence
Human Bcl-xL	288	H_2N–SQSNRELVVDFLSYKLSQK–COOH
Human Bcl-xL	288	H_2N–SQSNRELVVDFLSYKLSQK–$CONH_2$
Human CTL Granulysin	289	H_2N–QKLKKMVDKPTQRSVSN–COOH
Human CTL Granulysin	289	H_2N–QKLKKMVDKPTQRSVSN–$CONH_2$

Table 21: Novel Therapeutic Peptide Designs Based on Programmed Cell Death Effector Domains.

ID Name	Sequence	SEQ ID NO:	Length	Priority
BaxP-I-18	H_2N–alfyfasklvlkalytkv–$CONH_2$	264.	18	1
CidA-II-12	H_2N–alkysvkhkqrkgi–$CONH_2$	265.	14	2
Ncl-VIII-6	H_2N–irlvskygkskgiayi–$CONH_2$	152.	16	3
Csp3-II-12	H_2N–lkkitnfrgkryrsltgk–$CONH_2$	266.	18	4
Dnm2-II-4	H_2N–alrsklqsqllslrk–$CONH_2$	267.	15	5
Dnm1-IV-2	H_2N–atvkkqvqklk–$CONH_2$	80.	11	6
BclXb-I-2	H_2N–flsyklsqkgyswsqfs–$CONH_2$	26.	17	7
Hol-III-4	H_2N–sigslikrfaykkagv–$CONH_2$	268.	16	8
Mfn1-II-2	H_2N–kiqnnskllrnkavql–$CONH_2$	64.	16	9
BclWP-I-4	H_2N–tralvakfvgyklrqkgyv–$CONH_2$	269.	19	10
LrgA-I-4	H_2N–kvtsrskgdkvtkwiki–$CONH_2$	249.	17	11
BaxP-I-1	H_2N–nfnwgrvvalfyfasklvlkalytkv–$CONH_2$	270.	26	
BaxP-II-8	H_2N–tvtifvakvltasltiwkk–$CONH_2$	271.	19	
BclWP-II-6	H_2N–trfrrtfsklaaqlhvt–$CONH_2$	272.	17	
BclXb-IV-10	H_2N–gqrsptalslylfllywvivk–$CONH_2$	273.	21	
Mfn1-II-20	H_2N–kkievlkslqskakllrnkagwl–$CONH_2$	274.	23	
Dnm1-III-4	H_2N–glrnklqsqllsikk–$CONH_2$	275.	15	
Dnm2-I-4	H_2N–klakkvdpqglrtigvitkl–$CONH_2$	276.	21	
Dnm2-VI-16	H_2N–kskkytlplknlkir–$CONH_2$	277.	15	
Csp3-I-10	H_2N–sksiknlkpkiiyks–$CONH_2$	278.	15	
CidA-I-12	H_2N–laksivrlflfylglqfkiv–$CONH_2$	279.	20	

EXAMPLE II

In Vitro Antimicrobial Assay

The following assay is designed to measure the relative antimicrobial activity of peptides by determining zones of growth inhibition.

The top eleven prioritized target sequences identified in Example I (see Table 21) were synthesized by solid-phase chain extension synthesis using conventional techniques. Each

synthetic peptide was purified by RP-HPLC, and authenticated for purity and correct sequence by mass spectroscopy. Stock concentrations of the synthetic peptides were prepared at 1 mg/mL in 0.01% acetic acid and adjusted to pH 7.2. Synthetic peptides were assessed for antimicrobial efficacy, spectra, and conditional optima (pH 5.5 or 7.5) using the following modified radial diffusion assay, as detailed in Yount and Yeaman, *PNAS* 1010:7363-7368 (2004).

Media preparation

Molecular grade agarose (1.0%) in 10 mM $NaH_2PO_4H_2O$ was prepared, pH adjusted to 7.5 or 5.5, and autoclaved for 15 minutes at 121°C, then held in a waterbath set at 48°C until used. Mueller Hinton II overlay agarose was prepared by adding molecular grade agarose to Mueller Hinton II Broth at a final concentration of 1.0%, pH adjusted to 7.5 or 5.5, autoclaved for 10 minutes at 121°C, and then held at 48°C until used.

Inoculum Preparation

Trypticase Soy Broth (TSB) (10mL) we inoculated with an overnight growth of the test organism and incubated three to six hours until the organism reached log phase. The cells were collected by centrifugation, washed in PBS, then 0.01% acetic acid adjusted to pH 7.2. The pellet was resuspended in TSB and standardized to a 0.5 McFarland turbidity standard. A 10µl aliquot of the inoculum is added to 10 mL of the pH-adjusted 1.0 % molecular grade agarose cooled to 48°C resulting in a final inoculum concentration of 5 x 10^5 CFU/mL. The suspension is poured into a 15 x 100 mm Petri dish and allowed to solidify.

After solidification had occurred, five 4 mm diameter wells were bored into the agarose. The central well was used as the acetic acid control while 10 µl of peptide stock solution was added to each of the other wells resulting in a final concentration of 10 µg peptide/well. The plates were incubated upright for three hours at 37°C, then overlaid with 10 mL of Mueller Hinton II agarose. After the overlay solidified, the plates were inverted and incubated overnight at 37°C.

Activity Determination

The synthetic peptides identified in Table 22 were assayed for antimicrobial activity against known pathogenic microorganisms. These pathogenic microorganisms included five species

of bacteria (*Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa and Bacillus subtilis*) and one species of fungi (*Candida albicans*) (see Table 23). Zones of growth inhibition were measured and were grouped as complete and/or partial growth zones for the assayed microorganism. Zones were considered complete clearance when there was no visible growth (i.e. completely clear or free of growth). Zones were considered partial clearance when growth was impeded or partially cleared (i.e. reduction in microbial density as compared to adjacent confluent growth). The larger the zone size, the greater the antimicrobial activity of the test peptide. The lack of a zone is an indication of no antimicrobial activity of the test peptide against the target organism under the conditions tested.

Table 22: Peptide key for antimicrobial assay (Figures 30-35).

Template	Identifier *	Design Sequence	SEQ ID NO:	Length
Holin protein	Hol-III-4	H_2N–sigslikrfaykkagv–$CONH_2$	268.	16
Dynamin-2	Dnm2-II-4	H_2N–alrsklqsqllslrk–$CONH_2$	267.	15
BclW protein	BclWP-I-4	H_2N–tralvakfvgyklrqkgyv–$CONH_2$	269.	19
Caspase-3	Csp3-II-12	H_2N–lkkitnfrgkryrsltgk–$CONH_2$	266	18
LrgA protein	LrgA-I-4	H_2N–kvtsrskgdkvtkwiki–$CONH_2$	249.	17
Dynamin-1	Dnm1-IV-2	H_2N–atvkkqvqklk–$CONH_2$	80.	11
BclXb protein	BclXb-I-2	H_2N–flsyklsqkgyswsqfs–$CONH_2$	26.	17
Nucleolin	Ncl-VIII-6	H_2N–irlvskygkskgiayi–$CONH_2$	152.	16
Mitofusin-1	Mfn1-II-2	H_2N–kiqnnskllrnkavql–$CONH_2$	64.	16
Bax protein	BaxP-I-18	H_2N–alfyfasklvlkalytkv–$CONH_2$	264.	18
CidA protein	CidA-II-12	H_2N–alkysvkhkqrkgi–$CONH_2$	265.	14

*Note: identifier formula = [Template]–[Model Domain]–[Design No.]

Table 23: Microorganism key for antimicrobial assay (Figures 30-35).

Genus / Species	Identifier	Strain
Bacteria		
Staphylococcus aureus	SAISP479C	ISP479C
Escherichia coli	ECML-35	ML-35
Salmonella typhimurium	ST14028	14028
Pseudomonas aeruginosa	PA01	01
Bacillus subtilis	BS6633	ATCC 6633
Fungi		
Candida albicans	CA36082S	36082S

Results

85% (46/54) of all peptide and microorganism combinations tested at neutral pH showed antimicrobial activity (Figure 30, 32 and 34). The majority of peptides also maintained antimicrobial activity under acidic conditions, i.e. pH 5.5 (Figures 31, 33 and 35), albeit at a reduced level. The acidic pH reduced the antimicrobial activity of all peptides when tested against *Staphylococcus aureus*. Additionally, 64% (7/11), 73% (8/11) and 73% (8/11) of the peptides showed lesser antimicrobial activity when tested against *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium*, respectively (Figures 31, 33 and 35) at pH 5.5 versus pH 7.5. However, the acidic conditions did not appear to affect the activity of peptides Hol-III-4 (SEQ ID NO:268), Ncl-VIII-6 (SEQ ID NO:152) or BaxP-I-18 (SEQ ID NO:264) against *Bacillus subtilis, Escherichia coli, Salmonella typhimurium,* and *Pseudomonas aeruginosa*. Alternatively, the antimicrobial activity was increased for 82% (9/11) of the peptides when tested against the fungal pathogen *Candida albicans* under acidic conditions. Notably, all peptides assayed showed significant and consistently high antimicrobial activity against *Bacillus subtilis* regardless of the acidity of the agarose media (Figures 30-35, lane designation BS6633).

The above results show that the designed peptides exerted consistent *in vitro* efficacy against *Bacillus*. These results supports the concept that these molecules exploit a targetable evolutionary relationship between prokaryotic organisms and eukaryotic mitochondria. Evidence underscoring the bacterium-to-mitochondrial evolution is consistent with this concept (see Herrmann, *TRENDS Micro* 11(2):74-79 (2003)). For example, proteins that are believed to mediate ion-permeability transition in mitochondria are likely to have evolved from membrane targeting motifs such as helical pre-sequences present in ancestral Gram-positive organisms such as *Bacillus*, or other prokaryotes (see von Heijne, *EMBO Journal* 5:1335-1342 (1986)). Such molecules can target mitochondria, are often comprised of 20-60 amino acids, have the potential to form amphipathic α-helices that segregate hydrophobic and hydrophilic facets, and have one facet that is positively charged. The peptides described herein are highly consistent with such molecules. Therefore, the peptides described herein which have antimicrobial activity will also have anti-cancer, anti-inflammatory, anti-rheumatologic and other efficacy by virtue of their likelihood to target mitochondria and induce or cause dysfunctions in programmed cell death circuits.

EXAMPLE III

Antimicrobial Activity against *Pseudomonas aeruginos* and *Acinetobacter* spp. strains

Further to the methods disclosed in Example II, utilizing the same antimicrobial assay above, the antimicrobial activity of peptides Hol-III-4 (SEQ ID NO: 268) and Ncl-VIII-6 (SEQ ID NO: 152 was determined by identifying zones of growth inhibition.

Hol-III-4 and Ncl-VIII-6 peptides were assayted for antimicrobial activity against a panel of drug-resistant Gram-negative bacterial pathogens, specifically *Pseudomonas aeruginosa*, and various *Acinetobacter spp. strains*. The efficacies of these peptides were tested in the context of pH 5.5 (Firgures 36-46) and pH 7.5 (Figures 47-57) conditions, and compared with other peptides known to have antimicrobial acivity (e.g. RP-1, 6W-RP-1 (a 6-Trp variant of RP-1), IK, and PMP-2), in the radial diffusion assay. The RP-1 peptide is well known in the art to have antimicrobial activity, as illustrated in Yeaman et al., *Antimicrobial Agents and Chemotherapy*, 46(12):3883-3891 (2002). The 6W-RP-1 peptide is a 6-Trp variant of RP-1, which also has antimicrobial activity, as illustrated in Kilelee et al, *Antimicrobial Agents and Chemotherapy* 54(10):4476-4479 (2010). PMP is the C-terminal helix of the consensus molecule cPMP, as shown in Table 1 (bottom row; N—AALYKKKIIKKLLES—C; as shown in Yeaman et al., *Bichimica et Biophysica Acta*, 1768:609-619 (2007). The IK peptide is designed to have a nearly maximal polar angle (maximum angle is approximantely 180°). The results of the antimicrobial assay show that both Hol-III-4 and Ncl-VIII-6 consistently showed significant antimicrobial activity against all *Pseudomonas aeruginosa*, and all *Acinetobacter spp.* strains tested. Additionally, the antimicrobial activity of both Hol-III-4 and Ncl-VIII-6 agaist the various *Acinetobacter baumannii* isolates tested appeared to be pH dependent (see Figures 39, 40, 43-46 vs. Figures 50, 51, 54-57)

These results expand on and further substantiate the results shown in Example II regarding the antimicrobial efficacy of the peptides disclosed here. Additionally, the current data supports the conclusion that the PCD peptides may have a unique mechanism of action, but achieve generally equivalent efficacy as RP-1-like peptides against most organisms tested. The is evident by the following observations. The RP-1 and related antimicrobial peptides appear to target microbial cells enriched with electronegative constituents (e.g. phosphatidylglycerol, cardiolipin, etc.), and/or those having electronegative transmembrane potential. These features, in addition to conformational plasticity, inhibition of intracellular functions (e.g. macromolecular synthesis) and possibly superstructural assembly, are believed

to participate in the preferential microbial targeting and antimicrobial effects of such peptides. By comparison, the current peptides are designed from programmed cell death effector or modulating proteins. Without being bound by theory, one hypothesis is that such peptides induce microbial and other target cell death by inducing or dysregulating programmed cell death. Thus, it is possible that these peptides function via a mechanism that is not identical to the RP-1-like peptides. In balance, it is also possible that PCD peptides evolved (e.g. diverged) from antimicrobial peptide sequences, based on the view that mitochondria are modern day "bacteria"; this is one hypothesis we posed in the patent. If so, then the mechanisms may be conserved among antimicrobial helices, even if the helices come from proteins believed to have vastly divergent functions (e.g. helices from PMPs, PCD proteins, chemokines, etc).

Throughout this application various publications have been referenced. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains. Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

1. An isolated peptide comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263, wherein the amino acid residue represented by (x) is a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue, wherein the amino acid residue represented by (b) is a lysine, an arginine, an asparagine, a glutamine or a basic residue, wherein the amino acid residue represented by (j) is a cysteine or a thiol residue, wherein in the amino acid residue represented by (o) is an anthrylalanine or other non-natural amino acid and wherein said peptide induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

2. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 264, 270 and 271.

3. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 17, 18, 19, 21-25, 269 and 272.

4. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 30, 31-36 and 273.

5. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 39-47.

6. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 49-52.

7. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 54-57.

8. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 59-63 and 274.

9. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 66-75.

10. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 84-93 and 275.

11. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 102-106, 108-121, 267, 276 and 277.

12. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 132-175.

13. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 179-187, 266 and 278.

14. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NO: 191-199.

15. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 205-209 and 211-223.

16. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 227-235.

17. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 238-243, 245-247, 265 and 279.

18. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 249-251.

19. The isolated protein of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 253-256.

20. The isolated peptide of claim 1 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 260-263 and 268.

21. An isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9, 12, 14-16, 20, 26-29, 37, 38, 48, 53, 58, 64, 65, 72, 76-83, 94-101, 107, 114, 122-131, 170, 176-178, 188-190, 200-204, 210, 224-226, 236, 237, 244, 248, 252, 257-259 and 288-289, wherein said peptide induces

antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

22. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9 and 12.

23. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 14-16 and 20.

24. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-29.

25. The isolated peptide of claim 21 consisting of an amino acid sequence selected from SEQ ID NOS: 37 or 38.

26. The isolated peptide of claim 21 consisting of an amino acid sequence of SEQ ID NO: 48.

27. The isolated peptide of claim 21 consisting of an amino acid sequence of SEQ ID NO: 53.

28. The isolated peptide of claim 21 consisting of an amino acid sequence selected from SEQ ID NOS: 58 or 64.

29. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 65 or 72.

30. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 76-83.

31. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 94-101, 107 and 114.

32. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 122-131 and 170.

33. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 176-178.

34. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 188-190.

35. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 200-204 and 210.

36. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 224-226.

37. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 236, 237 and 244.

38. The isolated peptide of claim 21 consisting of an amino acid sequence of SEQ ID NO: 248.

39. The isolated peptide of claim 21 consisting of an amino acid sequence of SEQ ID NO: 252.

40. The isolated peptide of claim 21 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOS: 257-259 and 288-289.

41. The isolated peptide of any one of claims 1-40, wherein the C-terminus of the peptide comprises a carboxamide.

42. A context-activated protide comprising at least one activator site and two or more effectors, wherein at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263, wherein the amino acid residue represented by (x) is a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue, wherein the amino acid residue represented by (b) is a lysine, an arginine, an asparagine, a glutamine or a basic residue, wherein the amino acid residue represented by (j) is a cysteine or a thiol residue, wherein the amino acid residue represented by (o) is an anthrylalanine or other non-natural amino acid and wherein said at least one effector induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

43. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 264, 270 and 271.

44. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 17, 18, 19, 21-25, 269 and 272.

45. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 30, 31-36 and 273.

46. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 39-47.

47. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 49-52.

48. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 54-57.

49. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 59-63 and 274.

50. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 66-75.

51. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 84-93 and 275.

52. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 102-106, 108-121, 267, 276 and 277.

53. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 132-175.

54. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 179-187, 266 and 278.

55. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NO: 191-199.

56. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 205-209 and 211-223.

57. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 227-235.

58. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 238-243, 245-247, 265 and 279.

59. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 249-251.

60. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 253-256.

61. The protide of claim 42 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 260-263 and 268.

62. A context-activated protide comprising at least one activator site and two or more effectors, wherein at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9, 12, 14-16, 20, 26-29, 37, 38, 48, 53, 58, 64, 65, 72, 76-83, 94-101, 107, 114, 122-131, 170, 176-178, 188-190, 200-204, 210, 224-226, 236, 237, 244, 248, 252, 257-259 and 288-289, wherein said at least one effector induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

63. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9 and 12.

64. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 14-16 and 20.

65. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-29.

66. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from SEQ ID NOS: 37 or 38.

67. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence of SEQ ID NO: 48.

68. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence of SEQ ID NO: 53.

69. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from SEQ ID NOS: 58 or 64.

70. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 65 or 72.

71. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 76-83.

72. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 94-101, 107 and 114.

73. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 122-131 and 170.

74. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 176-178.

75. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 188-190.

76. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 200-204 and 210.

77. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 224-226.

78. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 236, 237 and 244.

79. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence of SEQ ID NO: 248.

80. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence of SEQ ID NO: 252.

81. The protide of claim 62, wherein said at least one effector comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 257-259 and 288-289.

82. The protide of any one of claims 42-81, wherein said activator site is context-activated.

83. The protide of any one of claims 42-81, wherein context-activation initiates programmed cell death.

84. The protide of any one of claims 42-81, wherein said context-activation results from a physiological condition.

85. The protide of claim 84, wherein said physiological condition is selected from the group consisting of acidity, alkalinity, ionic strength and osmotic strength.

86. The protide of any one of claims 42-81, wherein said context-activation results from association with an activator molecule.

87. The protide of claim 86, wherein said activator molecule modifies said activator site upon association.

88. The protide of claim 87, wherein said modification comprises cleavage of said activator site.

89. The protide of claim 86, wherein said activator molecule is an enzyme.

90. The protide of claim 89, wherein said activator molecule is selected from the group consisting of protease, esterase and lipase.

91. The protide of claim 90, wherein said activator molecule is a protease.

92. The protide of claim 91, wherein said activator is expressed by a pathogenic microorganism.

93. The protide of claim 92, wherein said pathogenic microorganism is *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Bacillus subtilis* or *Candida albicans*.

94. The protide of claim 86, wherein said activator is present in the context of tumor cells.

95. The protide of claim 94, wherein said activator is a tumor-specific protease.

96. The protide of claim 95, wherein said tumor-specific protease is matrix bound.

97. The protide of claim 96, wherein said tumor-specific protease is a matrix metalloproteinase.

98. The protide of claim 86, wherein said activator is present in the context of an inflammatory response.

99. The protide of claim 86, wherein said activator is a peptide selected from the group consisting of thrombin, bradykinin, elastase and metalloproteinase.

100. A conjugate comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 17, 18, 19, 21-25, 30, 31-36, 39-47, 49-52, 54-57, 59-63, 66-75, 84-93, 102-106, 108-121, 132-175, 179-187, 191-199, 205-209, 211-223, 227-235, 238-243, 245-247, 249-251, 253-256 and 260-263 and a moiety, wherein the amino acid residue represented by (x) is a serine, a threonine, a tryptophan, a H-bond donor residue or a H-bond acceptor residue, wherein the amino acid residue represented by (b) is a lysine, an arginine, an asparagine, a glutamine or a basic residue, wherein the amino acid residue represented by (j) is a cysteine or a thiol residue, wherein in the amino acid residue represented by (o) is an anthrylalanine or other non-natural amino acid and wherein said conjugate induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

101. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 4, 6, 8, 10, 11, 13, 264, 270 and 271.

102. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 17, 18, 19, 21-25, 269 and 272.

103. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 30, 31-36 and 273.

104. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 39-47.

105. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 49-52.

106. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 54-57.

107. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 59-63 and 274.

108. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 66-75.

109. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 84-93 and 275.

110. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 102-106, 108-121, 267, 276 and 277.

111. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 132-175.

112. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 179-187, 266 and 278.

113. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NO: 191-199.

114. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 205-209 and 211-223.

115. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 227-235.

116. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 238-243, 245-247, 265 and 279.

117. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 249-251.

118. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 253-256.

119. The conjugate of claim 100 comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 260-263 and 268.

120. A conjugate comprising one or more amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9, 12, 14-16, 20, 26-29, 37, 38, 48, 53, 58, 64, 65, 72, 76-83, 94-101, 107, 114, 122-131, 170, 176-178, 188-190, 200-204, 210, 224-226, 236, 237, 244, 248, 252, 257-259 and 288-289 and a moiety, wherein said conjugate induces antimicrobial, anti-cancer, anti-inflammatory, anti-proliferative or programmed cell death activity.

121. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 2, 5, 7, 9 and 12.

122. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 14-16 and 20.

123. The conjugate of claim 120 comprising of an amino acid sequence selected from the group consisting of SEQ ID NOS: 26-29.

124. The conjugate of claim 120 comprising an amino acid sequence selected from SEQ ID NOS: 37 or 38.

125. The conjugate of claim 120 comprising an amino acid sequence of SEQ ID NO: 48.

126. The conjugate of claim 120 comprising an amino acid sequence of SEQ ID NO: 53.

127. The conjugate of claim 120 comprising an amino acid sequence selected from SEQ ID NOS: 58 or 64.

128. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 65 or 72.

129. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 76-83.

130. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 94-101, 107 and 114.

131. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 122-131 and 170.

132. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 176-178.

133. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 188-190.

134. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 200-204 and 210.

135. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 224-226.

136. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 236, 237 and 244.

137. The conjugate of claim 120 comprising an amino acid sequence of SEQ ID NO: 248.

138. The conjugate of claim 120 comprising an amino acid sequence of SEQ ID NO: 252.

139. The conjugate of claim 120 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 257-259 and 288-289.

140. The conjugate of any one of claims 100-139, wherein said moiety comprises a therapeutic agent, a targeting peptide or a label.

141. The conjugate of claim 140, wherein said therapeutic agent is a cytotoxic agent.

142. The conjugate of claim 141, wherein said cytotoxic agent is an antibiotic.

143. The conjugate of claim 140, wherein said therapeutic agent is a chemotherapeutic agent.

144. The conjugate of claim 140, wherein said targeting peptide selectively homes said conjugate to a microorganism.

145. The conjugate of claim 140, wherein said targeting peptide selectively homes said conjugate to tumor tissue, tumor cell, or tumor vasculature.

146. The conjugate of claim 140, wherein said targeting peptide selectively homes said conjugate to an immune regulatory cell or an immune effector cell.

147. The conjugate of claim 140, wherein said targeting peptide is an antibody or a fragment thereof.

148. The conjugate of claim 140, wherein said label is a radioisotope.

149. The conjugate of claim 140, wherein said label is a dye.

150. A method of inducing programmed cell death in a cell, comprising contacting said cell with the isolated peptide of any one of claims 1-40.

151. The method of claim 150, wherein said cell is a microorganism.

152. The method of claim 151, wherein said microorganism is a pathogenic microorganism.

153. The method of claim 152, wherein said pathogenic microorganism is selected from the group consisting of *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus subtilis, Acinotobacter baumannii, Acinotobacter calcoaceticus, Acinotobacter haemolyticus, Pseudomonas aeruginosa* and *Candida albicans*.

154. The method of claim 150, wherein said cell is a tumor cell.

155. The method of claim 154, wherein said tumor cell is a malignant tumor cell.

156. The method of claim 150, wherein said cell is an immune regulatory cell or an immune effector cell.

157. A method of inducing programmed cell death in a cell, comprising contacting said cell with the protide of any one of claims 62-81.

158. The method of claim 157, wherein said cell is a microorganism.

159. The method of claim 158, wherein said microorganism is a pathogenic microorganism.

160. The method of claim 159, wherein said pathogenic microorganism is selected from the group consisting of *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Bacillus subtilis, Acinotobacter baumannii, Acinotobacter calcoaceticus, Acinotobacter haemolyticus, Pseudomonas aeruginosa* and *Candida albicans*.

161. The method of claim 157, wherein said cell is a tumor cell.

162. The method of claim 161, wherein said tumor cell is a malignant tumor cell.

163. The method of claim 157, wherein said cell is an immune regulatory cell or an immune effector cell.

164. A method of inducing programmed cell death in a cell, comprising contacting said cell with the conjugate of any one of claims 100-139.

165. The method of claim 164, wherein said cell is a microorganism.

166. The method of claim 165, wherein said microorganism is a pathogenic microorganism.

167. The method of claim 166, wherein said pathogenic microorganism is selected from the group consisting of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Bacillus subtilis, Acinotobacter baumannii, Acinotobacter calcoaceticus, Acinotobacter haemolyticus, Pseudomonas aeruginosa* and *Candida albicans*.

168. The method of claim 164, wherein said cell is a tumor cell.

169. The method of claim 168, wherein said tumor cell is a malignant tumor cell.

170. The method of claim 164, wherein said cell is an immune regulatory cell or an immune effector cell.

Human Holin-Like Proteins

Dnm-1 (dynamin-1 / pro-programmed cell death [apoptotic] holin-like protein)

```
  1   mgnrgmedli plvnrlqdaf saigqnadld lpqiavvggq sagkssvlen fvgrdflprg
 61   sgivtrrplv lqlvnattey aeflhckgkk ftdfeevrle ieaetdrvtg tnkgispvpi
121   nlrvysphvl nltlvdlpgm tkvpvgdqpp diefqirdml mqfvtkencl ilavspansd
181   lansdalkva kevdpqgqrt igvitkldlm degtdardvl enkllplrrg yigvvnrsqk
241   didgkkdita alaaerkffl shpsyrhlad rmgtpylqkv lnqqltnhir dtlpglrnkl
301   qsqllsieke veeyknfrpd dparktkall qmvqqfavdf ekriegsgdq idtyelsgga
361   rinrifherf pfelvkmefd ekelrreisy aiknihgirt glftpdlafe atvkkqvqkl
421   kepsikcvdm vvseltatir kcseklqqyp rlreemeriv tthireregr tkeqvmllid
481   ielaymntnh edfigfanaq qrsnqmnkkk tsgnqdeilv irkgwltinn igimkggske
541   ywfvltaenl swykddeeke kkymlsvdnl klrdvekgfm sskhifalfn teqrnvykdy
601   rqlelacetq eevdswkasf lragvyperv gdkekasete engsdsfmhs mdpqlerqve
661   tirnlvdsym aivnktvrdl mpktimhlmi nntkefifse llanlyscgd qntlmeesae
721   qaqrrdemlr myhalkeals iigdintttv stpmpppvdd swlqvqsvpa grrsptsspt
```

Bax (pro-apoptotic holin-like molecule from humans / apoptosis pathway) **1F16**

```
  1   mdgsgeqprg ggptsseqim ktgalllqgf iqdragrmgg eapelaldpv pqdastkkls
 61   eclkrigdel dsnmelqrmi aavdtdspre vffrvaadmf sdgnfnwgrv valfyfask
121   vlkalctkvp elirtimgwt ldflrerllg wiqdqggwdg llsyfgtptw qtvtifvagv
181   ltasltiwkk mg
```

Bcl-2 (anti-apoptotic holin-like protein from humans / apoptosis pathway) / **1G5M**

```
  1   mahagrtgyd nreivmkyih yklsqrgyew dagddveenr teapegtese vvhlalrqag
 61   ddfsrryrgd faemssqlhl tpftargrfa tvveelfrdg vnwgrivaff efggvmcves
181   vnremsplvd nialwmteyl nrhlhtwiqd nggwdafvel ygpsmx
```

Figure 1

S. aureus Holin-Like Proteins

CidA (pro-programmed cell death [apoptotic] holin-like protein)

```
  1   mhkvqliikl llqlgiiivi tyigteiqki fhlplagsiv glflfylllq fkivpltwve
 61   dganfllktm vfffipsvvg imdvaseitl nyilffavii igtcivalss gyiaekmsvk
121   hkqrkgiday e
```

LrgA (anti-programmed cell death [anti-apoptotic] protein)

```
  1   mvvkqqkdas kpahffhqvi vialvlfvsk iiesfmpipm pasviglvll fvllctgavk
 61   lgevekvgtt ltnnigllfv pagisvvnsl gvisqapfli igliivstil llictgyvtq iimkvtsrsk
121   gdkvtkkiki eeaqahd
```

Figure 2

>PERFORIN; PFN1_BOS
DTQRFLRPDGTCTLCRNALQKDVLQRLPLAITDWRAHGAGCKRRVVKLEGRSTF
DVAGEAANRIRNDWQVGLDVSPKPNANVRVTVAGSHSEDANFAAQKTHQDNY
RFSMDLVECRFYSFHLVHTPPVHPEFKRALKTLPPHFNTSTKPDYHRLISSYGTHF
IRSMELGGRISALTALRTCELALEGLTASEVEDCLAVEAEVSISDRASASPSFKAC
EEKKKNHKVGTSFHQAYRERHSNVDGGHHSTMHDLLFGSQAGPEQFSAWVASL
QDSPGLVDYTLEPLHMLVESQDPRREALRQAVSKYVTD

>BCL-2:BCL2_HUMAN
MAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQP
GHTPHPAASRDPVARTSPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRR
YRRDFAEMSSQLHLTPFTARGRFATVVEELFRDGVNWGRIVAFFEFGGVMCVES
VNREMSPLVDNIALWMTEYLNRHLHTWIQDNGGWDAFVELYGPSMRPLFDFSW
LSLKTLLSLALVGACITLGAYLGHK

>BCL-W:BCLW_HUMAN
MATPASAPDTRALVADFVGYKLRQKGYVCGAGPGEGPAADPLHQAMRAAGDE
FETRFRRTFSDLAAQLHVTPGSAQQRFTQVSDELFQGGPNWGRLVAFFVFGAAL
CAESVNKEMEPLVGQVQEWMVAYLETRLADWIHSSGGWAEFTALYGDGALEE
ARRLREGNWASVRTVLTGAVALGALVTVGAFFASK

Figure 3

Scores Table

Sort by | Alignment Score | View Output File

SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
3	BCL-2_BCL2_HUMAN	239	4	BCL-W_BCLW_HUMAN	193	43
5	AZUROCIDIN-1_AZN1_HUMAN	251	9	GRANZYME	246	29
1	PERFORIN1_PRF1_HUMAN	832	4	BCL-W_BCLW_HUMAN	193	22
4	BCL-W_BCLW_HUMAN	193	6	BAX-Beta_BAXb_HUMAN	218	17
3	BCL-2_BCL2_HUMAN	239	6	BAX-Beta_BAXb_HUMAN	218	16
8	GRANULYSIN_	129	12	CYTOPLASMIN	376	14
1	PERFORIN1_PRF1_HUMAN	832	6	BAX-Beta_BAXb_HUMAN	218	12
5	AZUROCIDIN-1_AZN1_HUMAN	251	8	GRANULYSIN_	129	12
5	AZUROCIDIN-1_AZN1_HUMAN	251	10	CXCL3	397	11
1	PERFORIN1_PRF1_HUMAN	832	2	PERFORIN_	309	10
1	PERFORIN1_PRF1_HUMAN	832	8	GRANULYSIN_	129	10
8	GRANULYSIN_	129	9	GRANZYME	246	10
1	PERFORIN1_PRF1_HUMAN	832	10	CXCL3	397	9
1	PERFORIN1_PRF1_HUMAN	832	11	FAS	281	9
4	BCL-W_BCLW_HUMAN	193	12	CYTOPLASMIN	376	9
2	PERFORIN_	309	10	CXCL3	397	8
7	CASPASE	303	8	GRANULYSIN_	129	8
8	GRANULYSIN_	129	11	FAS	281	8
1	PERFORIN1_PRF1_HUMAN	832	3	BCL-2_BCL2_HUMAN	239	7
1	PERFORIN1_PRF1_HUMAN	832	7	CASPASE	303	7
1	PERFORIN1_PRF1_HUMAN	832	12	CYTOPLASMIN	376	7
2	PERFORIN_	309	4	BCL-W_BCLW_HUMAN	193	7
2	PERFORIN_	309	6	BAX-Beta_BAXb_HUMAN	218	7
3	BCL-2_BCL2_HUMAN	239	10	CXCL3	397	7
9	GRANZYME	246	10	CXCL3	397	7
10	CXCL3	397	11	FAS	281	7
1	PERFORIN1_PRF1_HUMAN	832	9	GRANZYME	246	6
2	PERFORIN_	309	8	GRANULYSIN_	129	6
3	BCL-2_BCL2_HUMAN	239	12	CYTOPLASMIN	376	6
4	BCL-W_BCLW_HUMAN	193	8	GRANULYSIN_	129	6
6	BAX-Beta_BAXb_HUMAN	218	8	GRANULYSIN_	129	6
6	BAX-Beta_BAXb_HUMAN	218	11	FAS	281	6

Figure 4

```
BCL-2_BCL2_HUMAN  ----------FTARGRFATWWEELFRDG---------- 141
BCL-W_BCLW_HUMAN  ----------GSAQQRFTQWSDELFQGG---------- 90
BAX-Beta_BAXb_HUMAN ----------SPREVFFRVAADMFSDGN---------- 104
PERFORIN1_PRF1_HUMAN EEVYKRPLFLQPTYRYHRLPLPEQGSPLEAQLDAFVSVLRETPSLLQLRD 300
GRANULYSIN_       ----------EGPQGDLITKTQELGRDYR---------- 51
CYTOPLASMIN       ----------EVRAQLLELPYARKELSLILVLLPDDG---------- 236
PERFORIN_         ----------LISSYGTHFIRSMELGGRIS---------- 173
AZUROCIDIN-1_AZN1_HUMAN ----------LQNATVEAGTRCQVAGWGSQ---------- 157
GRANZYME          ----------SSKAQVKPGQLCSVAGWGYV---------- 150
CXCL3             ----------SAPHQPGPSLWAEAKTSEAP---------- 219
CASPASE           ----------KDGVTPIKDLTAHFRGDRCKT---------- 173
FAS               ----------PPPEKKELRKVAHLTGKSN---------- 154

BCL-2_BCL2_HUMAN  ----------VNWGRIVAFFEFGGVMCVESVNREMSPL 169
BCL-W_BCLW_HUMAN  ----------PNWGRLVAFFVFGAALCAESVNKEMEPL 118
BAX-Beta_BAXb_HUMAN ----------FNWGRVVALFYFASKLVLKALCTKVPEL 132
PERFORIN1_PRF1_HUMAN AHGPPPALVFSCQMGVGRTNLGMVLGTLILLHRSGTTSQPEAAPTQAKPL 350
GRANULYSIN_       ----------TCLTIVQKLKKMWDKPTQRSVSNAATRW 79
CYTOPLASMIN       ----------WELSTVEKSLTFEKLTAWTKPDCMKSTEVEW 267
PERFORIN_         ----------ALTALRTCELALEGLTASEVEDCLAVEAEVS 204
AZUROCIDIN-1_AZN1_HUMAN ----------RSGGRLSRFPRFVNVTVTPEDQCRPNNV 185
GRANZYME          ----------SMSTLATTLQEVLLTVQKDCQCERL 175
CXCL3             ----------STQDPSTQASTASSPAPEENAPSEGQRW 247
CASPASE           ----------LLEKPKLFFIQACRGTELDDGIQADSGPI 202
FAS               ----------SRSMPLEWEDTYGIVLLSGVKYKKGGL 181

BCL-2_BCL2_HUMAN  VDNIALWMTEYLNRH---------- 184
BCL-W_BCLW_HUMAN  VGQWQEWMVAYLETR---------- 133
BAX-Beta_BAXb_HUMAN IRTIMGWTLDFLRER---------- 147
PERFORIN1_PRF1_HUMAN PMEQFQVIQSFLRMVPQGRRMVEEVDRAITACAELHDLKEVVLENQKKLE 400
GRANULYSIN_       CRTGRSRWRDVCRNF---------- 94
CYTOPLASMIN       LLPKFKLQEDYDMESVLRHLG---------- 288
PERFORIN_         ISDRASASPSFKACEEKKKNHKVG---------- 228
AZUROCIDIN-1_AZN1_HUMAN CTGVLTRRGGICNGD---------- 200
GRANZYME          FHGNYSRATEICVGDPKKTQTGFK---------- 199
CXCL3             WGQGQSPRPENSLEREEMGPVPAHT---------- 272
CASPASE           NDTDANPRYKIPVEADFLFAYS---------- 224
FAS               VINETGLYFVYSKVYFRGQSCNNLP---------- 206
```

Figure 5

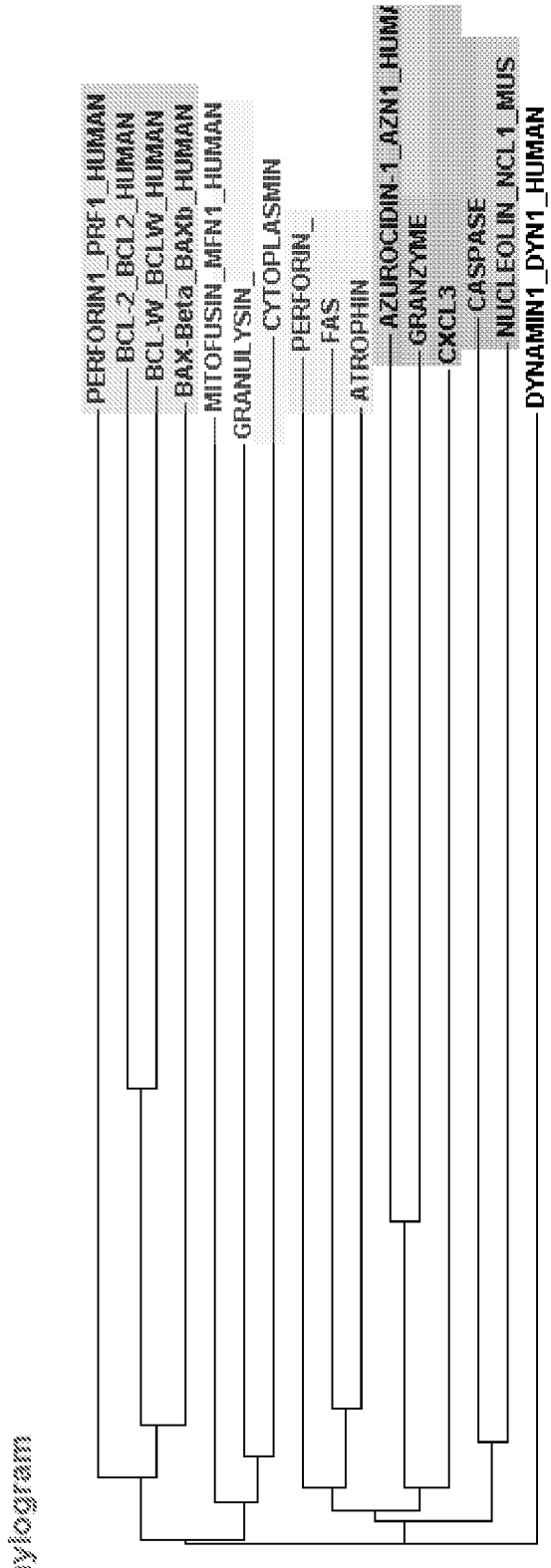

Phylogram
PERFORIN1_PRF1_HUMAN
BCL-2_BCL2_HUMAN
BCL_W_BCLW_HUMAN
BAX-Beta_BAXb_HUMAN
MITOFUSIN_MFN1_HUMAN
GRANULYSIN_
CYTOPLASMIN
PERFORIN_
FAS
ATROPHIN
AZUROCIDIN-1_AZN1_HUMA
GRANZYME
CXCL3
CASPASE
NUCLEOLIN_NCL1_MUS
DYNAMIN1_DYN1_HUMAN

Figure 6

430
440
450
460
470
480
490
500
510
BCL-2 BCL2 HUMAN/ 1-1190
BCL-W BCLW HUMAN/ 1-1190
BAX-Beta BAXb HUMAN/ 1-1190
PERFORIN1 PRF1 HUMAN/ 1-1190
GRANZYME /1-1190
MITOFUSIN MFN1 HUMAN/ 1-1190
CYTOPLASMIN/ 1-1190
DYNAMIN1 DYN1 HUMAN/ 1-1190
ATROPHIN/ 1-1190
FAS/ 1-1190
PERFORIN /1-1190
CXCL2/ 1-1190
AZUROCIDIN-1 AZN1 HUMAN/ 1-1190
GRAMZYME/ 1-1190
NUCLEOLIN NCL1 HUMAN/ 1-1190
CASPASE/ 1-1190
Quality/ 1-1190

Figure 7

Figure 8

570
580
590
600
610
620
630
640
650
BCL-2 BCL2 HUMAN/1-1190
BCL-G BCLG HUMAN/1-1190
BAX-Beta BAXB HUMAN/1-1190
FIBROBLAST FBT1 HUMAN/1-1190
GRANZYME B / 1-1190
MITOFUSIN MFN1 HUMAN/1-1190
CYTOPLASMIC/1-1190
DYNAMIN1 DYN1 HUMAN/1-1190
ATROPHIN/1-1190
FAS/1-1190
PERFORIN / 1-1190
CXCL3/1-1190
ASUBOCIDIN-1 ASN1 HUMAN/1-1190
GRANZYME/1-1190
NUCLEOLIN NCL1 HUS/1-1190
CASPASE/1-1190
Quality/1-1190

Figure 9

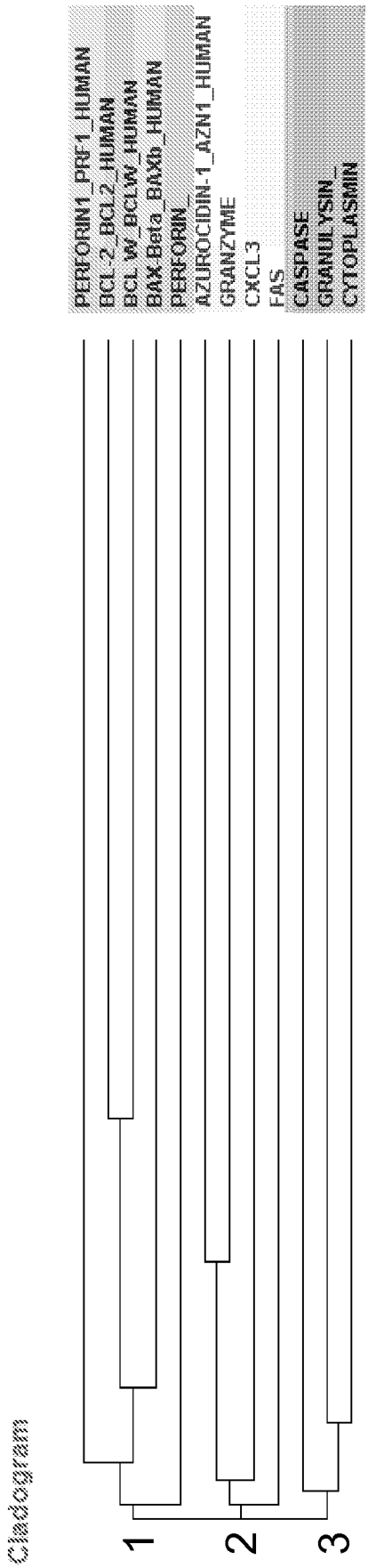

PERFORIN1_PRF1_HUMAN
BCL-2_BCL2_HUMAN
BCL-W_BCLW_HUMAN
BAX Beta_BAXb_HUMAN
PERFORIN
AZUROCIDIN-1_AZN1_HUMAN
GRANZYME
CXCL3
FAS
CASPASE
GRANULYSIN
CYTOPLASMIN
Cladogram
1
2
3

Figure 10

180
190
200
210
220
230
BCL-2 BCL2 HUMAN/ 1-832
BCL-W BCLW HUMAN/ 1-832
BAX-Beta BAXB HUMAN/ 1-832
PTPROIM1 PRT1 HUMAN/ 1-832
GRAMULYSIN /1-832
CYTOPLASMIN/ 1-832
PERFORIN /1-832
AZUROCIDIN-1 AZM1 HUMAN/ 1-832
GRANZYME/ 1-832
CXCL3/ 1-832
CASPASE/ 1-832
FAS/ 1-832
Quality/ 1-832

Figure 11

260
270
280
290
300
310
BCL-2 ECL2 HUMAN/ 1-822
BCL-W BCL2 HUMAN/ 1-822
BAX-Beta BAX HUMAN/ 1-822
PERFORIN1 PRF1 HUMAN/ 1-822
GRANZYME /1-822
CYTOPLASMIN/ 1-822
PERFORIN /1-822
AZUROCIDIN-1 AZU1 HUMAN/ 1-822
GRANZYME/ 1-822
CXCL2/ 1-822
CASPASE/ 1-822
FAS/ 1-822
Quality/ 1-822

Figure 12

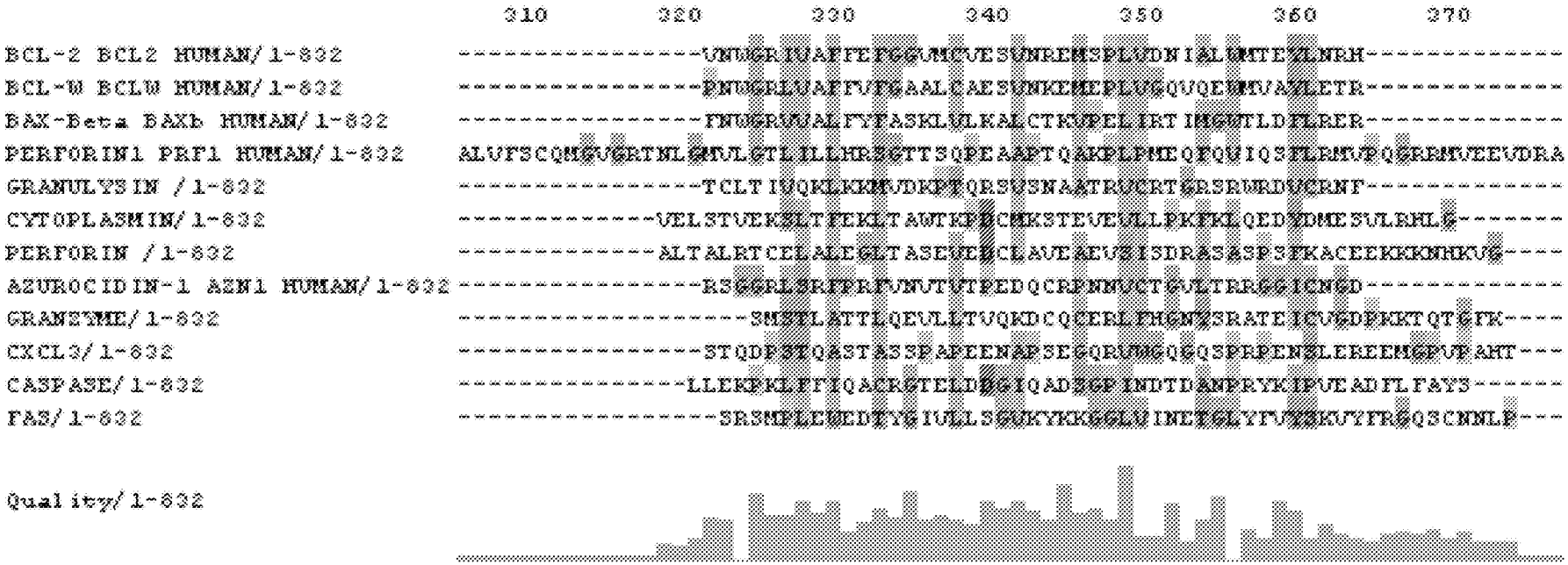

310
320
330
340
350
360
370
BCL-2_BCL2_HUMAN/1-832
BCL-W_BCLW_HUMAN/1-832
BAX-Beta_BAXb_HUMAN/1-832
PERFORIN1_PRF1_HUMAN/1-832
GRANULYSIN_/1-832
CYTOPLASMIN/1-832
PERFORIN_/1-832
AZUROCIDIN-1_AZN1_HUMAN/1-832
GRANZYME/1-832
CXCL3/1-832
CASPASE/1-832
FAS/1-832
Quality/1-832

Figure 13

470
480
490
500
510
520
530
BCL-2 BCL2_HUMAN/1-822
BCL-W BCL10_HUMAN/1-822
BAX-Beta BAXb_HUMAN/1-822
PERFORIN1 PERF1_HUMAN/1-822
GRANZYMB /1-822
CYTOPLASMIN/1-822
PERFORIN /1-822
AZUROCIDIN-1 AZU1_HUMAN/1-822
GRANZYME/1-822
CXCL3/1-822
CASPASE/1-822
FAS/1-822
Quality/1-822

Figure 14

Helix 1 of Human Bcl-2, Isoform 1 or 2: NREIVMKYIHYKLS

Figure 15

Helix 2 of Human Bcl-2, Isoform 1 or 2: HLALRQAGDDFSRRYR

Figure 16

Helix 2 of Human Bcl-X1: SQSNRELVVDFLSYKLSQK

Figure 17

Helix 2 of Human Bcl-W: TRALVADFVGYKLRQK

Figure 18

Helix 4 of Human Bax: RVVALFYFASKLVLKALCTK

Figure 19

Helix 1 of Human CTL Granulysin: RDYRTCLTIVQKLKKM

Figure 20

Helix 1-2 Span of Human CTL Granulysin: QKLKKMVDKPTQRSVSN

Figure 21

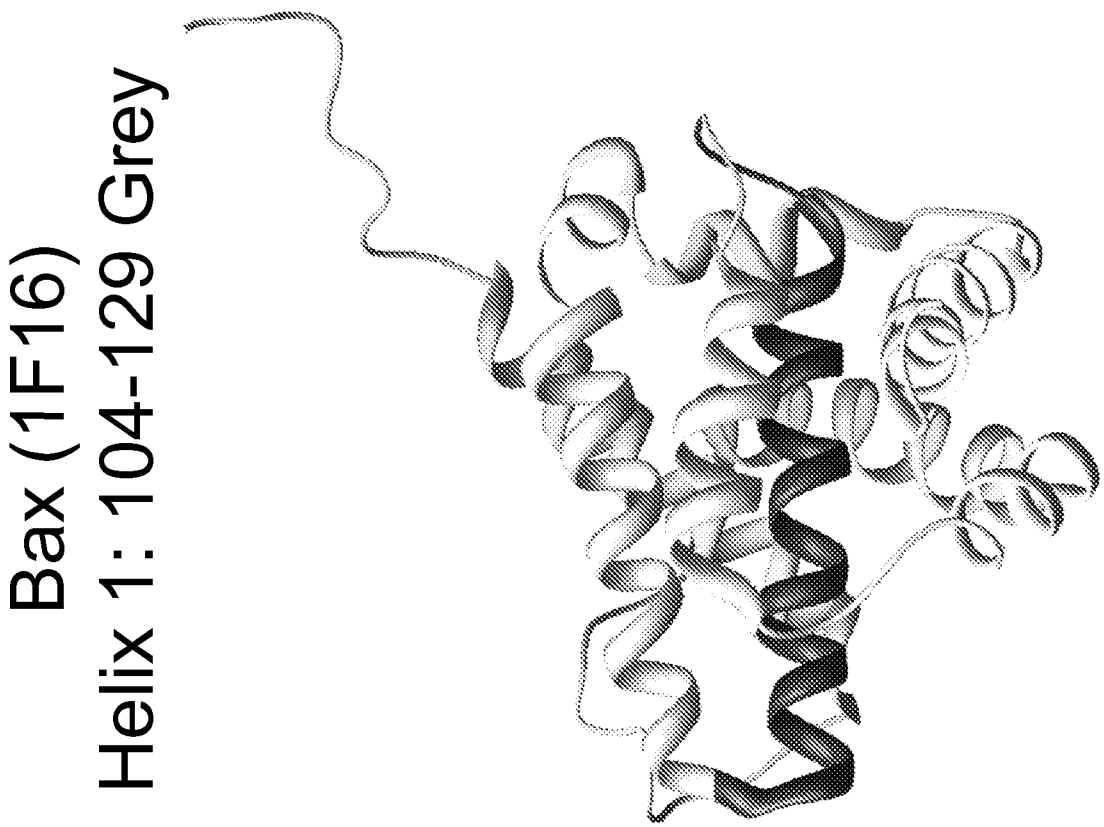
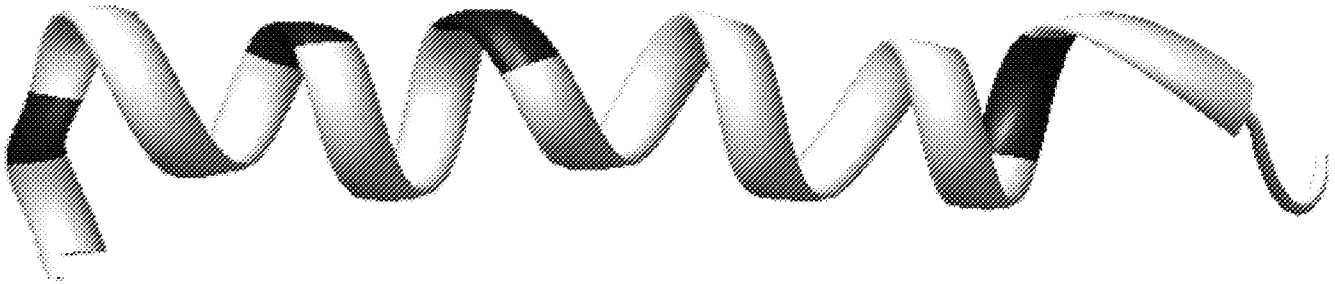


Figure 22

Bax Helix-1:104-129
positive dark grey

(A)



Bax Helix-1: 104-129
KD most hydrophilic dark grey ; most hydrophobic med. grey

(B)

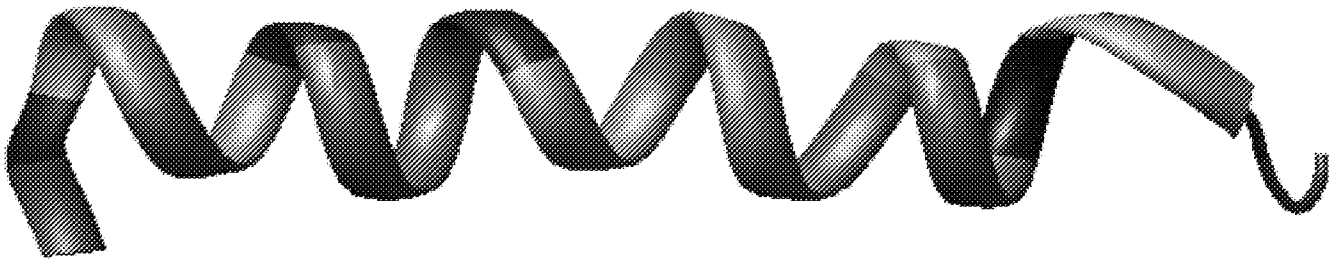


Figure 23

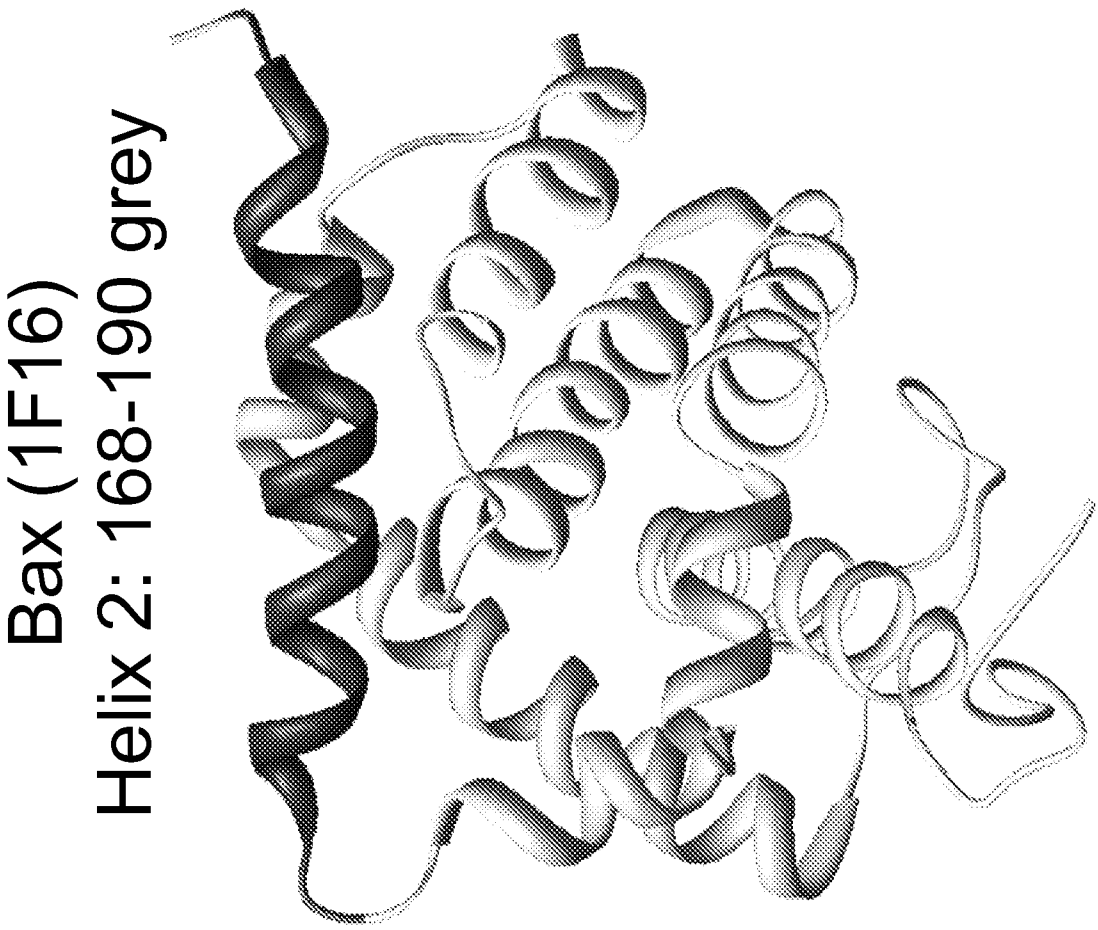
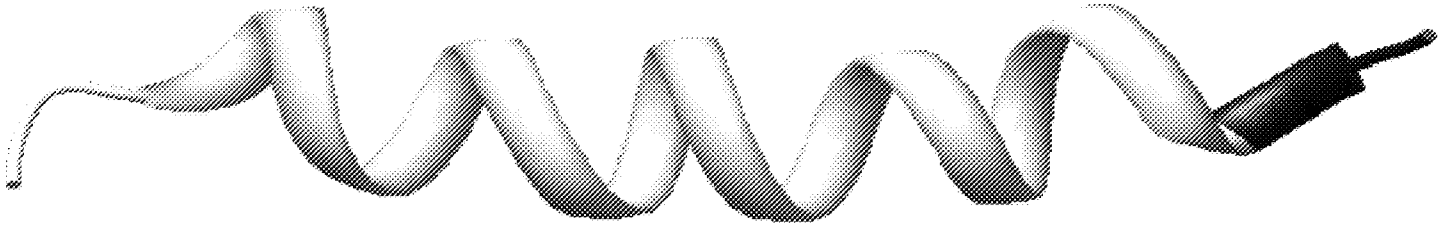


Figure 24

Bax Helix-2: 168-190
positive dark grey

(A)



Bax Helix-2: 168-190
KD most hydrophilic dark grey; most hydrophobic med. grey

(B)

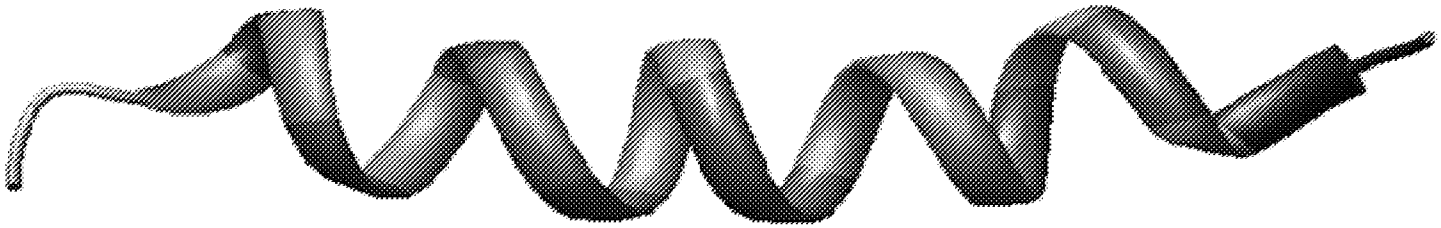


Figure 25

Bax helix 104-129 vs. IL-8 helix 54-72

(A)

Sequence alignment based on structure alignment.

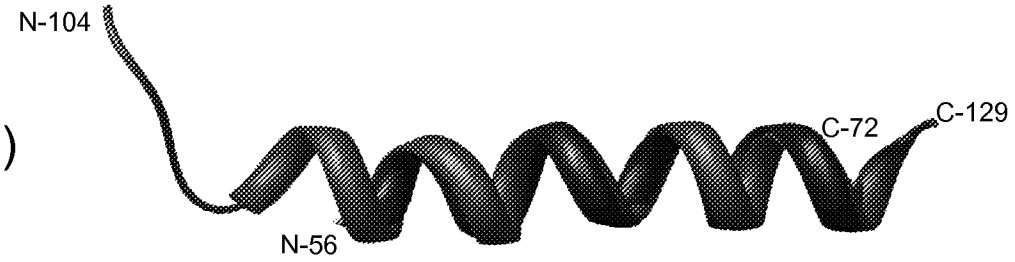
Sequence alignment based on structure alignment. Position numbers according to sequence (starting from 1) and according to PDB are given as SSSS/PPP. SSSS - sequence, PPP - PDB.

1IL8:A - INTERLEUKIN 8 (IL-8) (NEUTROPHIL ACTIVATION PROTEIN) /NAP8 (NMR8, MINIMIZED MEAN STRUCTURE)

1F16:A - MOL_ID: 1; MOLECULE: APOPTOSIS REGULATOR BAX, MEMBRANE ISOFORM ALPHA; CHAIN: A; ENGINEERED: YES

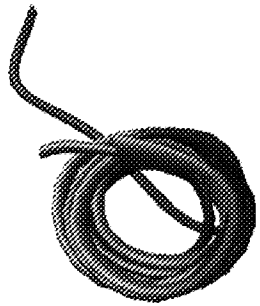
```
1IL8:A    55/56     ENEVQRVVEKFLKRAE
1F16:A   130/131    VVALFYFASELVLEAL
```

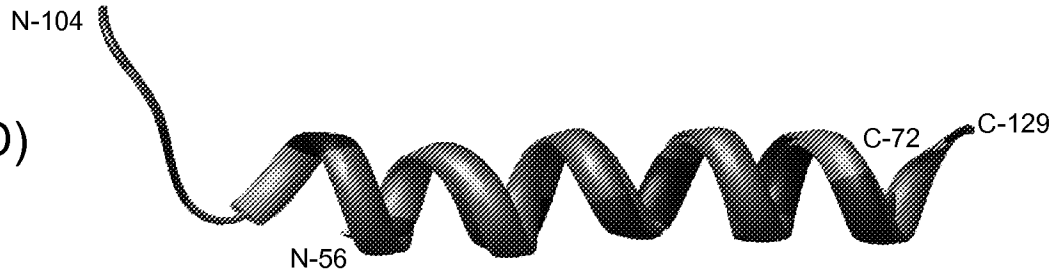
Rmsd = 0.4Å Z-Score = 2.8
Sequence identity = 12.5%
Aligned/gap positions = 16/0



(B)

(C)





(D)

Figure 26

Bax helix 168-190 vs. IL-8 helix 54-72

(A)

Sequence alignment based on structure alignment.

Sequence alignment based on structure alignment. Position numbers according to sequence (starting from 1) and according to PDB are given as SSSS/PPPP. SSSS - sequence, PPPP - PDB.

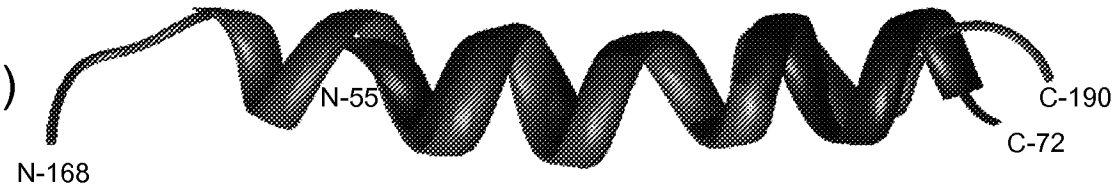
1IL8:A - INTERLEUKIN 8 (IL-8) (NEUTROPHIL ACTIVATION PROTEIN) /NAP8 (NMR8, MINIMIZED MEAN STRUCTURE)

1F16:A - MOL_ID: 1; MOLECULE: APOPTOSIS REGULATOR BAX, MEMBRANE ISOFORM ALPHA; CHAIN: A; ENGINEERED: YES

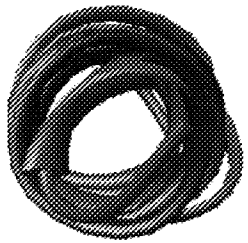
```
1IL8:A   55/56    EWVQRVVEKFLKRAE
1F16:A  174/175   TIFVAGVLTASLTIW
```

Rmsd = 0.7Å Z-Score = 2.6
Sequence identity = 18.8%
Aligned/gap positions = 16:0

(B)



(C)



(D)

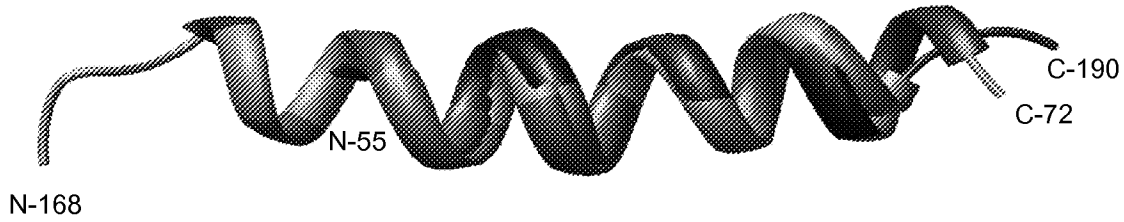


Figure 27

Bax helix 104-129 vs. Magainin 1-24

(A)

Sequence alignment based on structure alignment.

Sequence alignment based on structure alignment. Position numbers according to sequence starting from 1) and according to PDB are given as SSSSPPPP. SSSS - sequence, PPPP - PDB

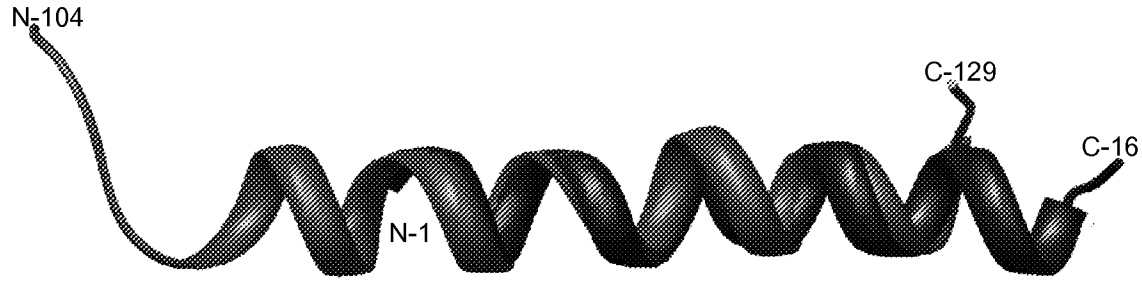
USR1:A - MOL_ID: 1; MOLECULE: MAGAININ 2; CHAIN: A; ENGINEERED: YES; OTHER_DETAILS: THE PEPTIDE WAS PREPARED BY SOLID-PHASE SYNTHESIS

1F16:A - MOL_ID: 1; MOLECULE: APOPTOSIS REGULATOR BAX, MEMBRANE ISOFORM ALPHA; CHAIN: A; ENGINEERED: YES

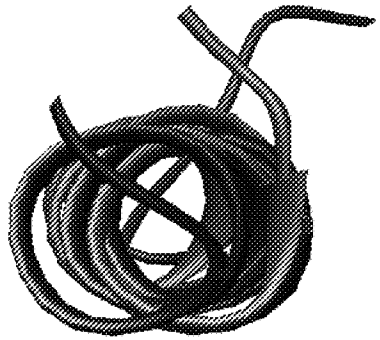
```
USR1:A     1/2      GIGKFLHSAKKFGKAF
1F16:A   120/111    VSALFTFAHELVLKAL
```

Rmsd = 0.6Å Z-Score = 2.8
Sequence identity = 25.0%
Aligned/gap positions = 16:0

(B)



(C)



(D)

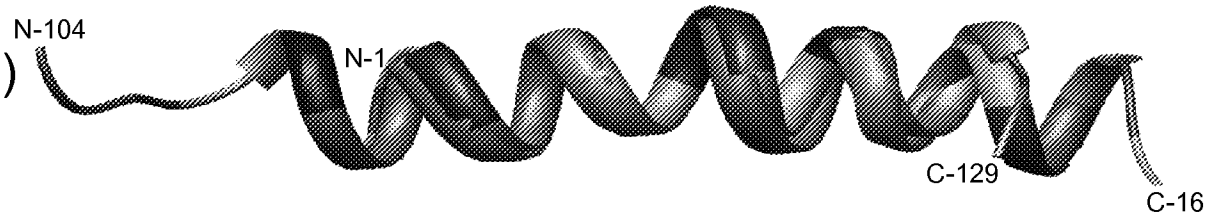


Figure 28

Bax helix 168-190 vs. Magainin 1-24

(A)

Sequence alignment based on structure alignment.

Sequence alignment based on structure alignment. Position numbers according to sequence (starting from 1) and according to PDB are given as SSSS/PPPP. SSSS - sequence, PPPP - PDB.

USER:A - MOL_ID: 1; MOLECULE: MAGAININ 2; CHAIN: A; ENGINEERED: YES; OTHER_DETAILS: THE PEPTIDE WAS PREPARED BY SOLID-PHASE SYNTHESIS

1F16:A - MOL_ID: 1; MOLECULE: APOPTOSIS REGULATOR BAX, MEMBRANE ISOFORM ALPHA; CHAIN: A; ENGINEERED: YES

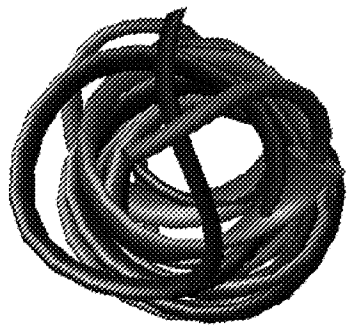
Rmsd = 0.7Å Z-Score = 2.8
Sequence identity = 0.0%
Aligned/gap positions = 16:0

```
USER:A     1/2      GIGKFLHSAKKFGKAF
1F16:A   168/168    GIVTIFVAGVLTASLT
```

(B)



(C)



(D)

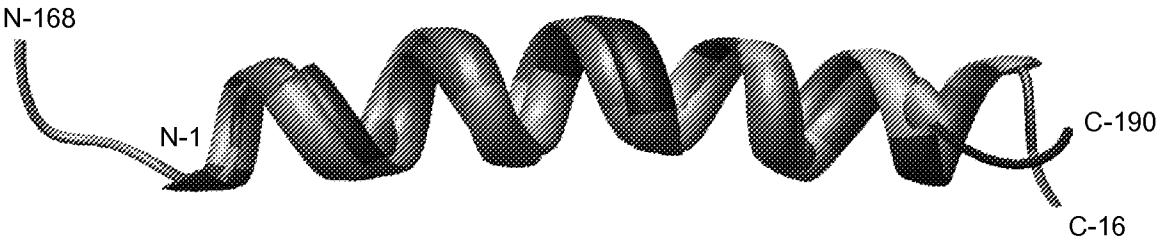


Figure 29

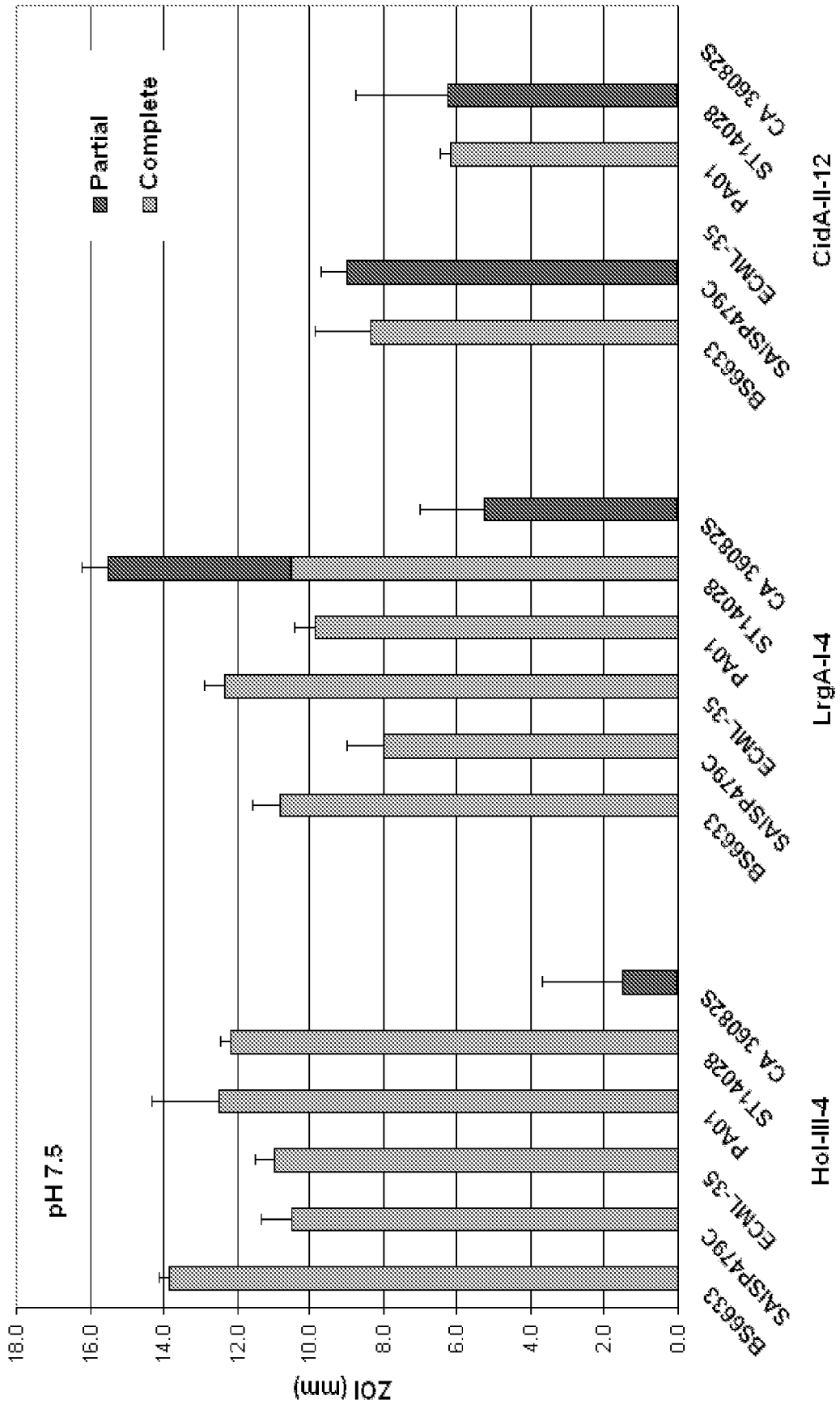

pH 7.5
Partial
Complete
ZOI (mm)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
BS6633
SAISP479C
ECML-35
PA01
ST14028
CA 360825
HolI-III-4
LrgA-I-4
CidA-II-12

Figure 30

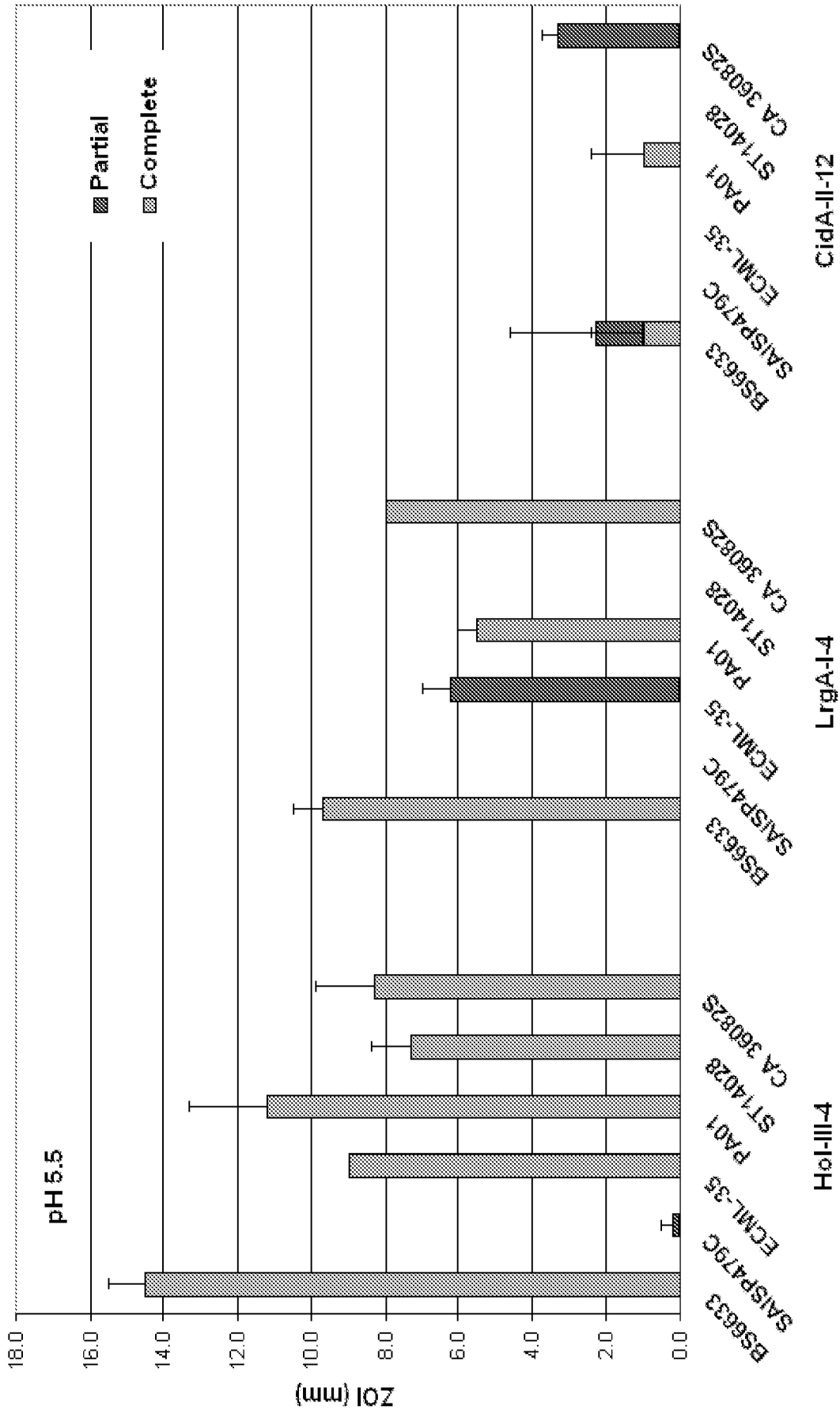

pH 5.5
Partial
Complete
ZOI (mm)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
BS6633
SAISPA479C
ECML-35
PA01
ST14028
CA 360825
HolI-III-4
LrgA-I-4
CidA-II-12

Figure 31

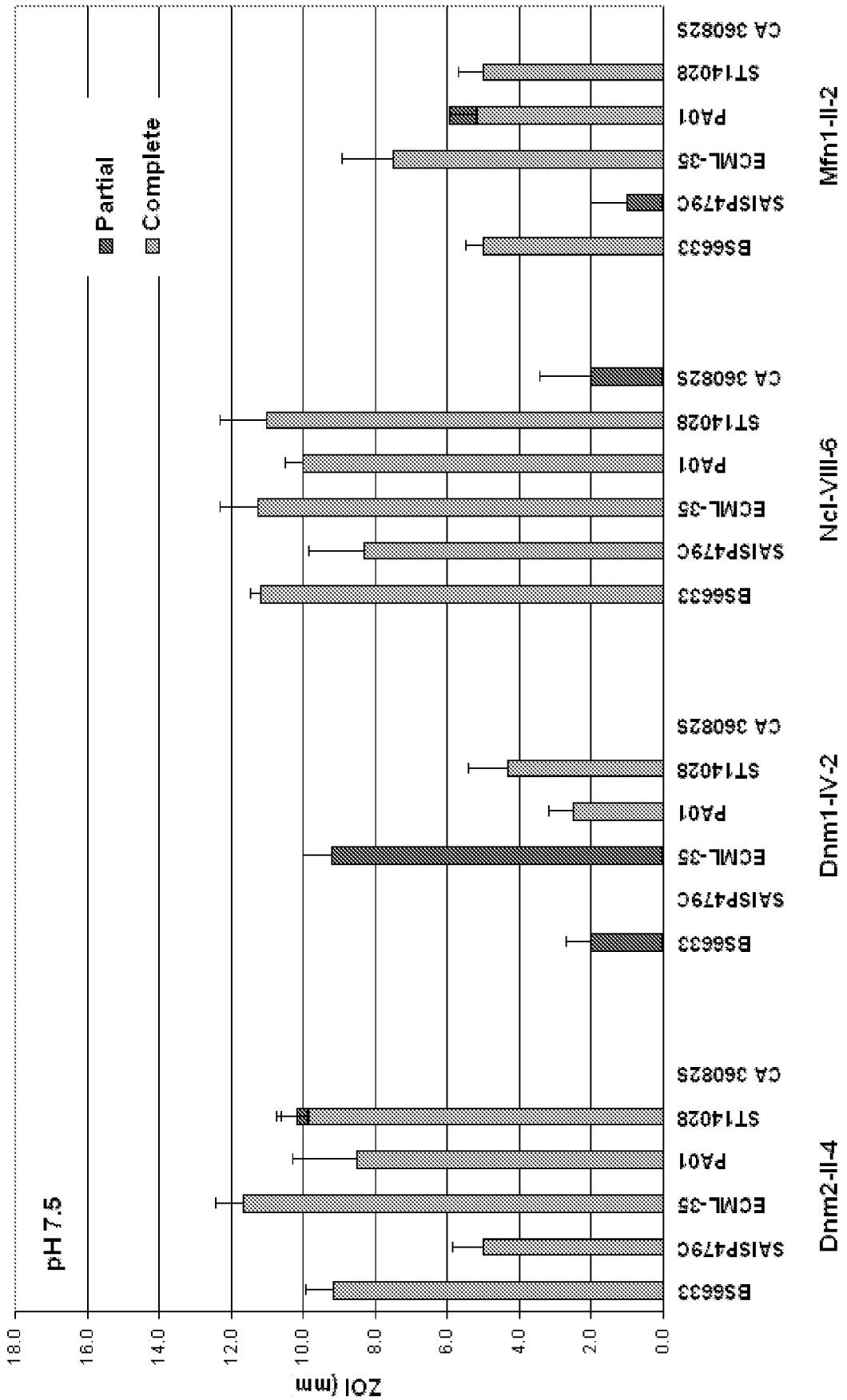

pH 7.5
Partial
Complete
ZOI (mm
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
BS6633
SAISP479C
ECML-35
PA01
ST1402B
CA 360825
Dnm2-II-4
BS6633
SAISP479C
ECML-35
PA01
ST1402B
CA 360825
Dnm1-IV-2
BS6633
SAISP479C
ECML-35
PA01
ST1402B
CA 360825
Ncl-VIII-6
BS6633
SAISP479C
ECML-35
PA01
ST1402B
CA 360825
Mfn1-II-2

Figure 32

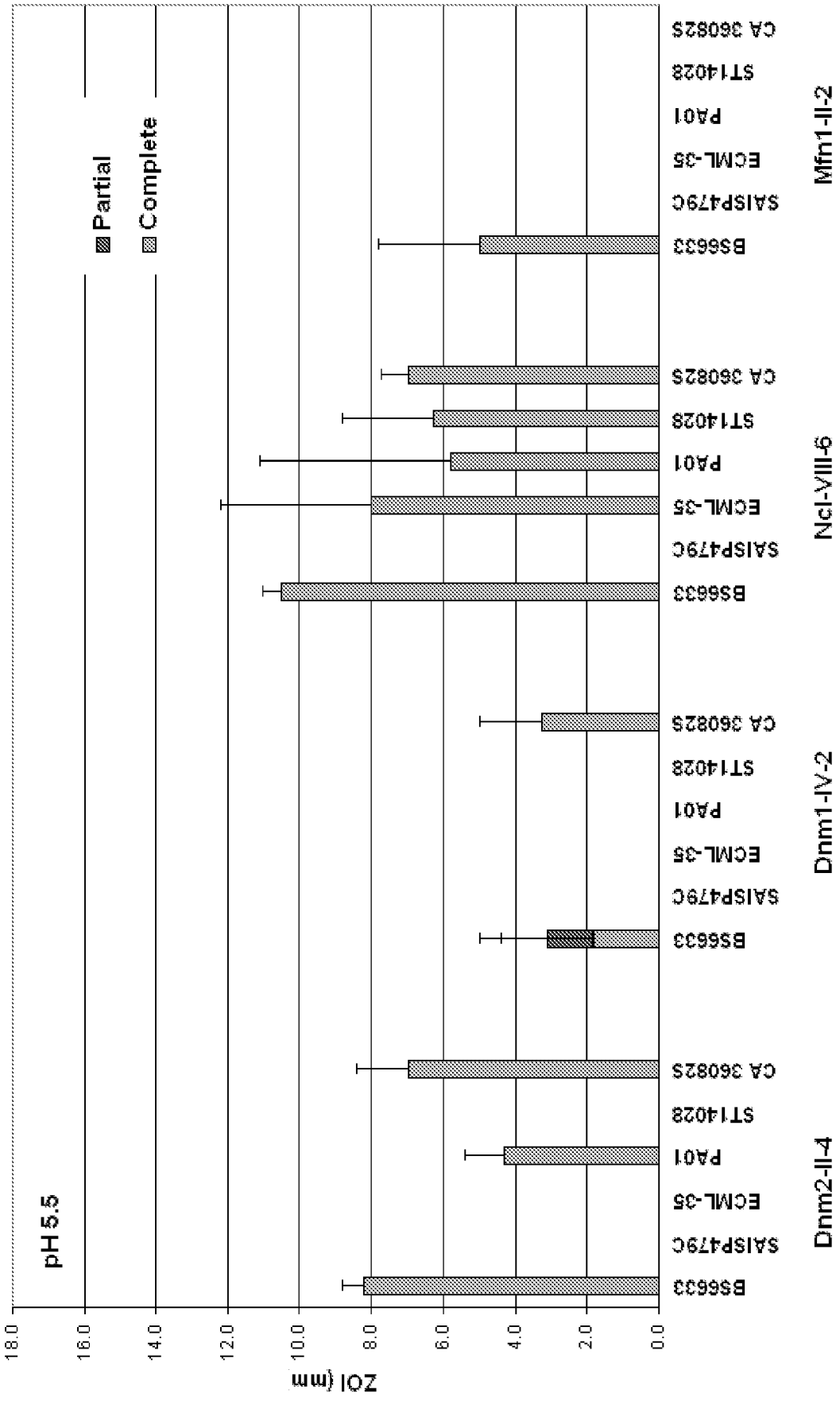

pH 5.5
Partial
Complete
ZOI (mm
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
BS6633
SAISP479C
ECML-35
PA01
ST14028
CA 360825
Dnm2-II-4
BS6633
SAISP479C
ECML-35
PA01
ST14028
CA 360825
Dnm1-IV-2
BS6633
SAISP479C
ECML-35
PA01
ST14028
CA 360825
Ncl-VIII-6
BS6633
SAISP479C
ECML-35
PA01
ST14028
CA 360825
Mfn1-II-2

Figure 33

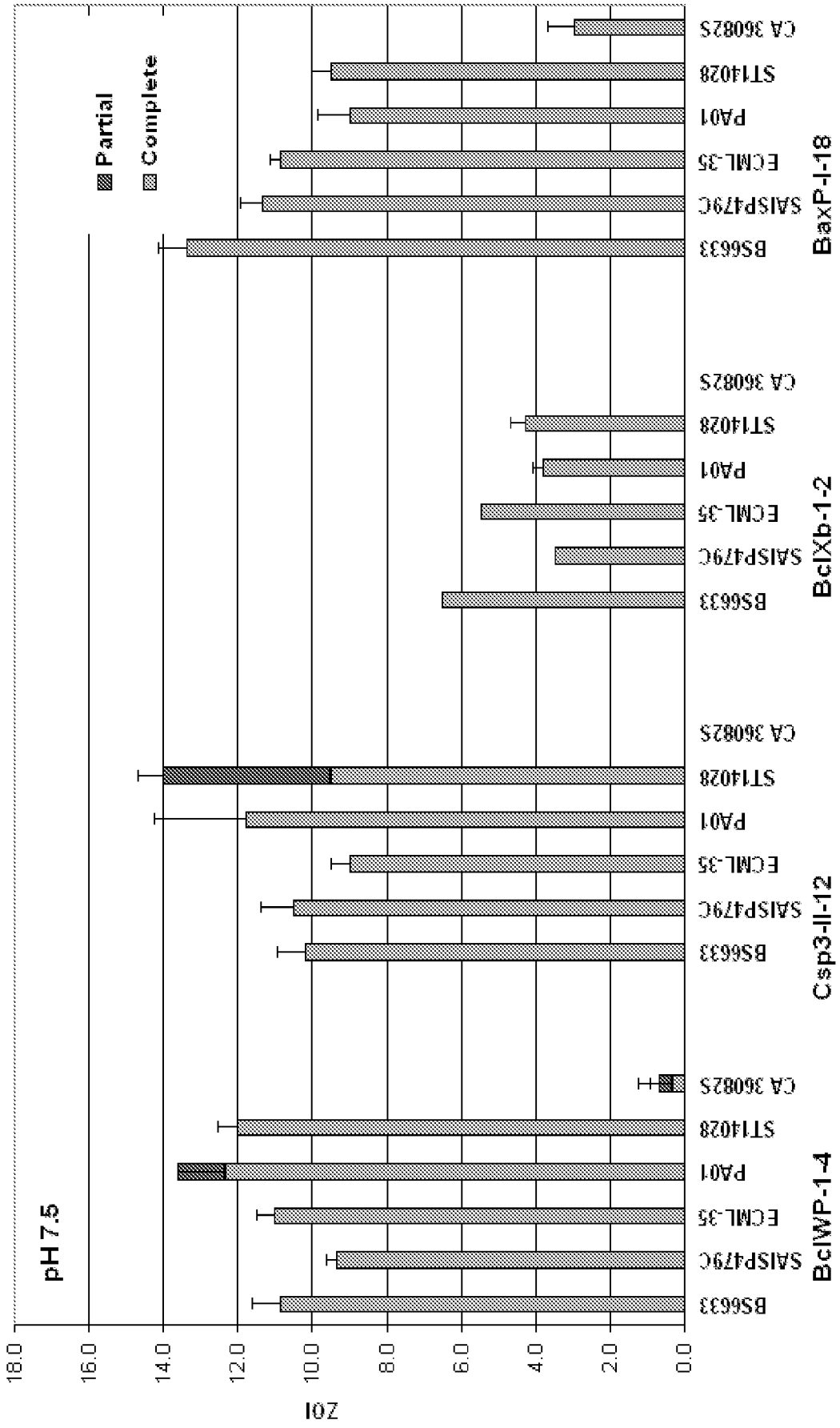

pH 7.5
Partial
Complete
ZOI
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
BclWP-1-4
Csp3-II-12
BclXb-1-2
BaxP-I-18
BS6633
SAISP479C
ECML35
PA01
ST14028
CA 360825

Figure 34

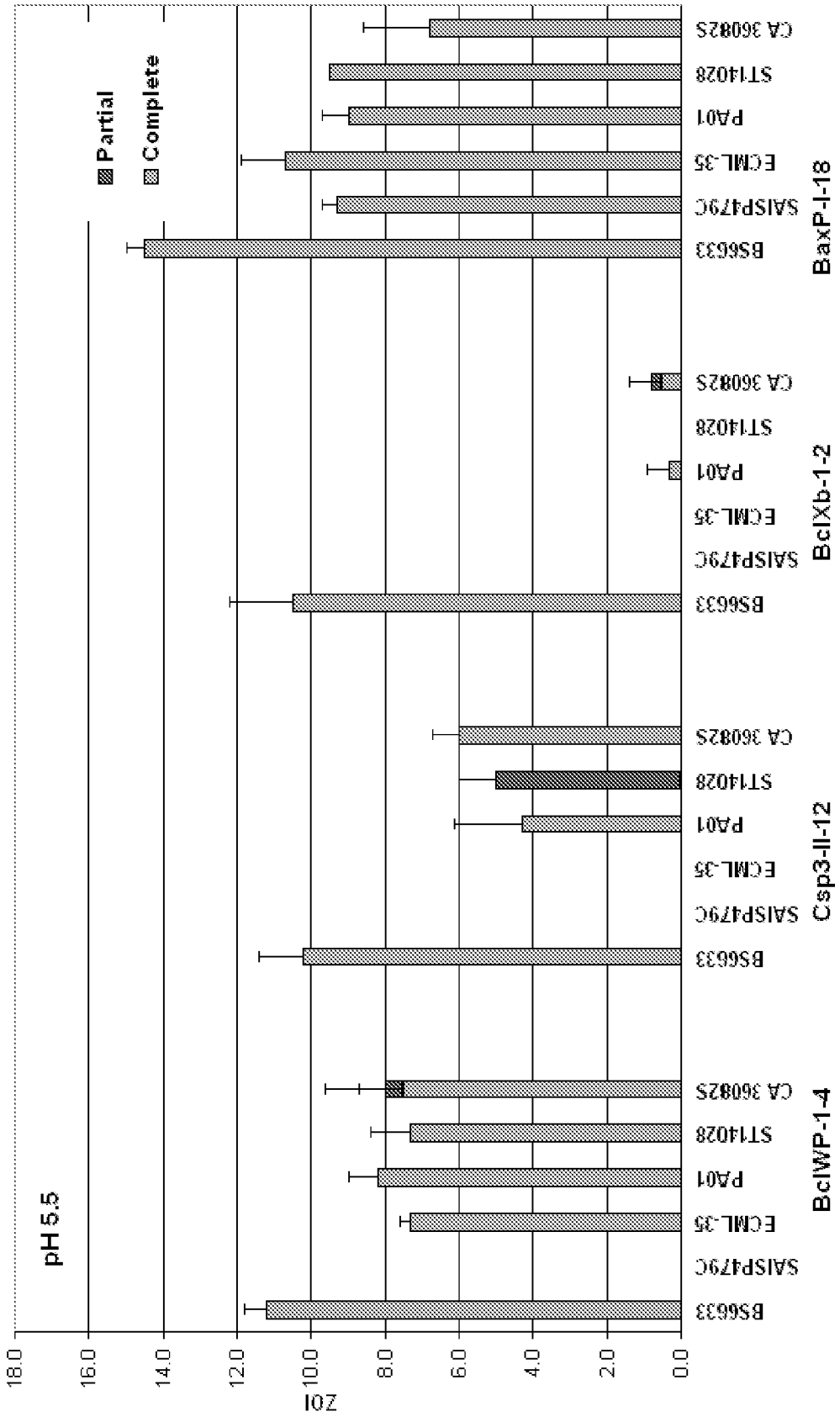

pH 5.5
Partial
Complete
ZOI
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
BclWP-1-4
Csp3-II-12
BclXb-1-2
BaxP-I-18
BS6633
SAISP479C
ECML-35
PA01
ST14028
CA 360825

Figure 35

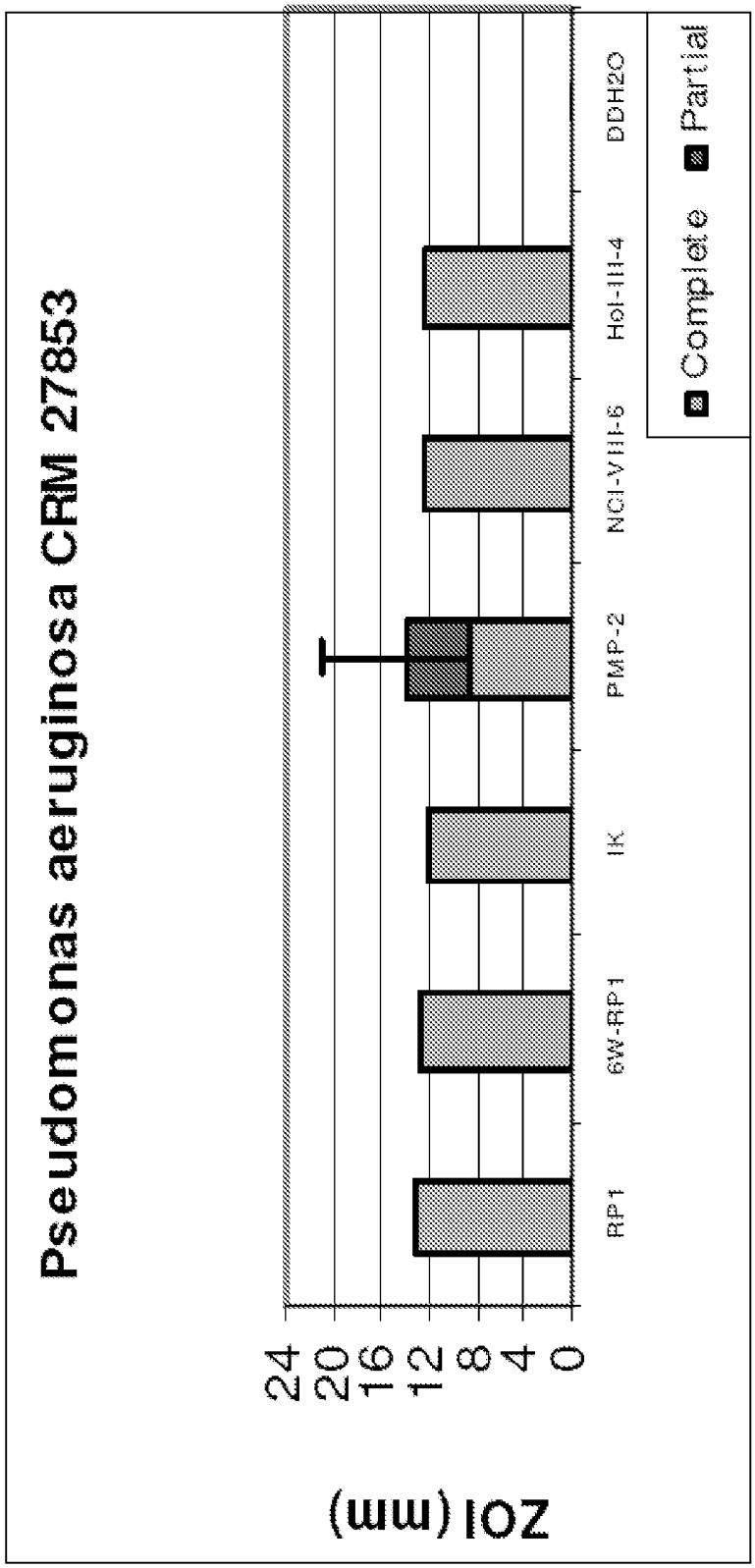

Pseudomonas aeruginosa CRM 27853
ZOI (mm)
24
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 36

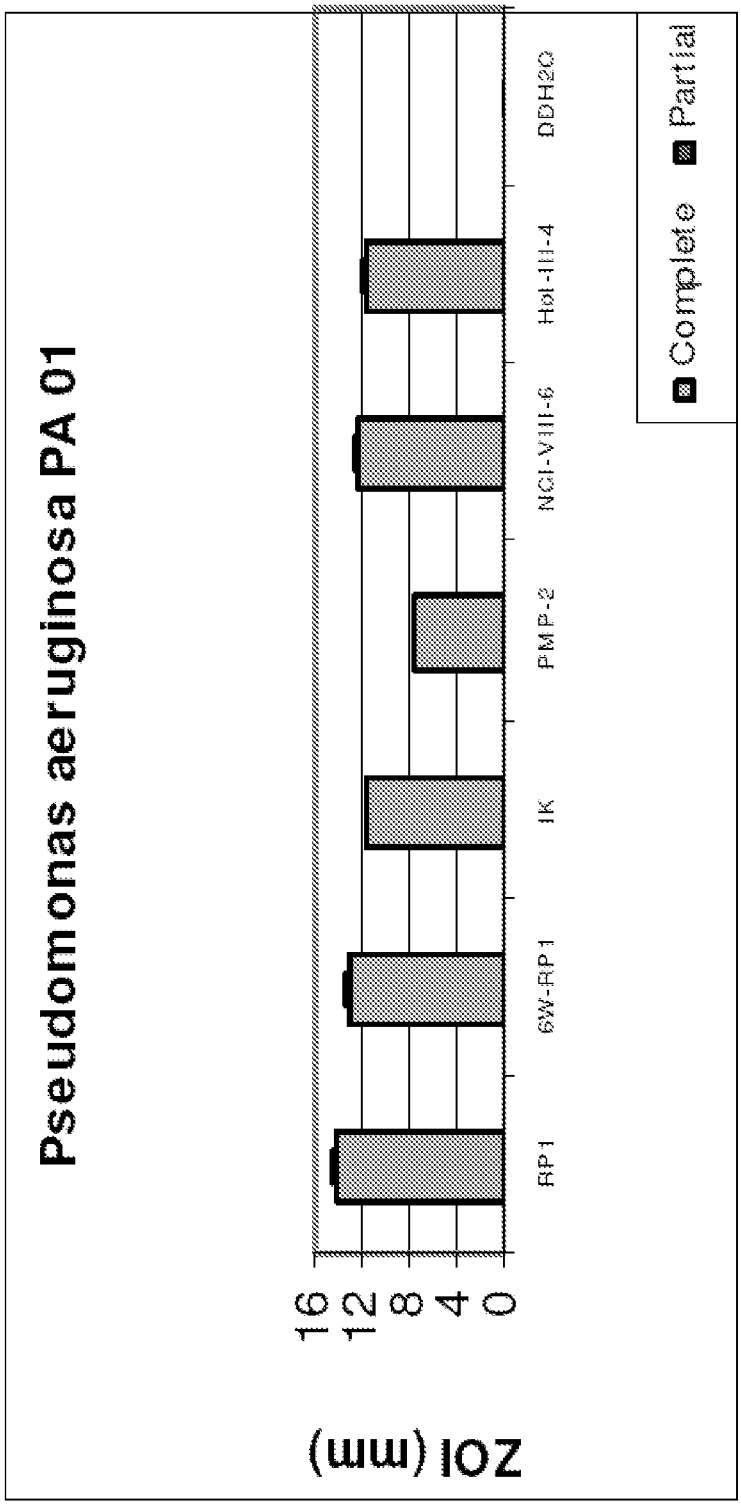

Pseudomonas aeruginosa PA 01
ZOI (mm)
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 37

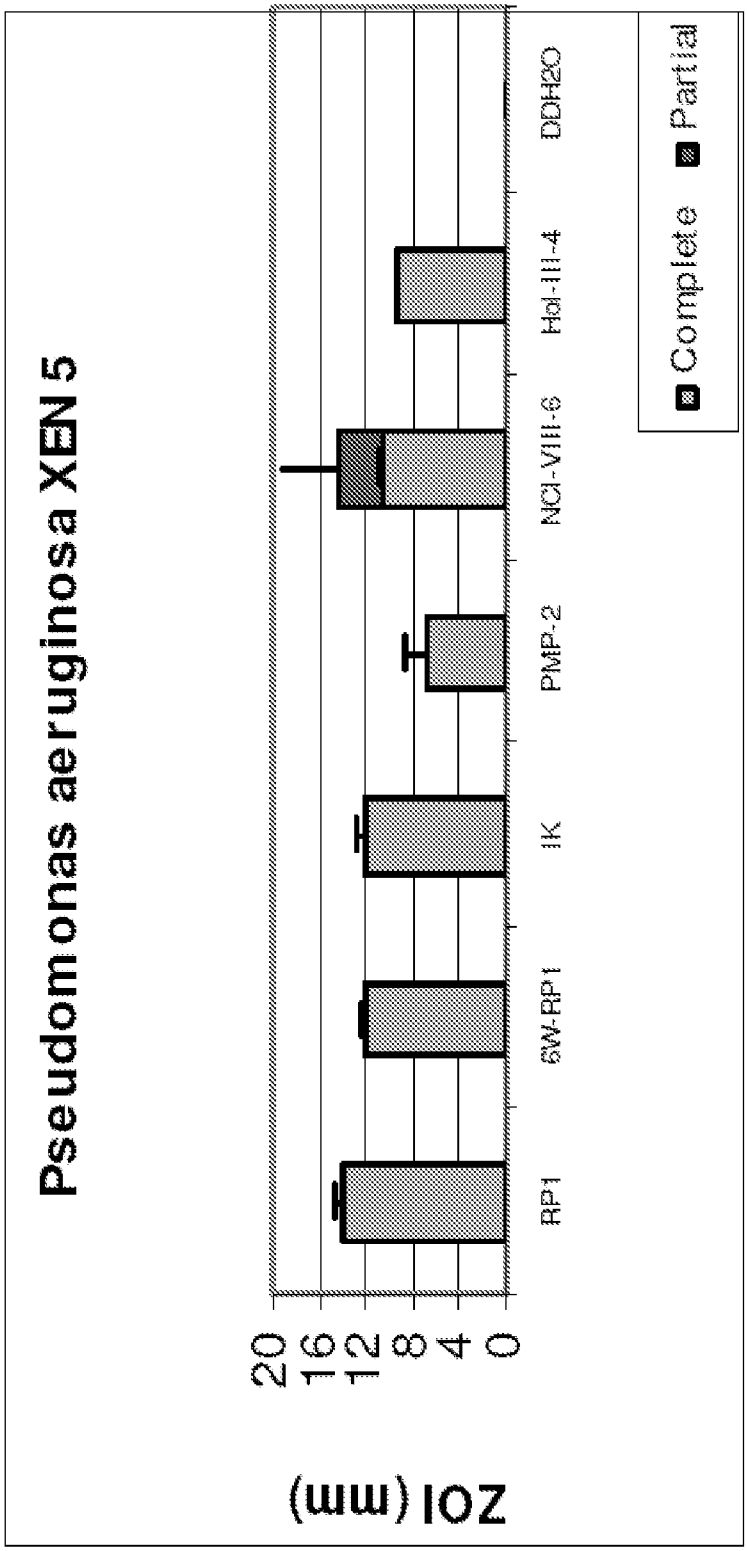

Pseudomonas aeruginosa XEN 5
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
1K
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 38

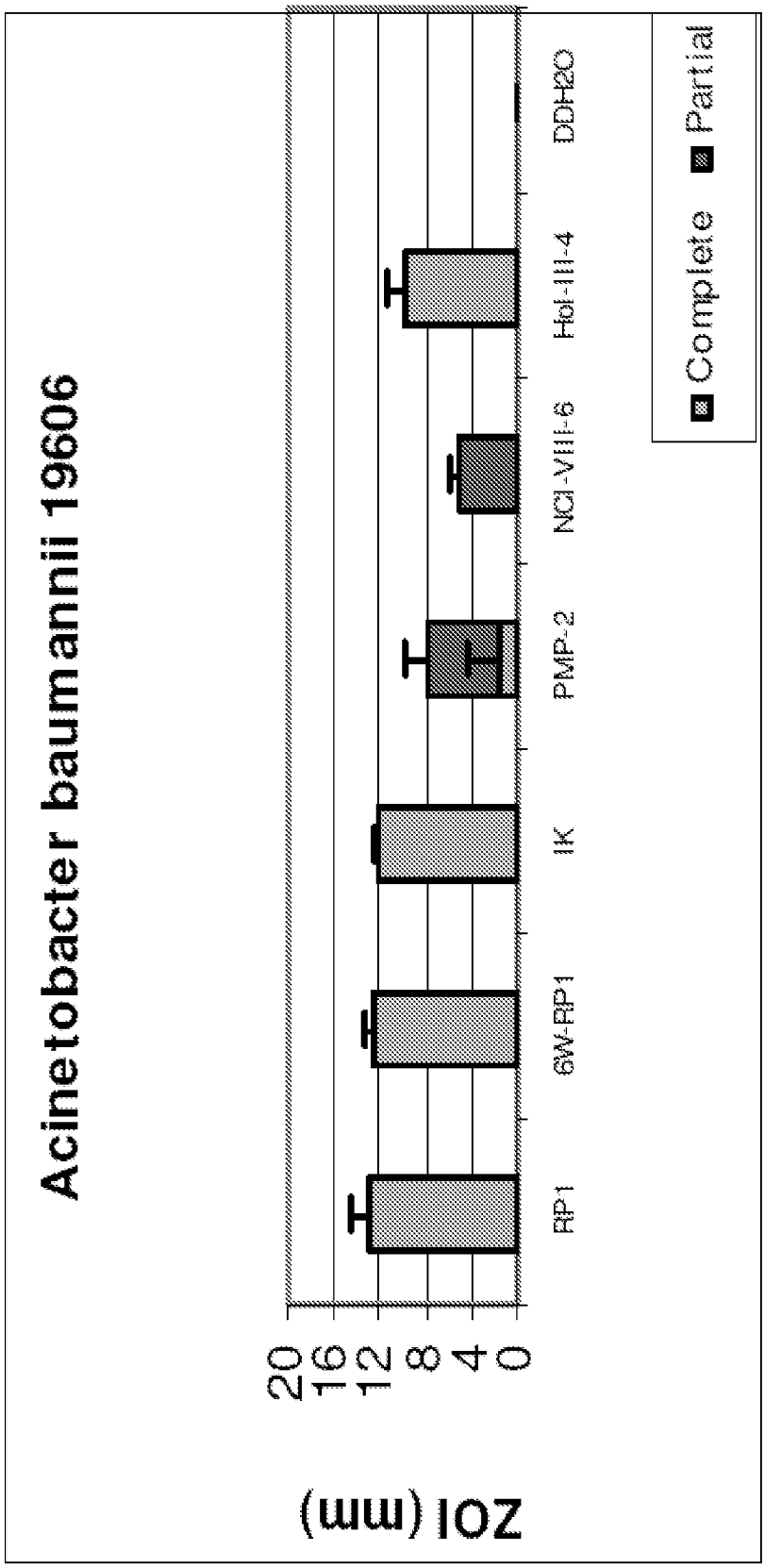

Acinetobacter baumannii 19606
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 39

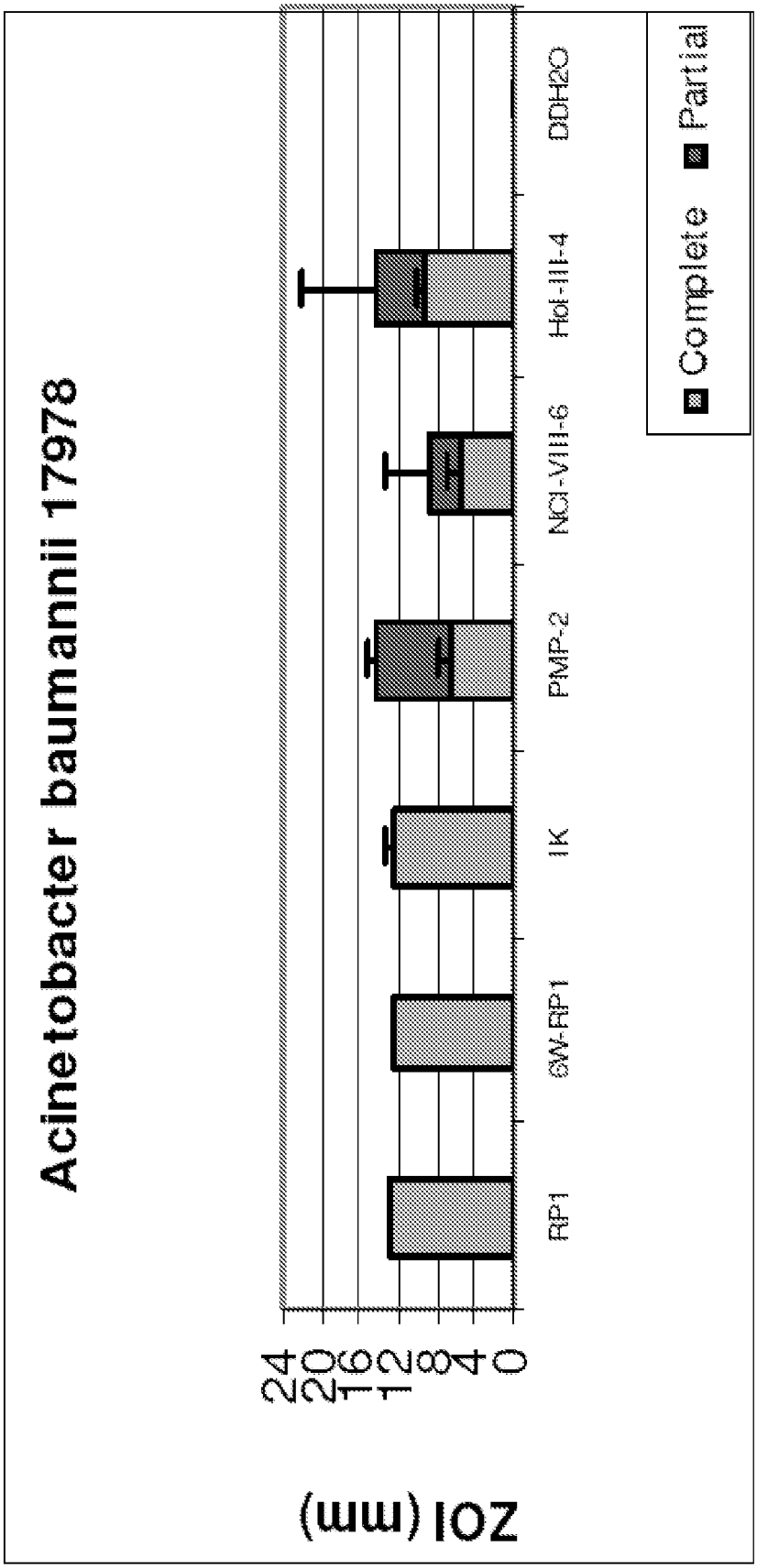

Acinetobacter baumannii 17978
ZOI (mm)
0
4
8
12
16
20
24
RP1
SW-RP1
1K
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 40

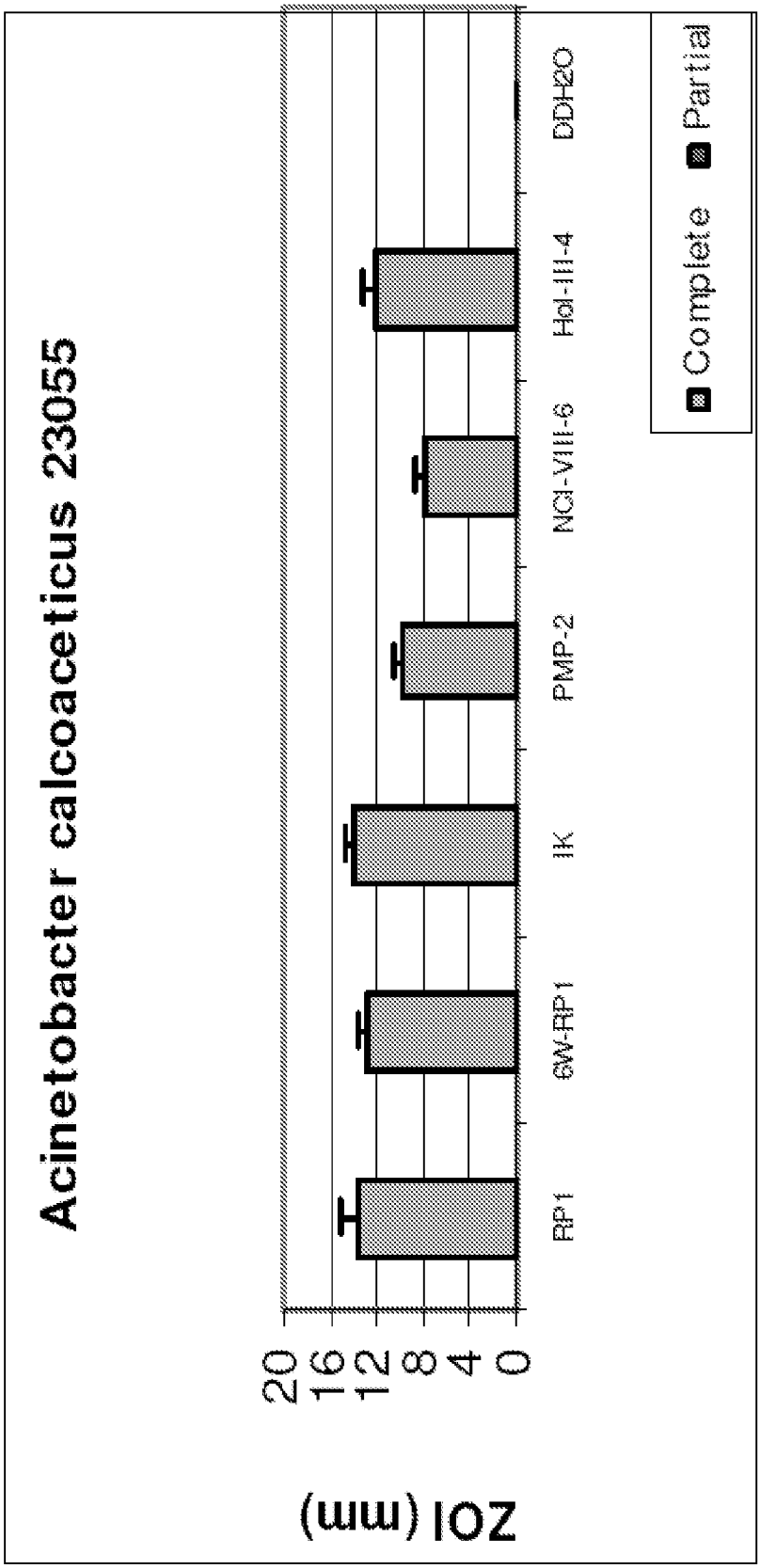

Acinetobacter calcoaceticus 23055
ZOI (mm)
0
4
8
12
16
20
RP1
6W-RP1
IK
PMP-2
NGI-VIII-6
Hal-III-4
DDH2O
Complete
Partial

Figure 41

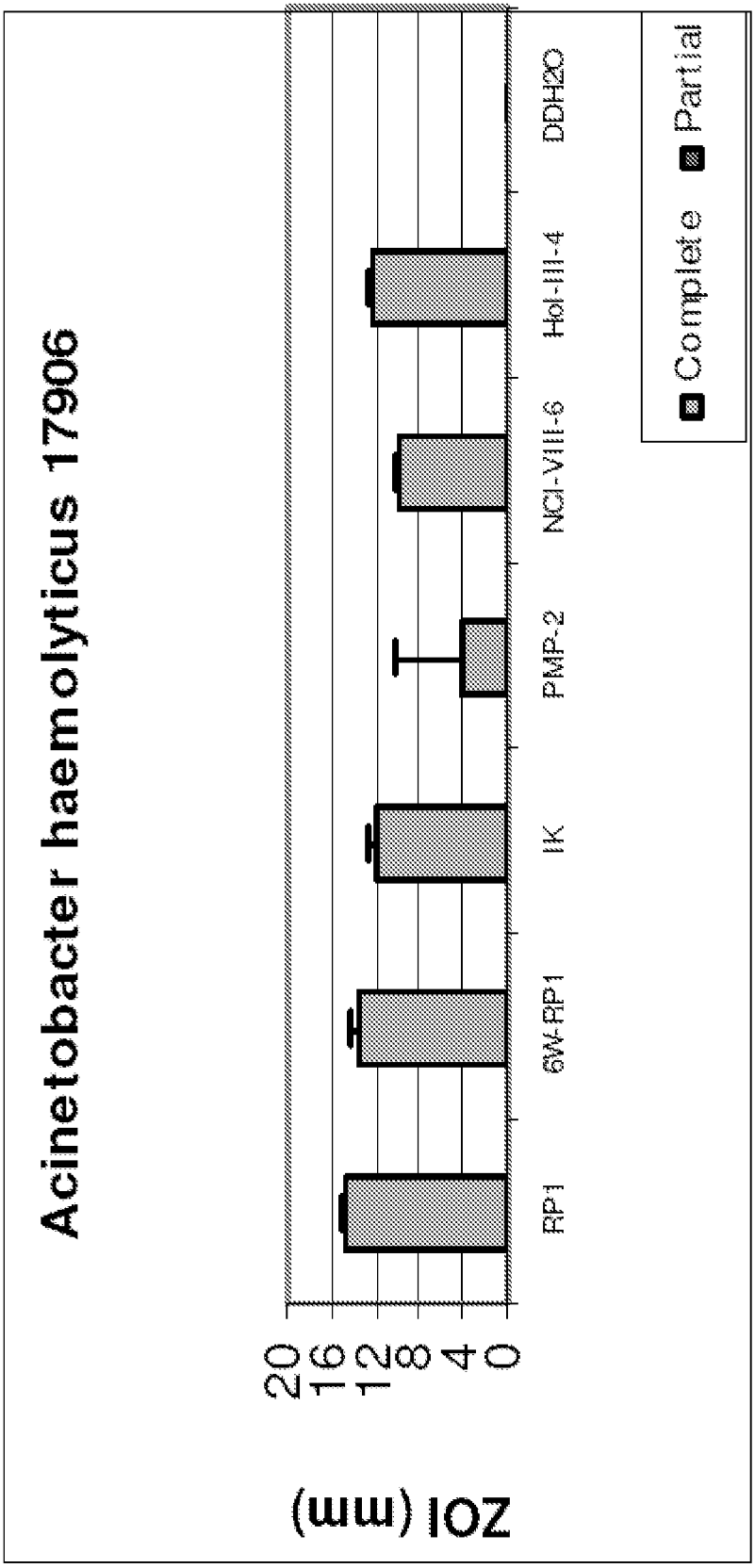

Acinetobacter haemolyticus 17906
ZOI (mm)
0
4
8
12
16
20
RP1
6W-PP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 42

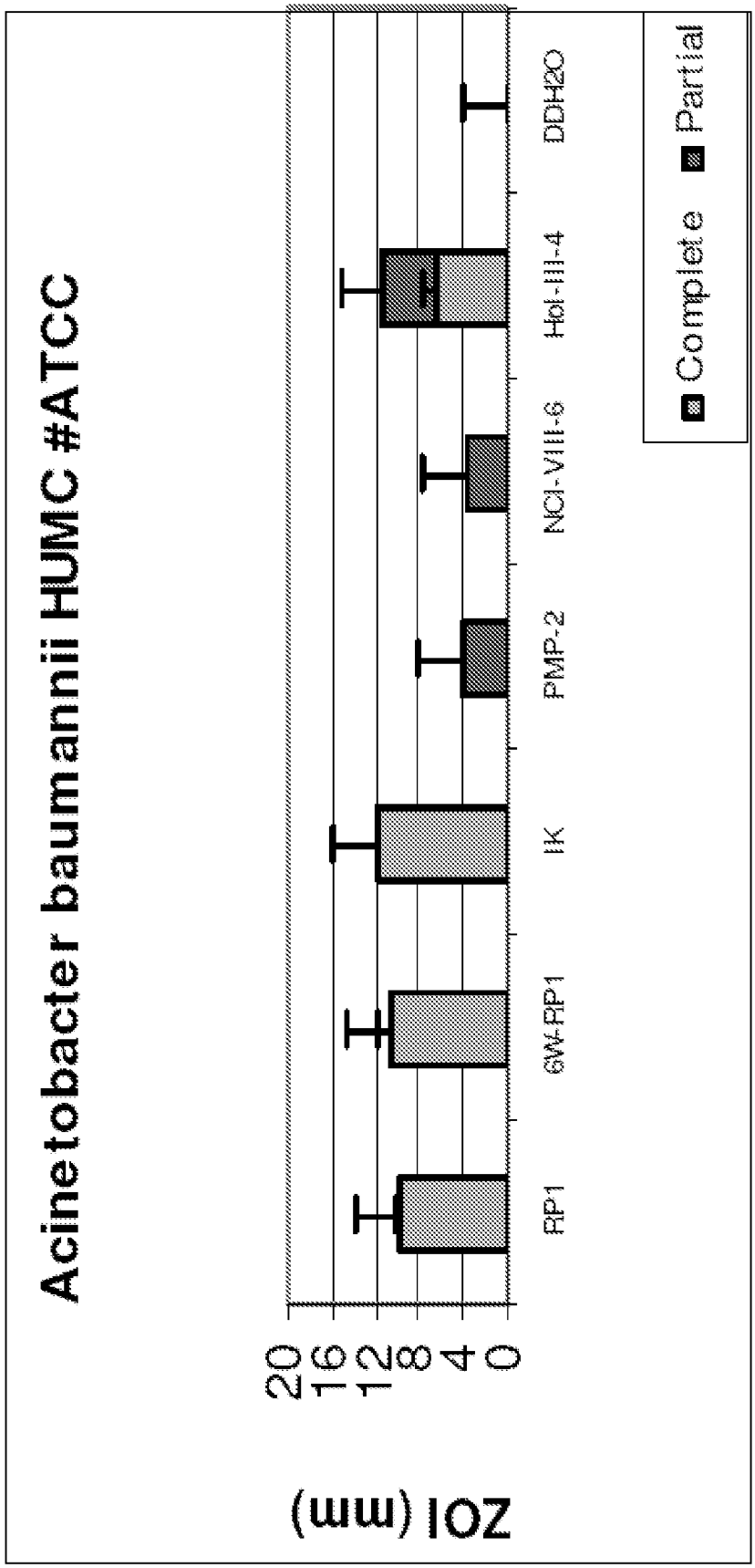

Acinetobacter baumannii HUMC #ATCC
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 43

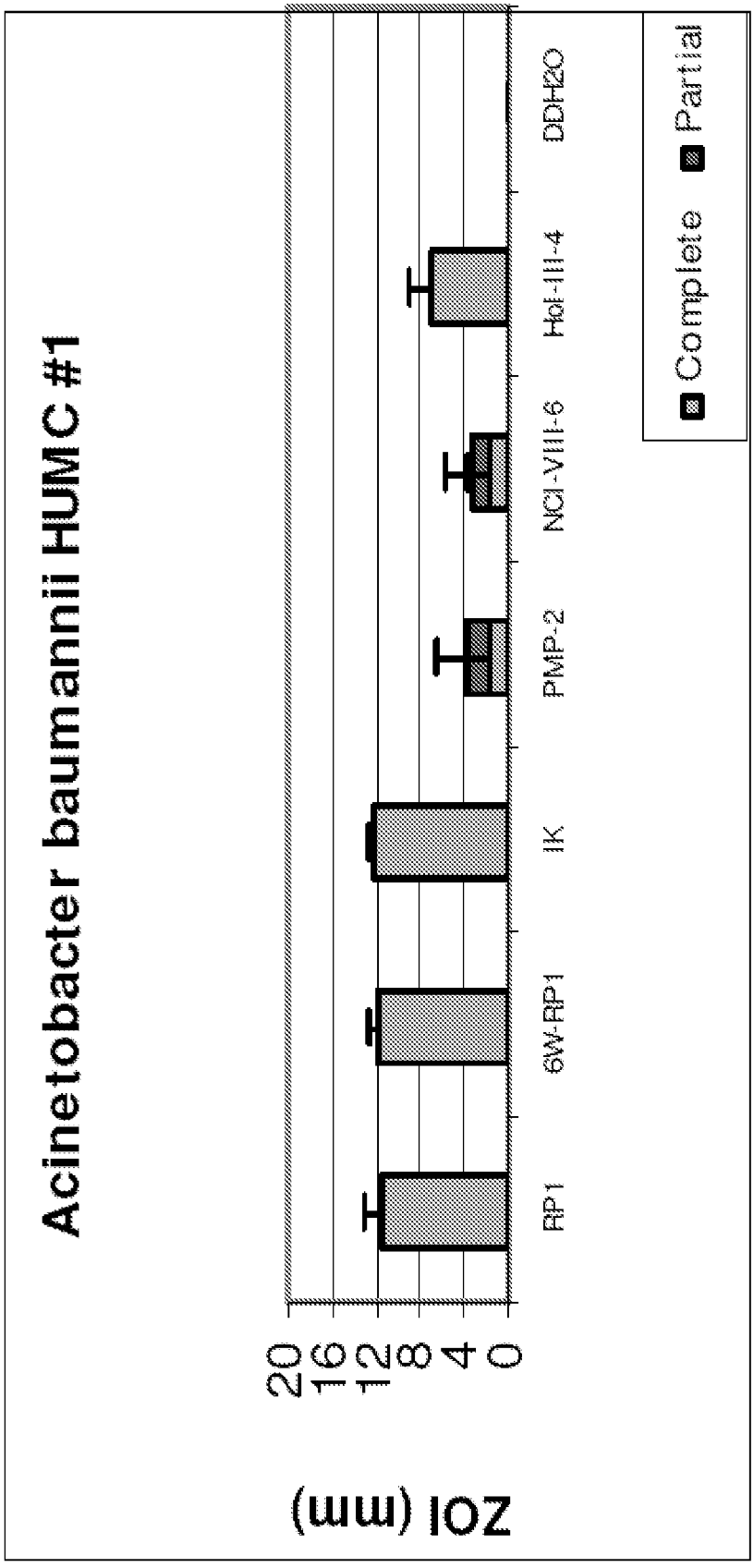

Acinetobacter baumannii HUMC #1
ZOI (mm)
0
4
8
12
16
20
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hal-III-4
DDH2O
Complete
Partial

Figure 44

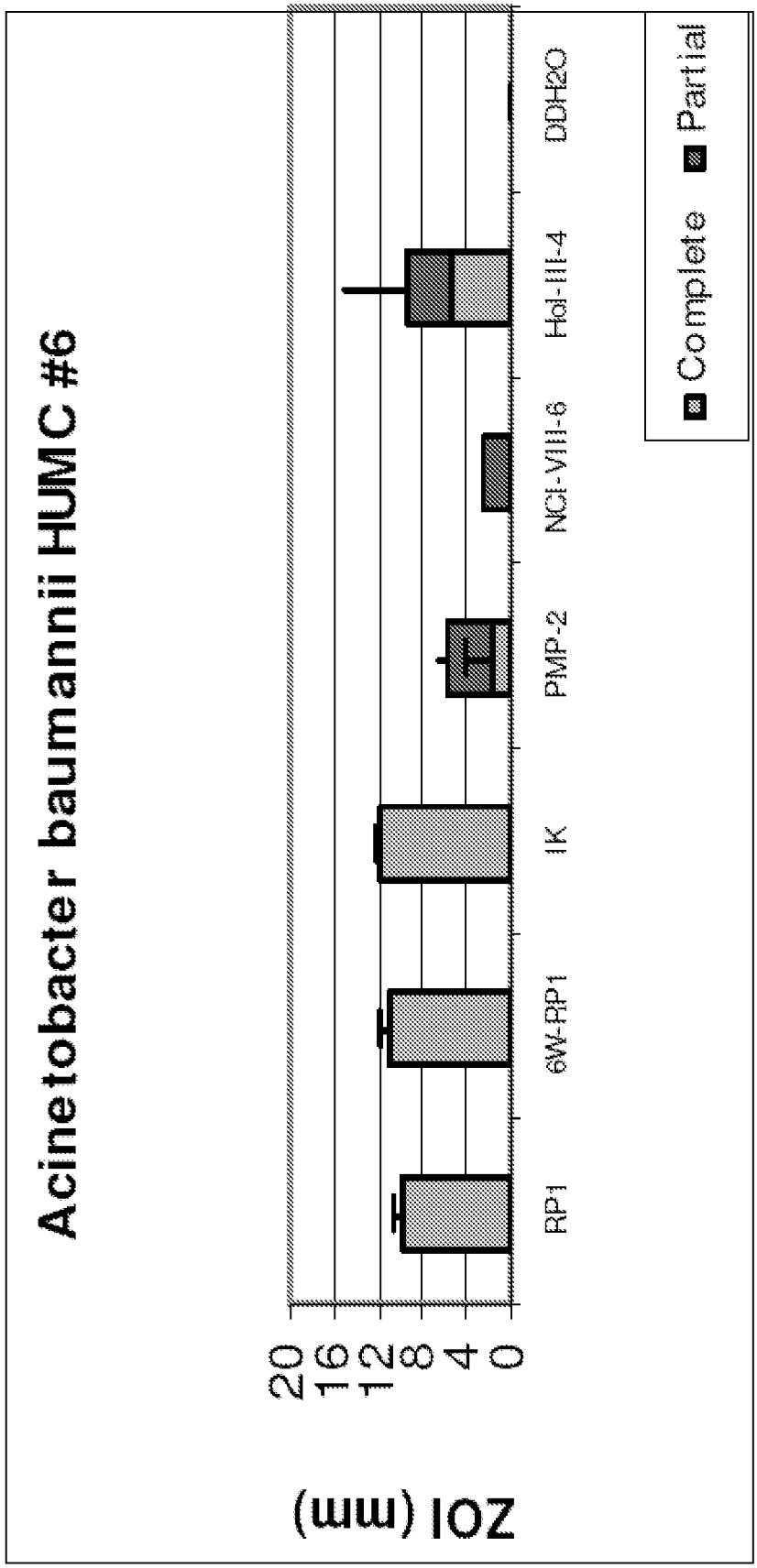

Acinetobacter baumannii HUMC #6
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 45

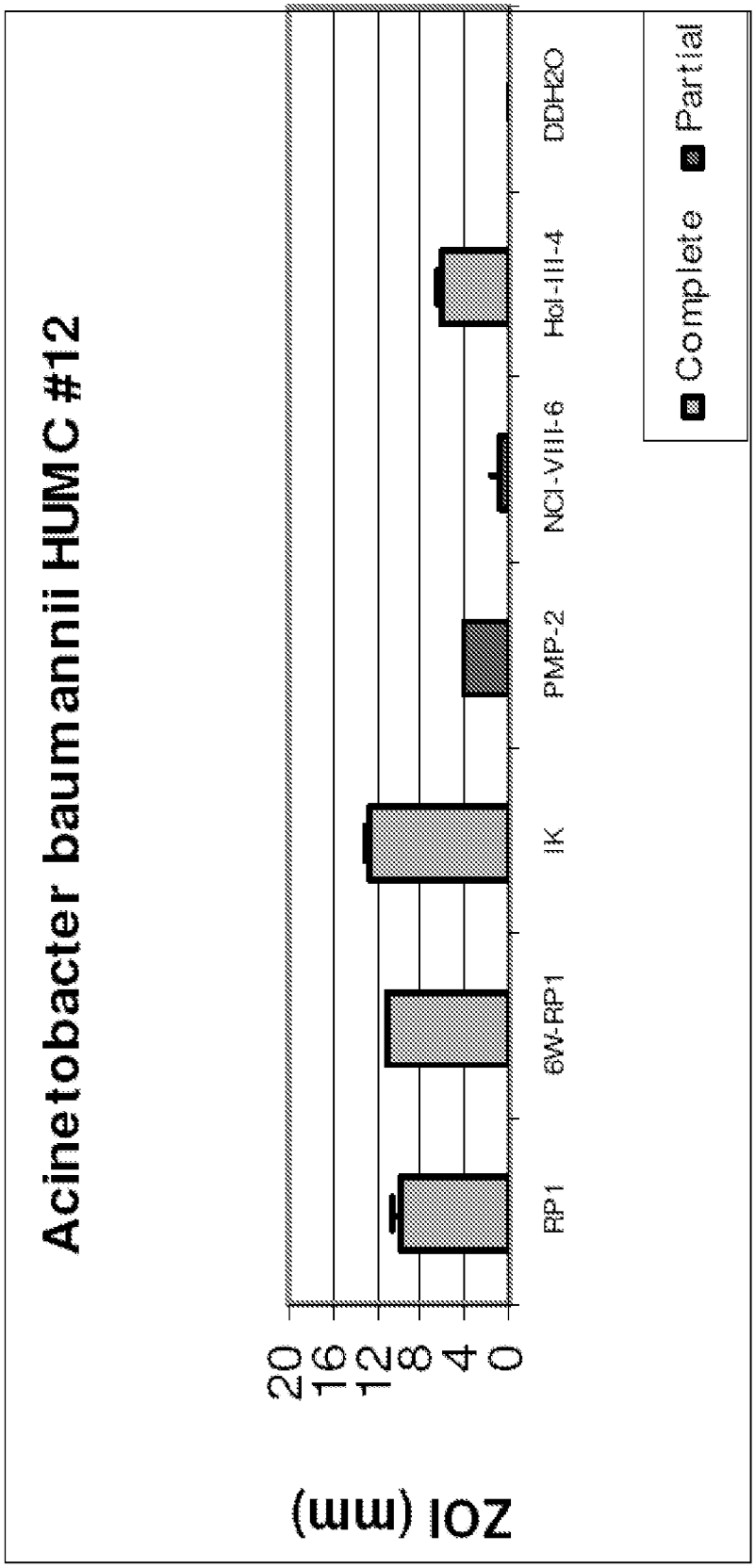

Acinetobacter baumannii HUMC #12
ZOI (mm)
0
4
8
12
16
20
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 46

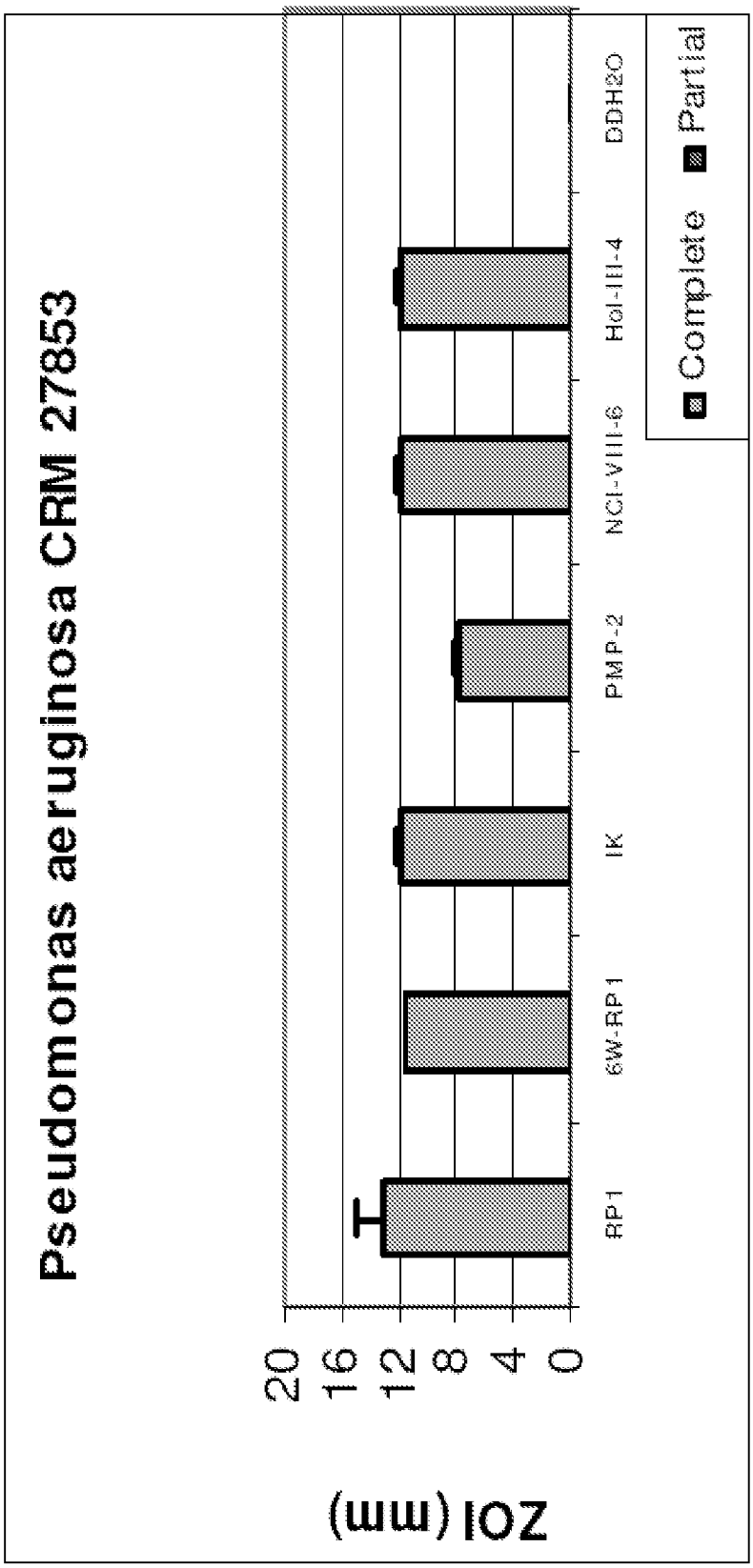

Pseudomonas aeruginosa CRM 27853
ZOI (mm)
0
4
8
12
16
20
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 47

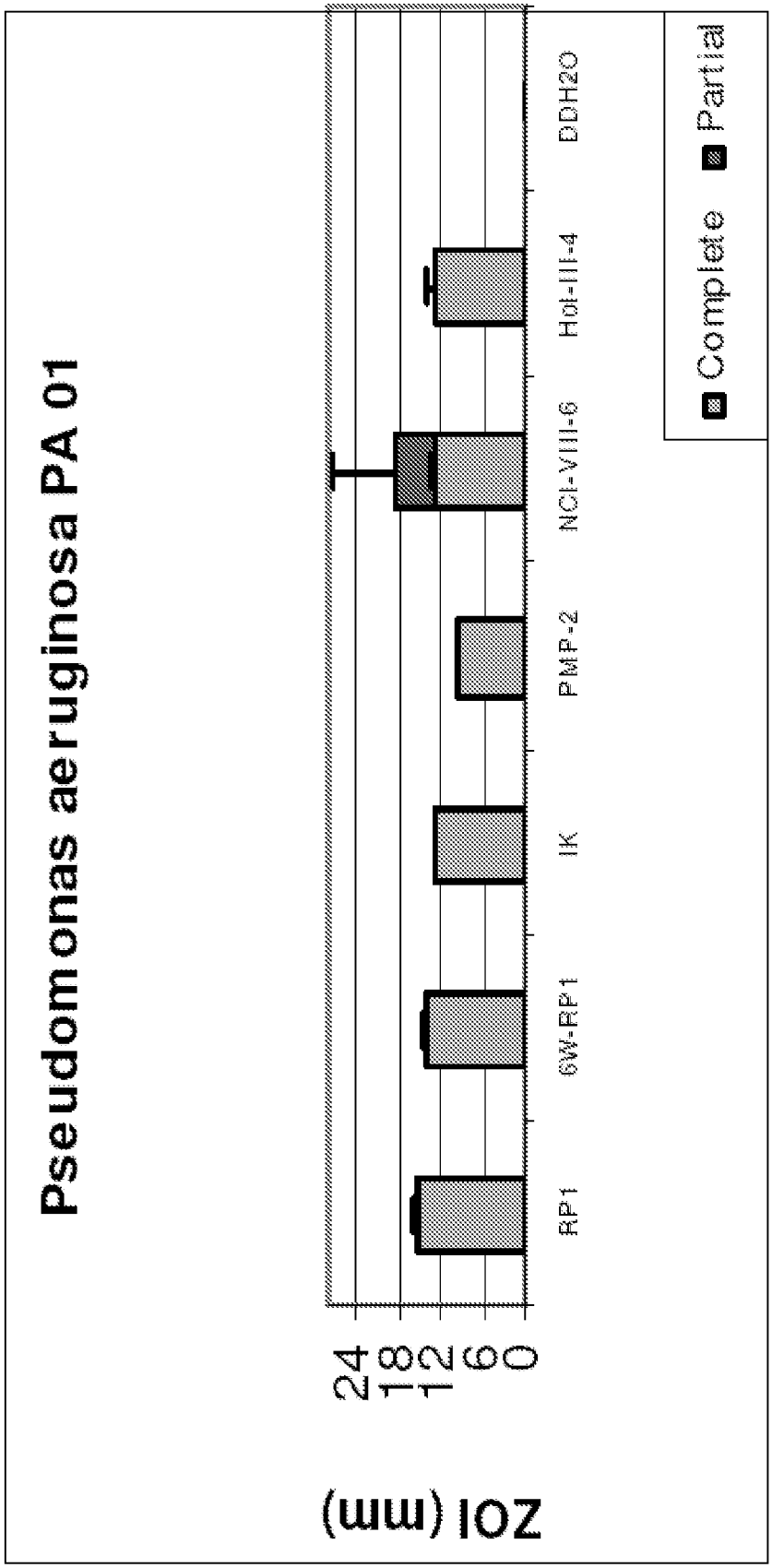

Pseudomonas aeruginosa PA 01
ZOI (mm)
24
18
12
6
0
RP1
GW-RP1
IK
PMP-2
NCI-VIII-6
HoI-III-4
DDH2O
Complete
Partial

Figure 48

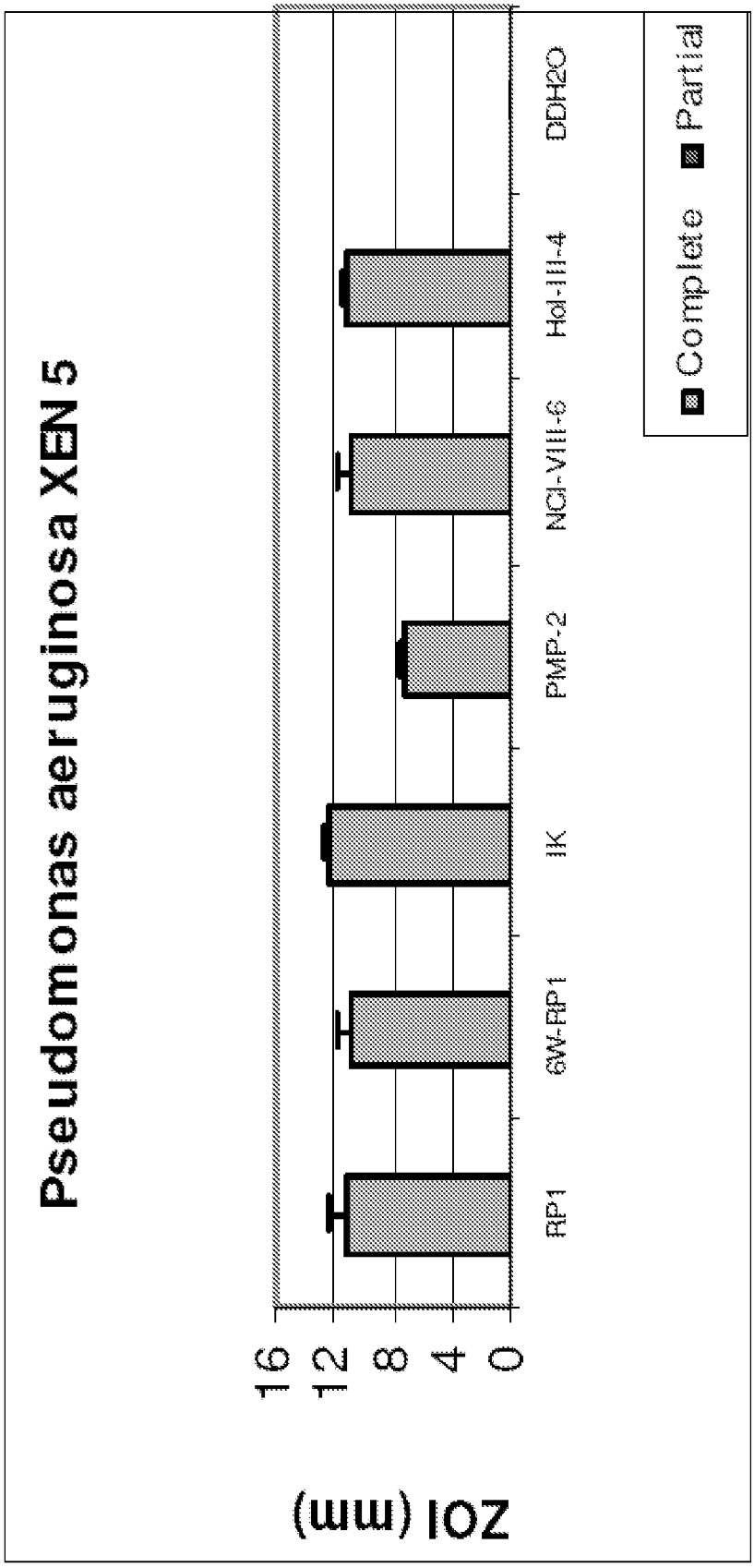

Pseudomonas aeruginosa XEN 5
ZOI (mm)
16
12
8
4
0
RP1
6W-BP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 49

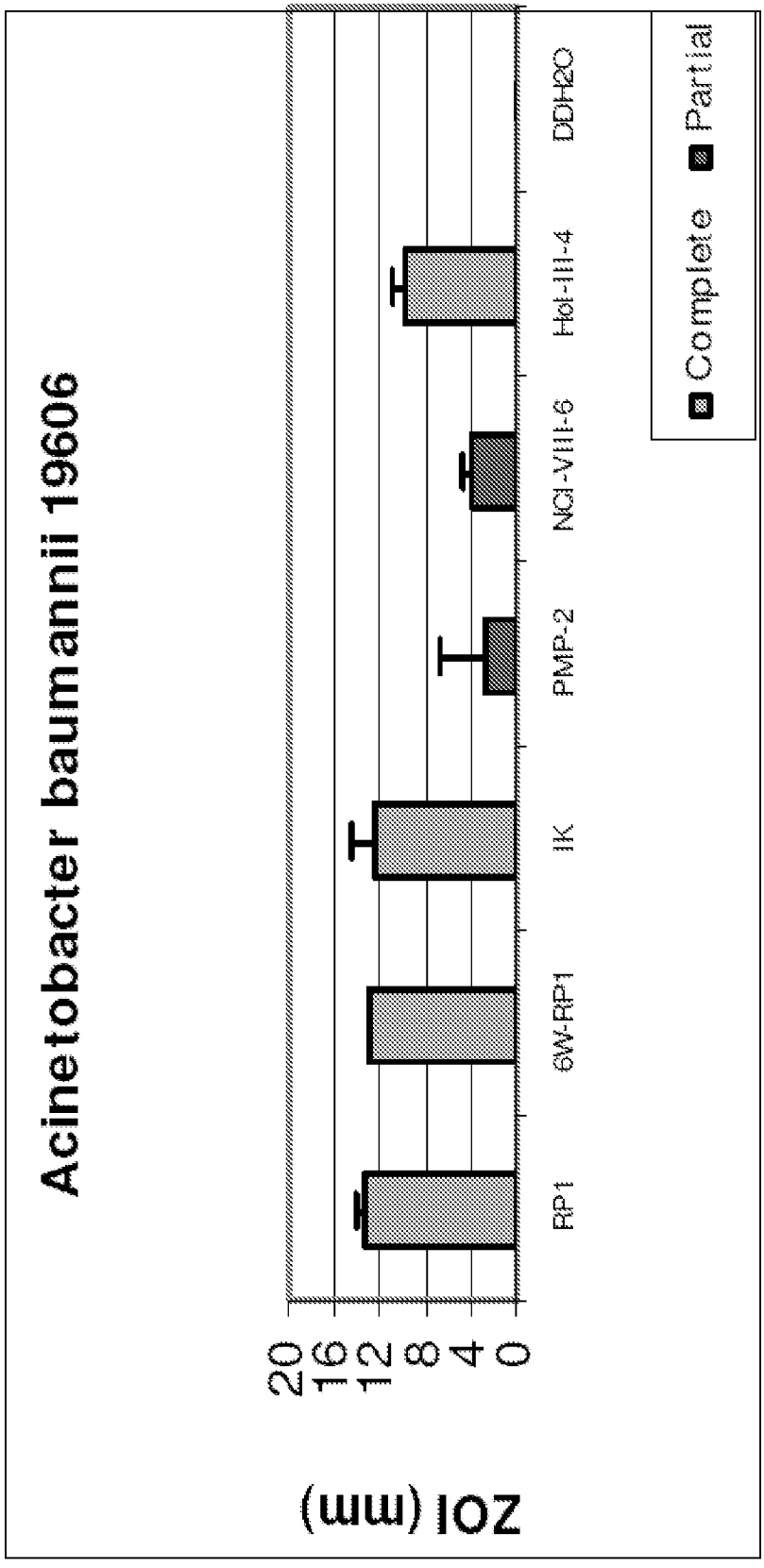

Acinetobacter baumannii 19606
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 50

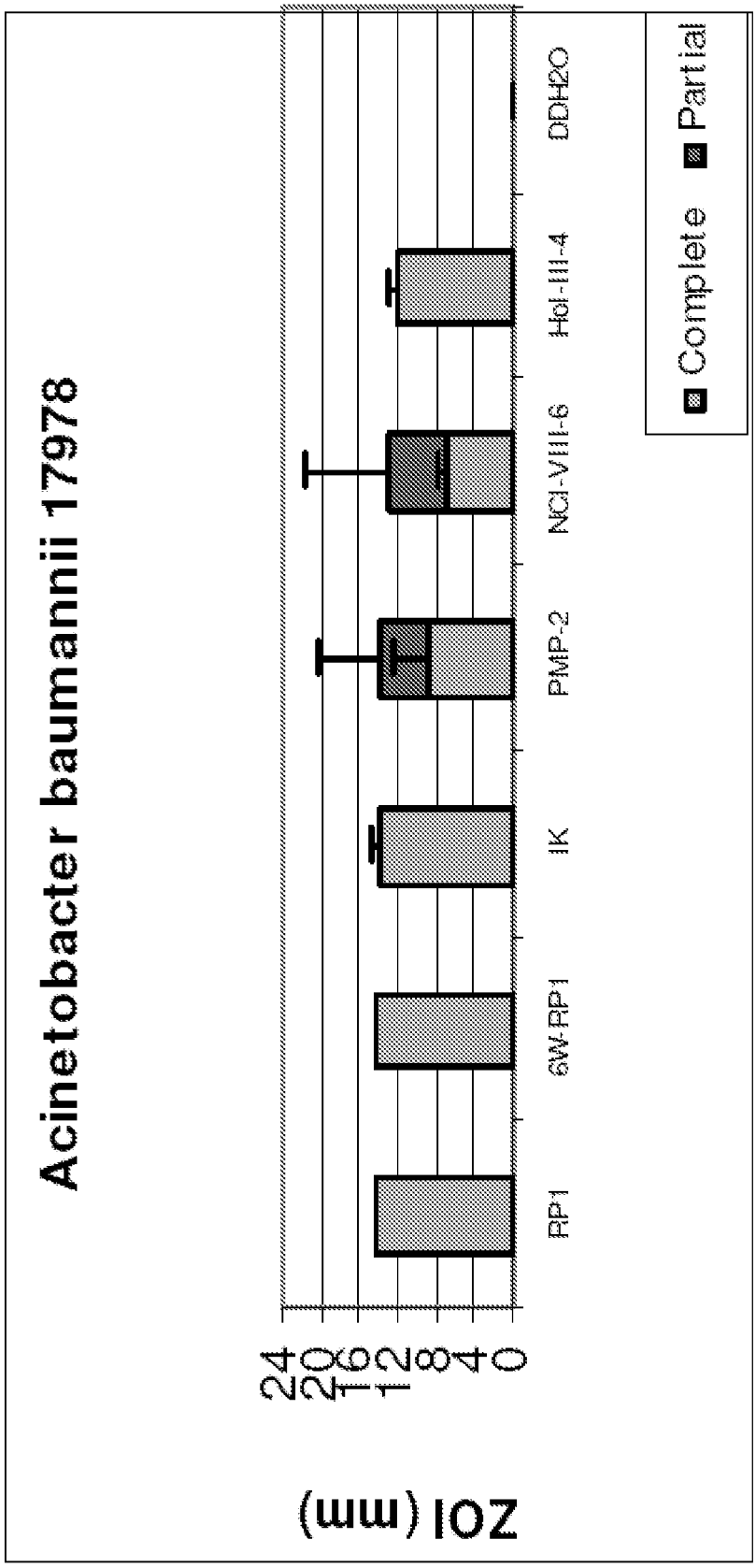

Acinetobacter baumannii 17978
ZOI (mm)
24
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 51

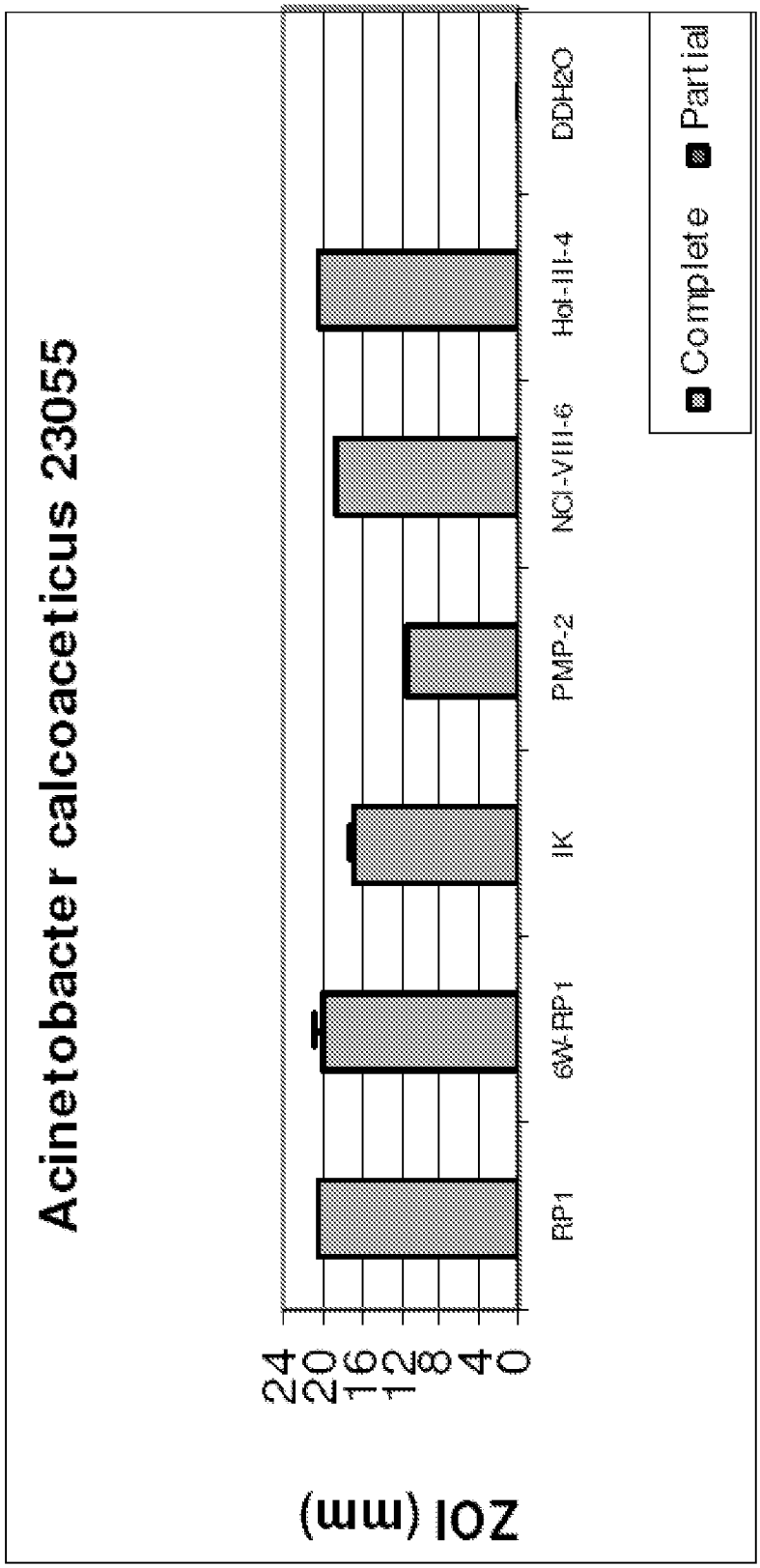

Acinetobacter calcoaceticus 23055
ZOI (mm)
24
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hot-III-4
DDH2O
Complete
Partial

Figure 52

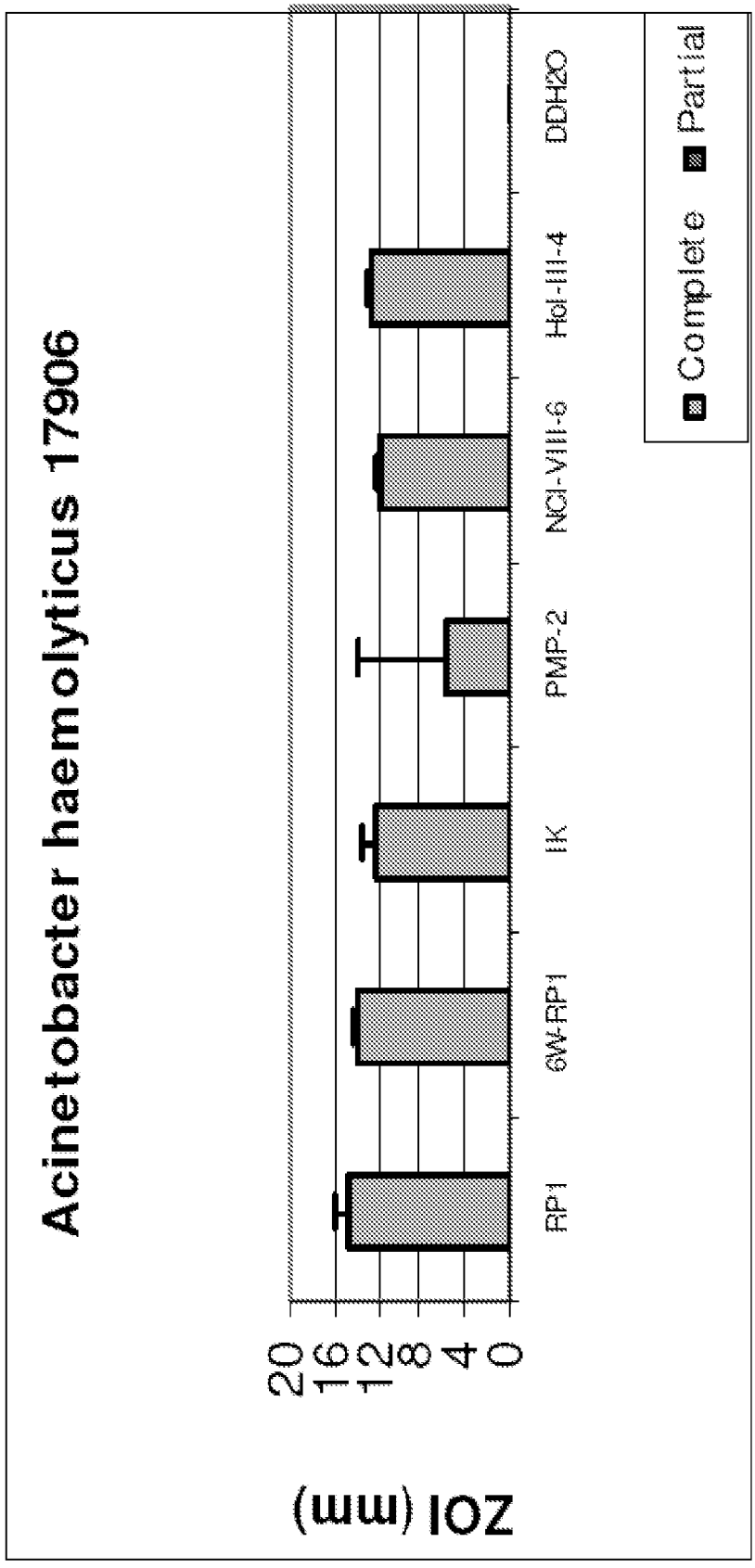

Acinetobacter haemolyticus 17906
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 53

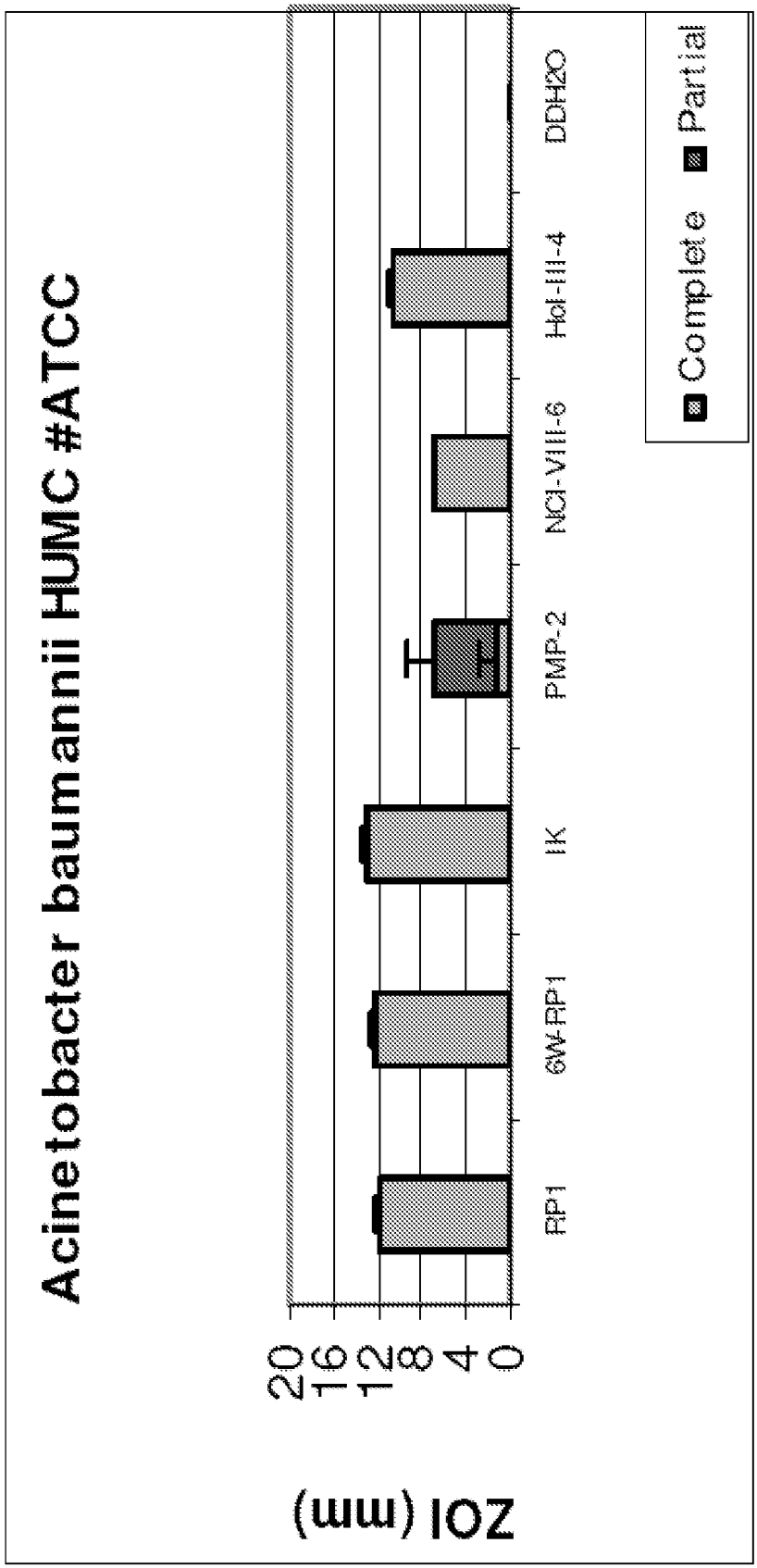

Acinetobacter baumannii HUMC #ATCC
ZOI (mm)
20
16
12
8
4
0
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hal-III-4
DDH2O
Complete
Partial

Figure 54

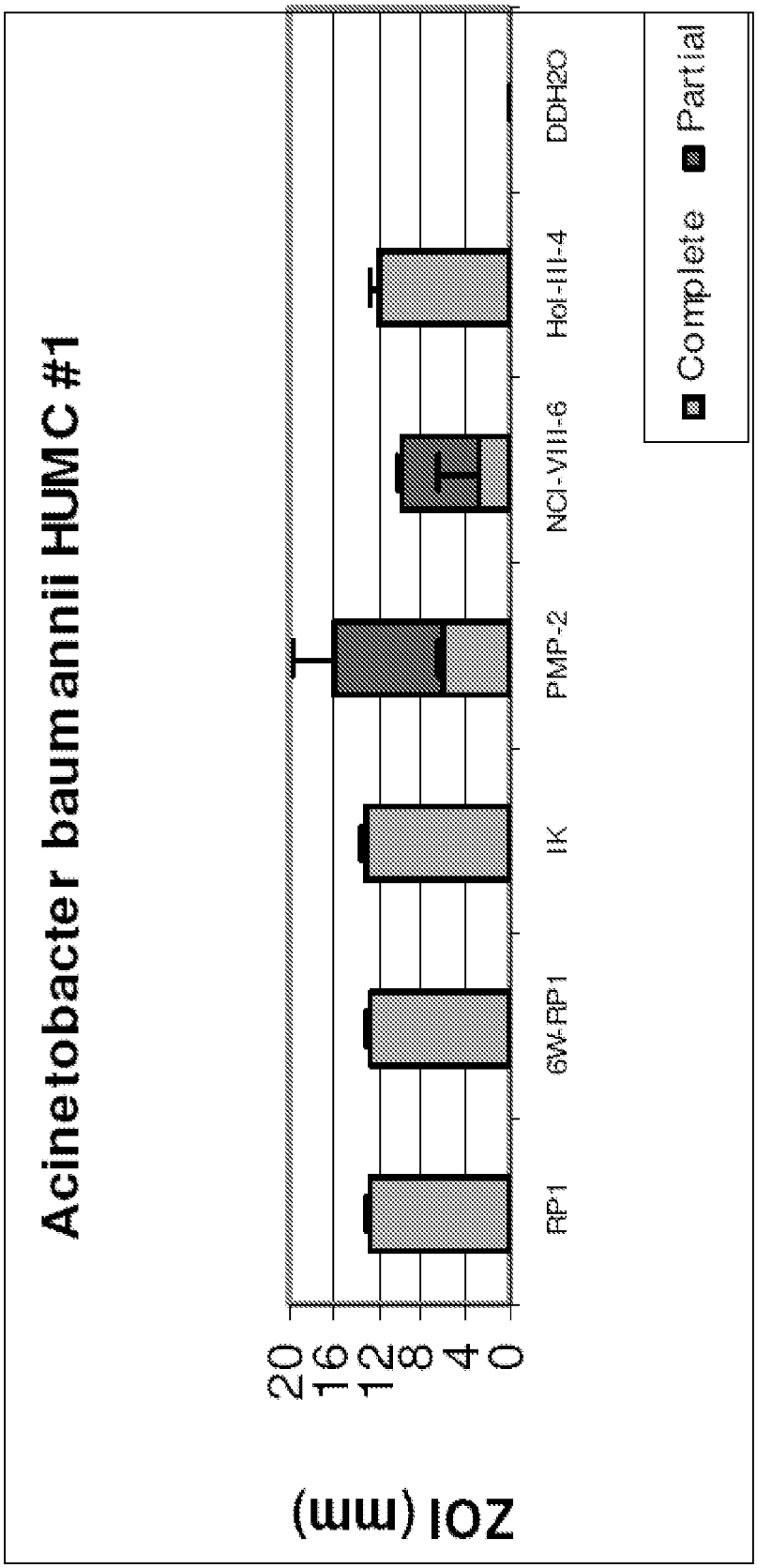

Acinetobacter baumannii HUMC #1
ZOI (mm)
20
16
12
8
4
0
RP1
GW-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 55

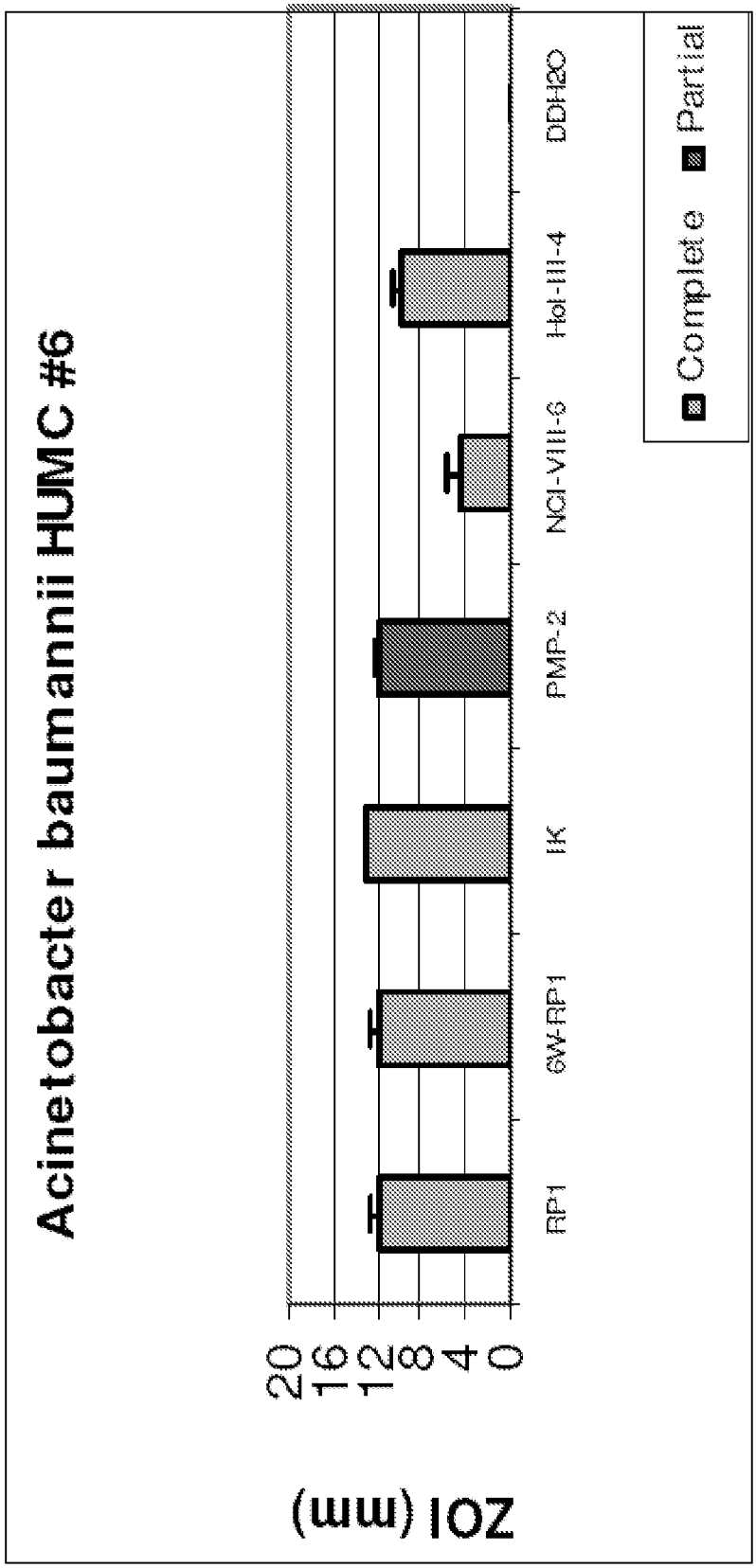

Acinetobacter baumannii HUMC #6
ZOI (mm)
0
4
8
12
16
20
RP1
SW-RP1
IK
PMP-2
NCI-VIII-6
Hol-III-4
DDH2O
Complete
Partial

Figure 56

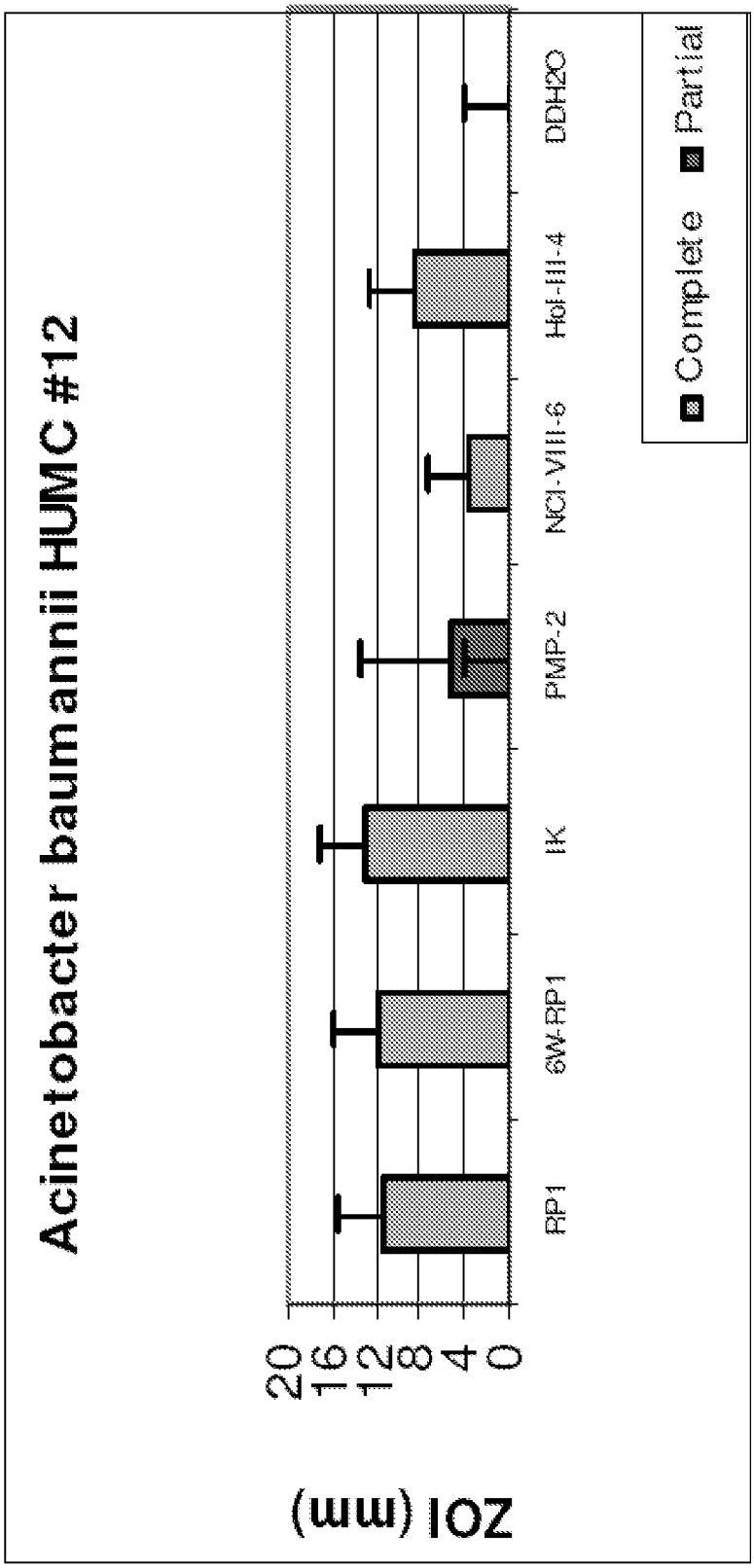

Acinetobacter baumannii HUMC #12
ZOI (mm)
0
4
8
12
16
20
RP1
6W-RP1
IK
PMP-2
NCI-VIII-6
Hof-III-4
DDH2O
Complete
Partial

Figure 57