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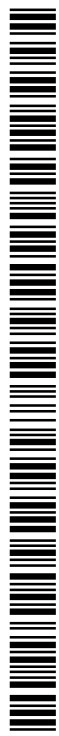
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(54) Title: ANTIMICROBIAL CATIONIC SYSTEMS AND METHODS OF USE

(57) Abstract: The present invention relates to antimicrobial cationic systems and methods of use thereof.



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ANTIMICROBIAL CATIONIC CONSTRUCTS AND METHODS OF USE**CROSS-REFERENCE TO RELATED APPLICATIONS**

The present application claims priority to U.S. Provisional Patent Application Serial
5 No. 62/365,168, filed July 21, 2016, the disclosure of which is hereby incorporated by
reference in its entirety.

FIELD OF THE INVENTION

The field of the invention is to antimicrobial cationic systems and their methods of
use.

BACKGROUND

The spread of antibiotic resistance among bacterial pathogens in both hospital and
community settings is occurring at an alarming rate, particularly to multi-drug resistant
(MDR) and extensively drug resistant (XDR) bacteria. These “superbugs” resistant to
multiple or to all antibiotic classes represent a serious threat to public health and require
15 immediate public attention to prevent a global health crisis. Therefore, there is an urgent
need for new paradigms in antibacterial drug development.

SUMMARY OF THE INVENTION

The present invention solves the issue of growing antibiotic resistance in a number of
bacteria through the creation and utilization of several antimicrobial cationic constructs. In
20 some embodiments, the cationic constructs are capable of binding to a bacterial cell and
delivering an antibacterial agent to a bacterium at a surprisingly high concentration, as
discussed herein, referred to as “cell poisoning” cationic constructs. In other embodiments,
the cationic constructs are capable of binding to and entrapping a bacterial cell, effectively
halting bacterial replication even without use of an antibiotic or other antibacterial agent, as
25 discussed herein, referred to as “cell entrapment” cationic constructs.

Accordingly, in some embodiments, the present invention is directed to a cationic
construct. In some embodiments, the cationic construct is comprised of a cationic polymer
core linked to a plurality of polymers. In some embodiments, each individual polymer in the
plurality of polymers is linked to a functional moiety. In some embodiments, each individual
30 polymer is linked to the functional moiety by a cleavable linker. In some embodiments, the
cleavable linker comprises a disulfide linkage. In some embodiments, the individual
polymers are linked to the functional moiety by click chemistry. In some embodiments, the

cationic polymer core comprises a positively charged peptide sequence. In some embodiments, the cationic polymer core is a homopolymer. In other embodiments, the cationic polymer core is a copolymer. In some embodiments, the cationic polymer core comprises between 10 and 40 repeating monomers. In some embodiments, the cationic polymer core comprises between 10 and 20 repeating monomers. In some embodiments, the cationic polymer core comprises between 20 and 40 repeating monomers. In some embodiments, the cationic polymer core comprises between 20 and 30 repeating monomers. In some embodiments, the cationic polymer core comprises between 10 and 30 repeating monomers. In some embodiments, the cationic polymer core comprises between 30 and 40 repeating monomers. In some embodiments, the cationic polymer core comprises a poly-lysine oligomer. In some embodiments, the cationic polymer core comprises between 10 and 40 repeat lysines. In some embodiments, the cationic polymer core comprises between 10 and 20 repeat lysines. In some embodiments, the cationic polymer core comprises between 20 and 40 repeat lysines. In some embodiments, the cationic polymer core comprises between 20 and 30 repeat lysines. In some embodiments, the cationic polymer core comprises between 10 and 30 repeat lysines. In some embodiments, the cationic polymer core comprises between 30 and 40 repeat lysines. In some embodiments, the cationic polymer core comprises one of 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 and 40 repeat lysines. In some embodiments, the cationic polymer core comprises less than 10 repeat lysines. In some embodiments, the cationic polymer core comprises more than 40 repeat lysines. In some embodiments, the cationic polymer core comprises Lys₂₀. In some embodiments, the cationic polymer core comprises poly-L-lysine. In some embodiments, the cationic polymer core comprises poly-R-lysine.

In some embodiments, the plurality of polymers is comprised of individual polymers. In some embodiments, the individual polymers are amphipathic. In some embodiments, the individual polymers are hydrophobic. In some embodiments, the individual polymers are hydrophilic. In some embodiments, the individual polymers are water-soluble. In some embodiments, the individual polymers comprise poly(ethylene) glycol. In some embodiments, the individual polymers comprise a derivative of poly(ethylene) glycol. In some embodiments, the poly(ethylene) glycol is substituted poly(ethylene glycol). In some embodiments, the poly(ethylene) glycol is di-substituted poly(ethylene glycol). In some embodiments, the substitution is terminally located. In some embodiments, the poly(ethylene) glycol is diamino-poly(ethylene) glycol. In some

embodiments, the poly(ethylene) glycol has a molecular weight between 1,000 and 10,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,000 and 5,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 5,000 and 10,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,000 and 2,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 2,000 and 10,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 2,000 and 5,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,000 and 3,000 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,500 and 2,500 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,800 and 2,200 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,900 and 2,200 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,900 and 2,100 MW. In some embodiments, the poly(ethylene) glycol has a molecular weight between 1,800 and 2,100 MW.

In some embodiments, each of the cationic constructs further comprises a detectable label. In some embodiments, the detectable label is bound to the cationic polymer core. In some embodiments, the detectable label comprises a fluorophore. In some embodiments, the fluorophore comprises a fluorescent protein. In some embodiments, the fluorescent protein comprises one of GFP, YFP, or RFP. In some embodiments, the fluorophore comprises BODIPY. In some embodiments, the detectable label comprises an enzyme. In some embodiments, the enzyme comprises a luciferase enzyme. In some embodiments, the enzyme comprises horseradish peroxidase. In some embodiments, the detectable label comprises a radioisotope. In some embodiments, the radioisotope is I-125.

In some embodiments, the functional moiety comprises a delivery agent. In some embodiments, the delivery agent is an antibacterial agent. In some embodiments, the antibacterial agent comprises a peptide. In some embodiments, the antibacterial agent comprises a lipopeptide. In some embodiments, the antibacterial agent comprises a plycopeptide. In some embodiments, the antibacterial agent is an antibiotic. In some embodiments, the antibiotic comprises a penicillin. In some embodiments, the antibiotic comprise a cephalosporin. In some embodiments, the antibiotic comprises a macrolide. In some embodiments, the antibiotic comprises a fluoroquinolone. In some embodiments, the antibiotic comprises a sulfonamide. In some embodiments, the antibiotic comprises a tetracycline. In some embodiments, the antibiotic comprises an aminoglycoside. In some

embodiments, the antibiotic comprises a nitrofurane. In some embodiments, the antibiotic comprises an oxazolidinone.

In some embodiments, the present invention comprises a system comprising a first cationic construct and a second cationic construct. In some embodiments, the first cationic construct is a cationic construct according to any aspect of this invention. In some
5 embodiments, the second cationic construct is a cationic construct according to any aspect of this invention. In some embodiments, the first cationic construct has a first linking moiety. In some embodiments, the second cationic construct has a second linking moiety. In some embodiments, the first linking moiety comprises an azide. In some embodiments, the first
10 linking moiety comprises an alkyne. In some embodiments, the first linking moiety comprises dibenzocyclooctyne (DBCO). In some embodiments, the second linking moiety comprises an azide. In some embodiments, the second linking moiety comprises an alkyne. In some embodiments, the second linking moiety comprises dibenzocyclooctyne (DBCO). In some embodiments, the first linking moiety is covalently linked to the second linking moiety.
15 In some embodiments, the first linking moiety binds to the second linking moiety through click chemistry. In some embodiments, the first linking moiety comprises an azide and the second linking moiety comprises dibenzocyclooctyne (DBCO). In some embodiments, the second linking moiety comprises an azide and the first linking moiety comprises
20 dibenzocyclooctyne (DBCO). In some embodiments, the first linking moiety comprises an azide and the second linking moiety comprises an alkyne. In some embodiments, the second linking moiety comprises an azide and the first linking moiety comprises an alkyne.

In some embodiments, the present invention is directed to a method of delivering a delivery agent to a target cell. In some embodiments the method comprises the steps of i. providing a target cell; and ii. contacting the target cell with a cationic construct having a
25 delivery agent under conditions allowing for the delivery agent to 1) internalize in the host cell or the host cell membrane and 2) release the delivery agent from the cationic construct. In some embodiments, release of the delivery agent occurs by cleavage of a cleavable linker. In some embodiments, the cleavable linker is a disulfide bond. In some embodiments, release of the delivery agent occurs by an oxidative reaction. In some embodiments, release
30 of the delivery agent occurs intracellularly. In some embodiments, release of the delivery agent occurs in a host cell membrane. In some embodiments, release of the delivery agent occurs under reductive conditions. In some embodiments, release of the delivery agent occurs under oxidative conditions.

In some embodiments, the delivery agent is an antibacterial agent. In some embodiments, the antibacterial agent is an antibiotic. In some embodiments, the antibiotic comprises a penicillin. In some embodiments, the antibiotic comprise a cephalosporin. In some embodiments, the antibiotic comprises a macrolide. In some embodiments, the antibiotic comprises a fluoroquinolone. In some embodiments, the antibiotic comprises a sulfonamide. In some embodiments, the antibiotic comprises a tetracycline. In some embodiments, the antibiotic comprises an aminoglycoside. In some embodiments, the antibiotic comprises a nitrofurantoin. In some embodiments, the antibiotic comprises an oxazolidinone.

In some embodiments, the present invention is directed to a method of entrapping a target cell comprising: i. providing a target cell; and ii. contacting the target cell with a plurality of first cationic constructs having first linking moieties and a plurality of second cationic constructs having second linking moieties under conditions allowing individual first cationic constructs to covalently link to individual second cationic constructs through covalently linking the first linking moieties to the second linking moieties in an amount sufficient to entrap the target cell.

In some embodiments, the first linking moieties comprise alkynes. In some embodiments, the first linking moieties comprise dibenzocyclooctyne (DBCO). In some embodiments, the second linking moieties comprise azides (*e.g.* N_3). In some embodiments, the second linking moieties comprise alkynes. In some embodiments, the first linking moieties bind to the second linking moieties through click chemistry. In some embodiments, the first cationic constructs are conjugated to the second cationic constructs *via* a triazole moiety. In some embodiments, the triazole moiety is formed *via* click chemistry. In some embodiments, the click chemistry proceeds under copper-free conditions. In some embodiments, the click chemistry proceeds *in vivo*. In some embodiments, the first linking moieties comprise azides and the second linking moiety comprise dibenzocyclooctyne (DBCO). In some embodiments, the second linking moieties comprises azides and the first linking moieties comprise dibenzocyclooctyne (DBCO). In some embodiments, the first linking moiety comprise azides and the second linking moieties comprise alkynes. In some embodiments, the second linking moieties comprise azides and the first linking moieties comprise alkynes.

In some embodiments, the target cell is a bacterial cell. In some embodiments, the bacterial cell is a Gram-positive bacterial cell. In some embodiments, the bacterial cell is a Gram-negative bacterial cell. In some embodiments, the bacterial cell is pathogenic. In some

embodiments, the bacterial cell is a multi-drug resistant (MDR) bacterial cell. In some embodiments, the bacterial cell is an extensively-drug resistant (XDR) bacterial cell.

In some embodiments, the present invention is directed to a method of treating an infection in a subject in need thereof. In some embodiments, the infection is a bacterial
5 infection. In some embodiments, the method comprises administering a cationic construct of the present invention to the patient in need thereof. In some embodiments, the cationic construct is a “cell poisoning” construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment” construct according to any aspect of the present invention. In some embodiments, the subject is an
10 immunocompromised subject. In some embodiments, the immunocompromised subject is infected with human immunodeficiency virus (HIV). In some embodiments, the cationic construct is co-administered with one or more antibacterial agents.

In some embodiments, the present invention is directed to a medical device coated with a cationic construct according to any aspect of the present invention. In some
15 embodiments, the medical device is an implant or prosthesis. In some embodiments, the medical device is infused with a cationic construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell poisoning” construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment” construct according to any aspect of the present invention.

In some embodiments, the present invention is directed to a packaging material coated with or containing a cationic construct according to any aspect of the present invention. In some
20 embodiments, the packaging material is a food packaging material. In some embodiments, the cationic construct is a “cell poisoning” construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment”
25 construct according to any aspect of the present invention.

In some embodiments, the present invention is directed to a method of disinfecting a surface comprising contacting the surface with a composition comprising a cationic construct according to any aspect of the present invention. In some embodiments, the surface is an environmental surface. In some embodiments, the cationic construct is a “cell poisoning”
30 construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment” construct according to any aspect of the present invention.

In some embodiments, the present invention is directed to a method of disinfecting a food or water source comprising contacting the food or water source with a cationic construct

according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell poisoning” construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment” construct according to any aspect of the present invention.

5 In some embodiments, the present invention is directed to a cationic construct for use in the treatment or prevention of a bacterial infection in a patient or subject in need thereof. In some embodiments, the cationic construct is a cationic construct according to any aspect of the present disclosure. In some embodiments, the cationic construct is a “cell poisoning” construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment” construct according to any aspect of the present invention.

In some embodiments, the present invention is directed to the use of a cationic construct in the manufacture of a medicament for the treatment or prevention of a bacterial infection in a patient or subject in need thereof. In some embodiments, the cationic construct is a cationic construct according to any aspect of the present disclosure. In some embodiments, the cationic construct is a “cell poisoning” construct according to any aspect of the present invention. In some embodiments, the cationic construct is a “cell entrapment” construct according to any aspect of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

20 FIG. 1 represents the structure of the cellular membrane in gram-negative and gram-positive bacteria.

FIG. 2 represents the structure of the lipopolysaccharide (LPS) unit found in the bacterial outer membrane (OM).

FIG. 3 represents an exemplary embodiment of the “cell poisoning” cationic construct. The positively-charged poly-lysine core attaches to the negatively-charged bacterial cell outer membrane *via* electrostatic attraction. Among other things, this greatly weakens the cellular membrane integrity of the bacterial cell. The poly(ethylene) glycol “arms” are bound to an antibacterial agent (*e.g.* rifampicin) and internalize into the bacterial cell due in part to the structurally compromised cellular membrane. The arms are bound to the payload through a cleavable disulfide bond. Once internalized into the bacterial cell, the reducing environment found within the cell cleaves the disulfide bond. The antibacterial agent is then free to act upon the bacterial cell.

FIG. 4 represents an exemplary embodiment of the “cell entrapment” cationic construct. The positively-charged poly-lysine core attaches to the negatively-charged bacterial cell outer membrane *via* electrostatic attraction. However, instead of being bound to an antibacterial agent, the poly(ethylene) glycol “arms” are bound to a linking moiety. There are two distinct linking moieties, a first linking moiety and a second linking moiety. As illustrated, the first linking moiety binds to the second linking moiety when brought into close contact with each other on the cellular membrane. This leads to an interlinking network of cationic constructs covalently linked to one another entrapping the bacterial cell.

FIGs. 5A and 5B represent an exemplary click-chemistry reaction.

FIGs. 6A and 6B represent a generic scheme for construct assembly. Figure 6A represents construction of the “cell poisoning” cationic constructs whereas Figure 6B represents construction of the “cell entrapment” cationic constructs.

FIG. 7 represents a synthetic strategy for generation of the “cell poisoning” constructs.

FIG 8 represents a synthesis of click-reactive rifampicin with a cleavable disulfide linker.

FIG. 9 represents a synthetic strategy for generation of the “cell entrapment” constructs.

FIGs. 10A, 10B and 10C represent the binding of molecular constructs to bacterial cells. Figure 10A shows fluorescence of labeled cationic constructs binding to *E. coli*. Figure 10B shows bound probes per cell for three different bacterial cell types. Figure 10C shows the relative binding for each.

FIGs. 11A and 11B represent the inhibition of *B. subtilis* growth by the “cell poisoning” constructs. Figure 11A represents that both full and partially assembled constructs completely blocked the growth of *B. subtilis* at 2 μ M concentration. Figure 11B represents that charging with rifampicin further increased growth inhibitory activity of the construct, illustrating that rifampicin was cleaved from the constructs and released into the cells.

FIG. 12 represents a proposed oxidative mechanism for releasing rifampicin from a cationic construct.

FIGs. 13A and 13B represent the measurement of a click chemistry reaction rate under biologically relevant conditions. Figure 13A represents the reaction. Figure 13B represents the product yield over time.

DETAILED DESCRIPTION OF THE INVENTION

The cationic construct of the present invention generally comprises a cationic polymer core that is linked to a plurality of polymers, each individual polymer in the plurality of the polymers being linked to a functional moiety. The connection of these components into a cationic construct can be performed via a linear synthetic route or a convergent route. A linear synthesis route starts from a single intermediate and subsequently incorporates different components to build the target compound. A convergent route incorporates several separately prepared components into the target. The constructs display high binding affinity and specificity towards bacteria with potent growth inhibitory activity demonstrated *in vitro*. The constructs are preferably constructed from non-toxic materials. The constructs represent a new paradigm in anti-bacterial drug design.

The cationic constructs of the present invention generally comprise a cationic polymer core that is linked to a plurality of polymers, each individual polymer in the plurality of the polymers being linked to a functional moiety. The cationic core can be any cationic polymer, *i.e.* a polymer having a net positive charge under biologically relevant conditions (*e.g.* within biologically relevant pH parameters. Purely by way of example, in exemplary embodiments, the cationic polymer is a poly-lysine polymer. At physiological pH, lysine has a net positive charge (*i.e.* cationic), with an isoelectric point around a pH of approximately 10. One of ordinary skill in the art will be familiar with how to calculate isoelectric points and net charges of different potential monomers and/or polymers for incorporation into the cationic constructs of the present invention. For example, arginine is a basic amino acid with an isoelectric point approximately around 11, and would therefore be suitable for incorporation into the present invention. Other cationic polymer cores do not necessarily need to be comprised of amino acid oligomers, for example other common cationic polymers may include amphiphilic cationic polymers, or polymers that have been aminated, such as aminated collagen, or chitosan (under acidic conditions), polyethylenimine (PEI), polyamidoamine, poly(amino-*co*-ester)s, poly[2-(*N,N*-dimethylamino)ethyl methacrylate] (PDMAEMA), and other cationic polymers. The cationic polymer core does not necessarily need to have every monomer be cationic, so long as the overall charge of the cationic polymer core remains positive. For example, a co-polymers, which are expressly considered part of the present invention, may include one (or more) cationic monomer(s) and one (or more) neutral monomer(s) in a repeating subunit, or may include two (or more) distinct cationic monomers. While not wishing to be bound by theory, a less cationic polymer core, while potentially (but not necessarily) having lower binding affinity to bacterial cells, may

have reduced toxicity *in vivo*. One of ordinary skill in the art will be able to appreciate such distinctions and where they may be applicable. For example, in some embodiments, the polymer core contains a single peptide. In some embodiments, the polymer core contains two or more peptides which may be linked to each other through a spacer. Non-limiting examples of the spacer include alkylene, amino acid, ester, amide or other inert moieties. In an exemplary embodiment, two lysine-containing peptides may be spaced from each other via a non-branched amino acid (*e.g.* glycine) or peptide.

The cationic polymer core is linked to a plurality of polymer linkers. The individual polymer linkers linked between the functional moiety and the cationic core can generally be prepared from one or more non-immunogenic polymers. Non-limiting examples of the polymers include dextrans, carbohydrate-based polymers, polyalkylene oxide, polyvinyl alcohols and other similar non-immunogenic polymers. Further examples include, linear or branched, poly(alkylene glycol), poly(olefinic alcohol), poly(vinylpyrrolidone) (PVP), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharide), poly(α -hydroxy acid), poly(acrylic acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), or copolymers or terpolymers thereof. The average molecular weight of the polymer ranges from about 100 to about 100,000 daltons, all subranges included.

In some embodiments, the individual polymer linkers linked between the functional moiety and the cationic core contain polyalkylene oxide. It should be understood that a wide variety of water soluble polymers including but not limited to poly(ethylene)glycol and other related polymers, including poly(dextran) and polypropylene glycol), are suitable for use in the practice of this invention and that the use of the term PEG or poly(ethylene glycol) is intended to encompass and include all such molecules. The term PEG includes, but is not limited to, poly(ethylene glycol) in any of its forms, including bifunctional PEG, multiarmed PEG, derivatized PEG, forked PEG, branched PEG, pendent PEG (*i.e.* PEG or related polymers having one or more functional groups pendent to the polymer backbone), or PEG with degradable linkages therein. Additional benefits of PEG include “stealth” protection from proteases that degrade poly-L-lysine to increase half-life.

The cationic polymer core may, in some embodiments, contain a terminal group that allows the core to be coupled to the individual polymer linkers. In some embodiments, the terminal group allows the cationic polymer core to be coupled to the individual polymer linkers through click chemistry. Non-limiting examples of the functional moieties include hydroxyl, alkoxyl, alkyne, active ester (*e.g.* N-hydroxysuccinimidyl esters and 1-

benzotriazolyl esters), active carbonate (*e.g.* N-hydroxysuccinimidyl carbonates and 1-benzotriazolyl carbonates), acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, amine, aminooxy, hydrazide, protected hydrazide, thiol, carboxylic acid, carboxylic acid, isocyanate, isothiocyanate, maleimide, aromatic or vinyl sulfone, acrylate, bromo or iodo acetamide, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, tresylate, alkene, dibenzocyclooctyl (DBCO), carbonyl, 2-amino-benzaldehyde or 2-amino-acetophenone group, oxime, potassium acyltrifluoroborate, O-carbamoylhydroxylamine, trans-cyclooctene, tetrazine, and azide. Various linkages resulting from the coupling with the polymer linker include, but are not limited to, amide, urea, thiourea, ester, amide, carbamate, heterocycles. These functional groups, if necessary, can be protected prior to coupling to prevent interference with other reactions. The procedures for the reaction between the polymer core and the polymer linker are well known in the field and can be readily practiced by one of ordinary skill in the art without undue experiment.

The cationic polymer core and the individual polymer linkers can be linear or branched. Branched polymer backbones are generally known in the art. Typically, a branched polymer has a central branch core moiety and a plurality of linear polymer chains linked to the central branch core. PEG is commonly used in branched forms that can be prepared by addition of ethylene oxide to various polyols, such as glycerol, glycerol oligomers, pentaerythritol and sorbitol. The central branch moiety can also be derived from several amino acids, such as lysine. Exemplary embodiments use poly-L-lysine. Additionally, poly-D-lysine is suitable for use in the cationic constructs of the present invention. In particular, poly D-lysine based constructs, as opposed to poly-L-lysine constructs, may have increased metabolic stability, by preventing proteolysis of the cationic polymer core. This could be advantageous depending on the particular application. While poly-L-lysine is known in the art as an anti-microbial compound, the use of such alone to impart anti-microbial effects is often bit sufficient due to rapid degradation. Therefore, in some embodiments, the present invention disclose use of, *e.g.*, PEG “arms” to functionalize the cationic constructs.

The individual polymer linkers may also contain one or more structural units, which include for example, branch or linear, substituted or unsubstituted, C₁₋₁₀ alkene, (CH₂)_aC(O)NR¹(CH₂)_b-, -(CH₂)_aO(CH₂CH₂O)_c-, -(CH₂)_aheterocyclyl-, -(CH₂)_aC(O)-, and -(CH₂)_a NR¹-, -CR¹=N-NR¹-, -CR¹=N-O-, -CR¹=N-NR²-CO-, -N=N-CO-, -S-S-, wherein a, b, and c are each an integer selected from 0 to 25, all subunits included; and R¹ and R²

independently represent hydrogen or a C₁-C₁₀ alkyl. One or more carbon atoms of the C₁₋₁₀ alkylene may be replaced with a heteroatom selected from O, S, and N. These structural units can be inserted into a polymer or connected to a terminal end of a polymer.

The polymer linker may also contain terminal groups for bonding to the cationic core and/or the functional moiety. In some embodiments, the terminal group allows the polymer linkers be coupled to the cationic core/and or the functional moieties through click chemistry. Non-limiting examples of the terminal groups include hydroxyl, alkoxyl, alkyne, active ester (*e.g.* N-hydroxysuccinimidyl esters and 1-benzotriazolyl esters), active carbonate (*e.g.* N-hydroxysuccinimidyl carbonates and 1-benzotriazolyl carbonates), acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, amine, aminoxy, hydrazide, protected hydrazide, thiol, carboxylic acid, carboxylic acid, isocyanate, isothiocyanate, maleimide, aromatic or vinyl sulfone, acrylate, bromo or iodo acetamide, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, tresylate, alkene, dibenzocyclooctyl (DBCO), carbonyl, 2-amino-benzaldehyde or 2-amino-acetophenone group, oxime, potassium acyltrifluoroborate, O-carbamoylhydroxylamine, trans-cyclooctene, tetrazine, and azide. These functional groups, if necessary, can be protected prior to coupling to prevent interference with other reactions.

One of ordinary skill in the art will readily recognize that a specific function group on the polymer linker needs to match with the reaction partner on the respective coupling moiety. For example, if the polymer linker bears a terminal isothiocyanate group, the respective cationic core preferably has an amino group to form a desired thiourea linkage. If the polymer linker bears a terminal DBCO group, the respective payload moiety should have an azido group to form a desired triazine linkage. The alkyne may also be placed on the payload moiety, which will require a matching azido on the polymer linker.

Various types of linkages may result from the coupling of a functional group with its respective reaction partner. Non-limiting examples of the linkages within a structural component or between different structural components include ester, amide, disulfide, ether, amino, carbamate, hydrazone, thioether, oxime, carbonate, urea, thiourea, carbocycles, and heterocycles. In further examples, a thioisocyanate may react with an amine to form a thiourea linkage between the polymer linker and the cationic core. A triazole is formed between the polymer linker and the payload moiety through the coupling between DBCO and azido group. In further examples, linkages can be formed through the reaction between carbonyl and hydrazide, and between trans-cyclooctene and tetrazine.

Each of the functional moiety, polymer linker, and cationic core may independently contain a spacer, which also plays an important role in adjusting the conformation, reactivity, bioavailability and other characteristics of the cationic construct. Non-limiting examples of the spacer include carbonyl, alkylene, alkenylene, amino, sulfide, sulfone, sulfoxide, ether, C₃-C₈ carbocyclyl, aryl, aryl, heteroaryl, heterocyclyl, poly-alkylene oxide, amino acid, peptide, and any combination of these groups.

The cationic constructs may also contain a detectable label. Non-limiting examples of the detectable label include fluorophores (e.g. DAPI, EBFP, BODIPY, cyanamides, etc.), radioisotopes (e.g. I-123, I-125, ^{99m}Tc etc.), fluorescent proteins (e.g. GFP, RFP, YFP), and enzymatic labels (e.g. horseradish peroxidase (HRP), alkaline phosphatase (AP), glucose oxidase, β -galactosidase, biotin, etc.). The labels may be attached to the cationic core and/or the individual polymer linker *via* a chemical linkage. Non-limiting examples of the linkage include amide, carbamate, ester, and ether. The procedure for attaching the detectable label is well known in the field and one of ordinary skill in the art can readily identify a suitable synthesis route without undue experiment.

The bacteria may be either a Gram positive or Gram negative bacteria. The bacteria may comprise any of the following, non limiting examples of bacteria: *Actinomyces spp.* (including but not limited to *A. israelii*), *Bacillus spp.* (including but not limited to *B. anthracis* and *B. cereus*), *Bacteroides spp.* (including but not limited to *B. fragilis*), *Bartonella spp.* (including but not limited to *B. henselae* and *B. quintana*), *Bordetella spp.* (including but not limited to *B. pertussis*), *Borrelia spp.* (including but not limited to *B. burgdorferi*, *B. garinii*, *B. afzelii*, and *B. recurrentis*), *Brucella spp.* (including but not limited to *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis*), *Campylobacter spp.* (including but not limited to *C. jejuni*), *Chlamydia spp.* (including but not limited to *C. pneumonia* and *C. trachomatis*), *Clamydophila spp.* (including but not limited to *C. psittaci*), *Clostridium spp.* (including but not limited to *C. botulinum*, *C. difficile*, *C. perfringens*, and *C. tetani*), *Corynebacterium spp.* (including but not limited to *C. diphtheriae*), *Ehrlichia spp.* (including but not limited to *E. canis* and *E. chaffeensis*), *Enterococcus spp.* (including but not limited to *E. faecalis* and *E. faecium*), *Escherichia spp.* (including but not limited to *E. coli*), *Francisella spp.* (including but not limited to *F. tularensis*), *Haemophilus spp.* (including but not limited to *H. influenzae*), *Helicobacter spp.* (including but not limited to *H. pylori*), *Klebsiella spp.* (including but not limited to *K. pneumoniae*), *Legionella spp.* (including but not limited to *L. pneumophila*), *Leptospira* (including but not limited to *L. interrogans*, *L. santarosai*, *L. weilii*, and *L. noguchii*), *Listeria spp.* (including but not limited to *L.*

monocytogenes), *Mycobacterium* spp. (including but not limited to *M. africanum*, *M. bovis*, *M. kansasii*, *M. leprae*, *M. microti*, *M. tuberculosis*, and *M. ulcerans*), *Mycoplasma* spp. (including but not limited to *M. pneumonia*), *Neisseria* spp. (including but not limited to *N. gonorrhoeae* and *N. meningitidis*), *Nocardia* spp. (including but not limited to *N. asteroides*),
 5 *Pseudomonas* spp. (including but not limited to *P. aeruginosa*), *Rickettsia* spp. (including but not limited to *R. rickettsii*), *Salmonella* spp. (including but not limited to *S. typhi* and *S. typhimurium*), *Shigella* spp. (including but not limited to *S. sonnei*), *Staphylococcus* spp. (including but not limited to *S. aureus*, *S. epidermidis*, and *S. saprophyticus*), *Streptococcus* spp. (including but not limited to *S. agalactiae*, *S. pneumonia*, and *S. pyogenes*), *Treponema*
 10 *spp.* (including but not limited to *T. pallidum*), *Ureaplasma* spp. (including but not limited to *U. urealyticum*), *Vibrio* spp. (including but not limited to *V. cholerae*), and *Yersinia* spp. (including but not limited to *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*).

The constructs of the present invention may be used to treat any conditions or pathologies (*e.g.* infection) associated with any of the bacteria species disclosed herein. For
 15 example, such non-limiting conditions or pathologies may include but are not limited to any of the following: opportunistic infections, actinomycosis, anthrax including pulmonary anthrax, gastrointestinal anthrax, and cutaneous anthrax, whooping cough, bronchitis, pneumonia including but not limited to atypical pneumonia, hospital-acquired pneumonia, neonatal pneumonia, and necrotizing pneumonia, sinus infection and/or upper respiratory
 20 tract infections, otitis including but not limited to inner ear infections, middle ear infections, and external otitis, fevers, Lyme disease, brucellosis, food poisoning, diarrhea, enteritis, dysentery, trachoma, conjunctivitis, urinary tract infections, kidney infections, urethritis including but not limited to nongonococcal urethritis and gonococcal urethritis, pelvic inflammatory disease, epididymitis, prostatitis, lymphogranuloma venereum, psittacosis,
 25 botulism, pseudomembranous colitis, anaerobic cellulitis, gangrene including but not limited to gas gangrene, sepsis including but not limited to hospital acquired sepsis and Waterhouse-Friderichsen syndrome, septicemia, tetanus, diphtheria, ehrlichiosis, bacterial endocarditis, biliary tract infections, meningococcal disease including but not limited to bacterial meningitis, encephalitis, hemolytic-uremic syndrome, tularemia, peptic ulcers, gastritis
 30 including but not limited to chronic gastritis, klebsiella pneumonia, Legionnaire's disease, Pontiac fever, leptospirosis, listeriosis, leprosy, tuberculosis, septic arthritis, osteomyelitis, nocardiosis, Rocky mountain spotted fever, Typhoid fever, salmonellosis, paratyphoid fever, shigellosis, impetigo, toxinoses including but not limited to scalded skin syndrome and toxic shock syndrome, cystitis, endometriosis, pharyngitis, scarlet fever, rheumatic fever,

erysipelas, puerperal fever, fasciitis including but not limited to necrotizing fasciitis, glomerulonephritis, syphilis including but not limited to congenital syphilis and neurosyphilis, cholera, plague including but not limited to bubonic plague and pneumonic plague, dental cavities/caries, abscesses or localized infection including of any organ or
 5 tissue, *e.g.* brain, brainstem, spinal cord, ventricular system, eye, ear, olfactory epithelium, tongue, skin including subcutaneous tissue, liver, kidney, stomach, lungs, gall bladder, mouth, pharynx, larynx, trachea, bronchi, esophagus, small intestine, large intestine, pancreas, diaphragm, ureters, bladder, urethra, ovaries, fallopian tubes, uterus, vagina, placenta, testes, epididymis, vas deferens, seminal vesicles, prostate, bulbourethral glands,
 10 penis, scrotum, pituitary gland, pineal gland, thyroid gland, parathyroid gland, adrenal gland, heart, arteries, veins, capillaries, lymphatic vessel, lymph nodes, bone marrow, thymus, spleen, tonsils, and combinations thereof.

In some embodiments, the functional moiety contains a delivery agent or a therapeutic agent, sometimes referred to as “cell poisoning” constructs. Synthesis of exemplary “cell
 15 entrapment” cationic constructs is shown in Example 1 *infra*. The delivery agent can be a cytotoxic or immunomodulatory agent. In exemplary embodiments, the delivery agent is an antibacterial agent. In some embodiments, the antibacterial agent is an antibiotic. The antibacterial agent can be bacteriostatic or it can be bacteriolytic. Common examples of antibiotics include aminoglycosides, beta lactams, amphenicols, antifolates, glycopeptides,
 20 rifamycins, macrolides, quinolones, fluoroquinolones, tetracyclines, sulfonamides, and trimethoprim. For example, the antibiotic can be any of the following non limiting examples: Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin, Paromomycin, Streptomycin, Spectinomycin, Gedlanamycin, Herbimycin, Rifaximin, Loracarbef, Ertapenem, Doripenem, Imipenem, Cilastatin, Meropenem, Cefadroxil,
 25 Cefazolin, Cefalotin, Cefalothin, Cefalexin, Cefaclor, Cefamandole, Cefoxitin, Cefprozil, Cefuroxime, Cefixime, Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, Ceftriaxone, Cefepime, Ceftaroline fosamil, Ceftobiprole, Teicoplanin, Vancomycin, Telavancin, Dalbavancin, Ortiavancin, Clindamycin, Lincomycin, Daptomycin, Azithromycin, Clarithromycin, Dirithromycin, Erythromycin,
 30 Roxithromycin, Troleandomycin, Telithromycin, Spiramycin, Aztreonam, Furazolidone, Nitrofurantoin, Linezolid, Posizolid, Radezolid, Torezolid, Amoxocillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Diclocacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin, Piperacillin, Temocillin, Ticarcillin, Bactricin, Colistin, Polymixin B, Ciprofloxacin, Enoxacin, Gatifloxacin, Levofloxacin, Lomefloxacin,

Moxifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Sparfloxacin, Temafloxacin, Mafenide, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Trimethoprim-Sulfamethoxazole, Sulfonamido-chrysoidine, Demeclocycline, Doxycycline, 5 Minocycline, Oxytetracycline, Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol, Ethionamide, Isoiazid, Pyrazinamide, Rifampicin, Rifabutin, Rifapentine, Streptomycin, Araphenamine, Chloramphenicol, Fosfomycin, Fusidic acid, Metronidazole, Mupirocin, Platensimycin, Quinupristin, Dalfopristin, Thiamphenicol, Tigecycline, Tinidazole, Trimethoprim, and combinations thereof. In an exemplary embodiment, the 10 antibiotic is Rifampicin.

Antibiotics that may be particularly effective against methicillin-resistant *Staphylococcus aureus* (MRSA) may include Ceftobiprole, Ceftriaxone, Clindamycin, Dalbavancin, Daptomycin, Linezolid, Mupirocin, Oritavancin, Tedizolid, Telavancin, Tigecycline, and Vancomycin. Antibiotics that may be particularly effective against 15 vancomycin-resistant *Enterococcus* (VRE) may include Linezolid, Streptogramins, Tigecycline, and Daptomycin. Drugs effective against *Mycobacteria* including *Mycobacterium tuberculosis* may include Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol, Ethionamide, Isoiazid, Pyrazinamide, Rifampicin, Rifabutin, Rifapentine, and Streptomycin.

20 The cationic constructs of the present invention may be particularly effective at treating antibiotic resistant bacterial infections, particularly where the mechanisms of resistance involve membrane permeability in the case of “cell poisoning” constructs. The current major anti-bacterial drugs on the market (aminoglycosides, beta lactams, tetracyclines, sulfonamides, quinolones, macrolides, phenicols, etc.) all target synthesis of 25 functional biomolecules (cell wall, RNA, DNA, protein/enzymes) necessary for bacteria cell function and survival. However, inefficient delivery of the drug is an issue that compromises their efficacy. An overview of antibiotic resistance can be found in Munita *et al.* Microbiol Spectr. PMC 2016 Oct 1, *Mechanisms of Antibiotic Resistance*, hereby incorporated by reference in its entirety. Common mechanisms of drug resistance include decreased influx, 30 increased efflux (“drug pumps”), target site alterations, target amplification, and antibiotic inactivation. Aminoglycosides typically are subject to decreased influx, inactivation, target site alteration, and increased efflux. Beta lactams are typically subject to decreased influx, increased efflux, target site alteration, and antibiotic inactivation. Amphenicols are typically subject to inactivation, as so are antifolates. Glycopeptides are typically subject to

inactivation and target site alteration, as are glycopeptides and rifamycins. Quinolones are typically subject to increased efflux, and fluoroquinolones are typically subject to target site alteration but may also be affected by membrane permeability. Macrolides are typically subject to increased efflux and target site alteration, as are tetracyclines. Sulfonamides and trimethoprim are typically subject to target amplification.

Inactivation can include either destruction or modification of the antibiotic molecule. For example, the main mechanism of β -lactam resistance relies on the action of β -lactamases which destroy the amide bond of the β -lactam ring, rendering the antimicrobial ineffective. Aminoglycosides on the other hand are often modified by aminoglycoside modifying enzymes (AMEs) that covalently modify the hydroxyl or amino groups of the aminoglycoside molecule. Decreased membrane permeability is particularly common in Gram negative bacteria. Hydrophilic molecules such as tetracyclines, β -lactams and some fluoroquinolones are often affected by changes in permeability of the outer membrane since they often use porins to cross the membrane barrier. Efflux systems may be substrate-specific or may possess broad substrate specificity, which are usually found in MDR bacteria. Efflux pumps have five major families: i) major facilitator superfamily (MFS), ii) small multidrug resistance family (SMR), iii) resistance-nodulation-cell-division family (RND), iv) ATP-binding cassette family (ABC), and v) multidrug and toxic compound extrusion family (MATE). Tetracycline resistance is a classic example of efflux-mediated resistance. Target changes include i) point mutations in the target site, ii) enzymatic alterations (*e.g.* methylation) of the binding site and/or iii) replacement/bypass of the original target. Rifampicin resistance caused by mutation in the *rpo β* gene encoding the β subunit of RNA polymerase (binding site of Rifampicin) is a classic example of a target change.

Preferably, in the “cell poisoning” constructs, the functional moiety also contains a cleavable linker. Cleavable linkers are linkers that can be cleaved chemically and / or enzymatically under *in vivo* conditions. The cleavage may involve oxidation, reduction, hydrolysis, or any combination of these processes. For example, some disulfide containing linkers are cleavable through disulfide exchange, which can occur under physiological conditions. The linker may contain an enzymatically cleavable peptide moiety. Specifically, the linker may include a peptide containing a cleavage recognition sequence for a particular protease. A cleavage recognition sequence for an enzyme is a specific amino acid sequence recognized by the protease during enzymatic cleavage. Many enzymatic cleavage sites are known in the art, and these and other cleavage sites can be included in the linker moiety. Acid-labile linkers may be cleavable at suitable acidic conditions. Certain intracellular

compartments, such as endosomes and lysosomes, have an acidic pH (pH 4-5), and provide conditions suitable to cleave acid-labile linkers. Linkers that are photo-labile are useful at the body surface and in many body cavities that are accessible to light. Furthermore, infrared light can penetrate tissue. Some linkers can be cleaved enzymatically via enzymes such as

5 lipases, esterases, amidases, proteases and glycosidases. Exemplary cleavable groups in the linker include, but are not limited to peptides, amino acids, esters, carbamates, hydroquinones, disulfides, and a combination of these groups. **FIG. 8** illustrates an example of a disulfide linker which can be cleaved enzymatically in a cell. **FIG. 12** further provides an example of a linker containing a hydroquinone moiety. Oxidation of the hydroquinone

10 leads to the formation of a benzoquinone and subsequent hydrolysis releases the therapeutic agent from the cationic construct.

“Cell entrapment” constructs form a network structure enclosing target cells. The functional moiety of a first cationic construct contains a first linking moiety, which forms a linkage with a second linking moiety on a second cationic construct. Because the formation

15 of multiple linkages between two cationic takes place on the surface of the target cells, cell proliferation is effectively halted. Synthesis of exemplary “cell entrapment” cationic constructs is shown in Example 2 *infra*.

A key element in such a cell entrapment construct lies in the reactivity of the linking moieties. The functional moieties must efficiently form a new linkage or a structural

20 component after the constructs attach to the surface of the cells but before substantial cell growth or proliferation has taken place. In non-limiting examples, the functional moiety pairs include alkyne – azide pair and strained alkene – azide pair.

The present invention may employ cycloaddition reaction between a 1,3-dipole and a dipolarophile (*e.g.* azide and alkyne), also known as click chemistry, to form a five-

25 membered ring. The azide and alkyne functional moieties are largely inert towards biological molecules and aqueous environments, which allows the use of the azide-alkyne cycloaddition to form cell entrapment constructs. The triazole has similarities to the ubiquitous amide moiety found in nature, but unlike amides, is not susceptible to cleavage. Additionally, triazoles are nearly impossible to oxidize or reduce. While copper catalyst is needed in some

30 cycloaddition reactions, the increased reactivity resulting from a strained alkyne allows the cycloaddition reaction with azide to proceed smoothly at room temperature without the use of copper catalysts. Reports and procedures on cycloaddition reactions between a 1,3-dipole and a dipolarophile are readily available to one of ordinary skill in the art. Literatures in the relevant filed include Jewett, *et al.*, Chem Soc Rev. 2010, 39(4), 1272-9 and Schultz *et al.*,

Org. Lett. 2010, 12 (10), 2398–401, the entire disclosure of which is herein incorporated by reference.

Besides the cycloaddition reaction between an alkyne and an azide, the reaction between a strained alkene and a tetrazine can also be utilized in the cell entrapment constructs. The ring strain of the alkene provides a driving force for the 4+2 cycloaddition to proceed under mild conditions to provide a 6-membered ring. Similar to the alkyne-azide coupling the reaction procedures between a strained alkene and a tetrazine are well within the knowledge of a person of ordinary skill in the art without undue experiment. Literatures in the relevant field include Wang, *et al.*, Nature Chem. 2014, 6, 393–403; Seitchik, *et al.*, J. Am. Chem. Soc. 2012, 134(6), 2898–2901; and Taylor, *et al.*, J. Am. Chem. Soc. 2011, 133, 9646, the entire disclosure of which is herein incorporated by reference.

Other cleavable linkers are known in the art and may comprise any of the following. Most known cleavable linkers fall into categories of hydrazones, peptide linkers, disulfide linkers, and thioester linkers. Hydrazone linkers are designed for serum stability and degradation within the cytoplasm. Peptide linkers are typically designed to be enzymatically hydrolyzed by lysosomal proteases. Exemplary proteases include *cathepsin B*. Disulfide linkers are typically (but not necessarily) designed to be cleaved through disulfide exchange with an intracellular thiol, for example, a glutathione. Thioesters are non-reducible linkers and are designed for intracellular proteolytic degradation. Hydrazones and disulfide bridges are generally recognized as chemically labile linkers. Hydrazones are easily synthesized and have a plasma half-life of approximately 183 hours at pH 7 and approximately 4.4 hours at pH 5. This suggests that they are selectively cleavable under acidic conditions such as those found in the lysosome. Acidic conditions, however, are often found in various places in the body, which increases the potential for nonspecific release. Disulfide bridges take advantage of the cellular reducing environment as discussed herein. Steric hindrance enhances the plasma stability of disulfide-linked conjugates, however the factors governing disulfide-linked metabolite processing remain poorly understood.

The other main category of cleavable linkers, aside from chemically labile linkers, include enzymatically cleavable linkers. An overview of cleavable linking moieties which may be suitable for the present invention is provided in Leriche *et al.*, Cleavable linkers in chemical biology Bioorg Med Chem. 2012 Jan 15;20(2):571-82, hereby incorporated by reference in its entirety. The most popular enzymatic cleavage sequence is the dipeptide valine-citrulline (Val-Cit linkers), combined with a self-immolative linker *p*-aminobenzyl alcohol (PAB). The cleavage of an amide-linked PAB triggers a 1,6-elimination of carbon

dioxide and concomitant release of the free drug in parent amine form. Other dipeptide linkers besides Val-Cit linkers include Phe-Lys linkers. It was found in Leriche *et al.* that Phe-Lys was cleaved with a half-life of 8 min, followed by Val-Lys with a half-life of 9 min. Val-Cit linkers, however, showed a half-life of 240 min. Removal of the PAB group was found to reduce the cleavage rate, likely through steric interference with enzyme binding. However, substituting the PAB group for a glycine residue provides spacing for cleavage. Importantly, however, it does not allow the release of free payload. Val-Cit linkers have been shown to be over 100 times as stable as hydrazine linkers. Enzymatically cleavable linkers do not necessarily need to comprise peptide linkers. For example, a glucuronide linker incorporating a hydrophilic sugar group cleaved by the lysosomal enzyme beta glucuronidase has been investigated. Briefly, once the sugar is cleaved from the phenolic backbone, self-immolation of the PAB group releases the payload.

The cationic constructs of the present invention have a range of potential uses, including, but not limited to, *in vivo* therapeutic use. Notably, as shown in Example 3, having detected only background fluorescence, the cationic constructs do not bind to human red blood cells under physiologically relevant conditions, illustrating that the cationic constructs are suitable for *in vivo* use. The cationic constructs are capable of adhering to the surface of a bacterium, including both Gram positive and Gram negative bacteria, at a high concentration. For example, **FIG. 10B** shows the cationic constructs binding to several different bacterial strains at a concentration of 10^6 bound constructs per cell, as measured by the fluorescence intensity of the bound detectable label. This is because, as illustrated in **FIG. 1** and **FIG. 2**, the outer membrane (OM) of bacteria possesses a net negative charge. The cationic polymer core is attracted through electrostatic interaction to the surface of the bacterium and adheres to the surface.

The “cell poisoning” constructs of the present invention can be used to deliver a delivery agent to a target cell. Generally, after binding to the surface of the bacterium, the plurality of polymer linkers linked to the delivery agent penetrate the outer membrane of the bacterium (Gram negative) or the bacterial cell wall (Gram positive) to deliver the delivery agent to the target cell. As described above, the delivery agent can be a cytotoxic, immunomodulatory agent, or an antibacterial agent, and is preferably an antibiotic. The delivery agent is released from the polymer linker once it has penetrated the outer membrane of the bacterium. Aside from just binding to the surface of the bacterium, the cationic polymer core serves to weaken the structural integrity of the outer membrane/cell wall to allow for penetration by the polymer linkers containing the delivery payload. For example, in

those embodiments where the delivery agent is bound *via* a disulfide linker, the cytoplasm of the bacterium serves to reduce the disulfide linkage and release the delivery agent once the delivery agent linked to the polymer linkers has penetrated into the cytoplasm. This delivery mechanism is exemplified in **FIG. 3** and ensures that the delivery agent will not be released
5 prematurely and will be internalized into the bacterium; furthermore, since the cationic constructs are capable of binding at such a high concentration, a high degree of internalization is possible. This may be particularly effective at combating antibiotic resistance, especially in those circumstances where antibiotic resistance occurs *via* mechanisms such as, for example, drug efflux pumps, enzymatic degradation of antibiotics,
10 or reduced membrane permeability to an antibiotic. Regarding efflux pumps and enzymatic degradation, without wishing to be bound by theory, such a high amount of antibiotic is internalized that the drug is effective even with the counter-measures. Regarding reduced membrane permeability, as described above, the cationic constructs serve to weaken the outer membrane/cell wall and allow for penetration, including into the cytosol, and subsequent
15 release of the delivery agent.

An alternative delivery mechanism may be an oxidative release of the delivery agent, for example as shown in Example 3 and the mechanism of action in **FIG. 12**. In some embodiments, the delivery agent is released in an outer membrane (*e.g.* with Gram negative bacteria) without having penetrated through to the cytosol. This is an alternative release
20 mechanism which does not rely a cleavable disulfide linker or a reductive environment. Depending on the membrane/cell wall thickness of a particular bacteria, this provides an alternative exemplary embodiment for release of a delivery agent into the target cell.

While the structure of the “cell entrapment” constructs of the present invention are generally similar in structure to the “cell poisoning” constructs of the present invention, the methods of use are different. The “cell entrapment” cationic constructs work as a system to
25 covalently link to one another when brought in close proximity, *i.e.* when bound to the surface of a bacterium. Like the “cell poisoning” constructs, the “cell entrapment” constructs rely on a cationic polymer core to adhere the constructs to the surface of the bacteria. This process is exemplified in **FIG. 4** and is generally as follows. Individual cationic constructs
30 each have one type of linking moiety as the functional moiety linked to the polymer linkers, *e.g.* a first linking moiety and a second linking moiety. The first linking moiety and second linking moiety will covalently link to one another when brought into close proximity, for example, but explicitly not limited to, through click chemistry reactions. In an exemplary embodiment, the first linking moiety is DBCO and the second linking moiety is an alkyne.

This allows for copper-free click chemistry reactions that proceed at a fast rate, for example (but not necessarily) in under 30 seconds as described in Example 3. The cationic constructs link to one another to form a “network” or “mesh” around the bacterium, entrapping the bacterium and preventing it from replicating or interacting with other cells. Like the “cell poisoning” constructs, the “cell entrapment” constructs may be effective even in circumstances where the bacteria is resistant to antibiotics, but the “cell poisoning” constructs may, but not necessarily, have even greater effectiveness. This is because the “cell entrapment” constructs do not rely on delivery of an antibiotic agent to prevent bacterial growth, the mechanism of action relies on forming a network on the surface of the bacteria and not on penetrating the membrane to deliver a delivery agent, *e.g.* an antibiotic.

In some embodiments, the cationic constructs of the present invention may be provided in a pharmaceutical composition. The pharmaceutical composition may be formulated as powders, granules, solutions, suspensions, aerosols, solids, pills, tablets, capsules, gels, topical crèmes, suppositories, transdermal patches, etc. Pharmaceutically acceptable salts are intended to include any art recognized pharmaceutically acceptable salts including organic and inorganic acids and/or bases. Examples of salts include sodium, potassium, lithium, ammonium, calcium, as well as primary, secondary, and tertiary amines, esters of lower hydrocarbons, such as methyl, ethyl, and propyl. Other salts include organic acids, such as acetic acid, propionic acid, pyruvic acid, maleic acid, succinic acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, salicylic acid, etc.

The pharmaceutically acceptable composition may be in liquid form or solid form. A solid formulation is generally, but not necessarily, lyophilized and brought into solution prior to administration for either single or multiple dosing. The formulations should not be exposed to extreme temperature or pH so as to avoid thermal denaturation. Thus, it may be important to formulate a composition of the present invention within a biologically relevant pH range. A solution buffered to maintain a proper pH range during storage is often necessary, especially for liquid formulations stored for longer periods of time between formulation and administration. Typically, both liquid and solid formulations require storage at lower temperatures (usually, but not necessarily, between 2-8 °C.) in order to retain stability for longer periods. Formulated compositions, especially liquid formulations, may contain an additional bacteriostat beyond the cationic constructs to prevent or minimize proteolysis during storage, including but not limited to effective concentrations (usually <1% w/v) of benzyl alcohol, phenol, m-cresol, chlorobutanol, methylparaben, and/or propylparaben. A bacteriostat may be contraindicated for some patients. Therefore, a

lyophilized formulation may be reconstituted in a solution either containing or not containing such a component.

Additional components may be added to either a buffered liquid or solid formulation, including but not limited to sugars as a cryoprotectant (including but not necessarily limited to polyhydroxy hydrocarbons such as sorbitol, mannitol, glycerol and dulcitol and/or disaccharides such as sucrose, lactose, maltose or trehalose) and, in some instances, a relevant salt (including but not limited to NaCl, KCl or LiCl). Such formulations, especially liquid formulations slated for long term storage, will rely on a useful range of total osmolarity to both promote long term stability at temperature of, *e.g.* 2-8 °C., or higher, while also making the formulation useful for parenteral injection. For example, but not necessarily, an effective range of total osmolarity (the total number of molecules in solution) may be from about 200 mOs/L to about 800 mOs/L. It will be apparent that the amount of a cyroprotectant, such as sucrose or sorbitol, may depend upon the amount of salt in the formulation in order for the total osmolarity of the solution to remain within an appropriate range. Therefore a salt free formulation may, but not necessarily, contain from about 5% to about 25% sucrose.

Alternatively, a salt free sorbitol-based formulation may, but not necessarily, contain sorbitol within a range from about 3% to about 12%. Salt-free formulations may warrant increased ranges of the respective cryoprotectant in order to maintain effective osmolarity levels. These formulation may also contain a divalent cation (including but not necessarily limited to MgCl₂, CaCl₂ and MnCl₂); and a non-ionic surfactant (including but not necessarily limited to Polysorbate-80 (Tween 80®), Polysorbate-60 (Tween 60®), Polysorbate-40 (Tween 40®) and Polysorbate-20 (Tween 20®), polyoxyethylene alkyl ethers, including but not limited to Brij 58®, Brij 35®, as well as others such as Triton X-100®, Triton X 114®, NP40®, Span 85 and the Pluronic series of non-ionic surfactants (*e.g.*, Pluronic 121)). The compositions of the present invention may also be a “chemical derivative”, which describes compositions that contain additional chemical moieties which are not normally a part of the original compound (*e.g.*, pegylation). Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule.

Suitable formulations may be found in, among others, Remington's Pharmaceutical Sciences, 17th edition, *Mack Publishing Co.*, Philadelphia, Pa., 1985 and Handbook of Pharmaceutical Excipients, 3rd Ed, Kibbe, A. H. ed., Washington D.C., American

Pharmaceutical Association, 2000; hereby incorporated by reference in their entirety. The pharmaceutical compositions described herein can be made in a manner well known to those skilled in the art (*e.g.*, by means conventional in the art, including mixing, dissolving, granulating, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes).

5 For the purposes of this invention, the method of administration is chosen depending on the condition being treated, the form of the subject compositions, and the pharmaceutical composition. Administration of the oligopeptides can be done in a variety of ways, including, but not limited to, cutaneously, subcutaneously, intravenously, orally, topically, transdermally, intraperitoneally, intramuscularly, nasally, and rectally (*e.g.*, colonic
10 administration). For example, microparticle, microsphere, and microencapsulate formulations are useful for oral, intramuscular, or subcutaneous administrations. Liposomes and nanoparticles are additionally suitable for intravenous administrations. Administration of the pharmaceutical compositions may be through a single route or concurrently by several routes. For instance, oral administration can be accompanied by rectal or topical administration to
15 the affected area. Alternatively, oral administration is used in conjunction with intravenous or parenteral injections.

The delivery systems also include sustained release or long term delivery methods, which are well known to those skilled in the art. By “sustained release or” “long term release” as used herein is meant that the delivery system administers a pharmaceutically
20 therapeutic amount of subject compounds for more than a day, preferably more than a week, and most preferable at least about 30 days to 60 days, or longer. Long term release systems may comprise implantable solids or gels containing the subject peptide, such as biodegradable polymers described above; pumps, including peristaltic pumps and fluorocarbon propellant pumps; osmotic and mini-osmotic pumps; and the like. Peristaltic
25 pumps deliver a set amount of drug with each activation of the pump, and the reservoir can be refilled, preferably percutaneously through a port. A controller sets the dosage and can also provide a readout on dosage delivered, dosage remaining, and frequency of delivery. Fluorocarbon propellant pumps utilize a fluorocarbon liquid to operate the pump. The fluorocarbon liquid exerts a vapor pressure above atmospheric pressure and compresses a
30 chamber containing the drug to release the drug. Osmotic pumps (and mini-osmotic pumps) utilize osmotic pressure to release the drug at a constant rate. The drug is contained in an impermeable diaphragm, which is surrounded by the osmotic agent. A semipermeable membrane contains the osmotic agent, and the entire pump is housed in a casing. Diffusion of water through the semipermeable membrane squeezes the diaphragm holding the drug,

forcing the drug into bloodstream, organ, or tissue. These and other such implants are particularly useful in treating a disease condition, especially those manifesting recurring episodes or which are progressive in nature, by delivering the oligopeptides of the invention via systemic (*e.g.*, intravenous or subcutaneous) or localized doses (*e.g.*,

5 intracerebroventricular) in a sustained, long term manner.

In one embodiment, the method of administration is by oral delivery, in the form of a powder, tablet, pill, or capsule. Pharmaceutical formulations for oral administration may be made by combining one or more peptide with suitable excipients, such as sugars (*e.g.*, lactose, sucrose, mannitol, or sorbitol), cellulose (*e.g.*, starch, methyl cellulose,

10 hydroxymethyl cellulose, carboxymethyl cellulose, etc.), gelatin, glycine, saccharin, magnesium carbonate, calcium carbonate, polymers such as polyethylene glycol or polyvinylpyrrolidone, and the like. The pills, tablets, or capsules may have an enteric coating, which remains intact in the stomach but dissolves in the intestine. Various enteric coating are known in the art, a number of which are commercially available, including, but not limited to,

15 methacrylic acid-methacrylic acid ester copolymers, polymer cellulose ether, cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropyl methyl cellulose phthalate, and the like. Alternatively, oral formulations of the peptides are in prepared in a suitable diluent. Suitable diluents include various liquid form (*e.g.*, syrups, slurries, suspensions, etc.) in aqueous diluents such as water, saline, phosphate buffered saline, aqueous ethanol, solutions

20 of sugars (*e.g.* sucrose, mannitol, or sorbitol), glycerol, aqueous suspensions of gelatin, methyl cellulose, hydroxymethyl cellulose, cyclodextrins, and the like. As used herein, diluent or aqueous solutions also include infant formula. In some embodiments, lipophilic solvents are used, including oils, for instance vegetable oils, peanut oil, sesame oil, olive oil, corn oil, safflower oil, soybean oil, etc.); fatty acid esters, such as oleates, triglycerides, etc.;

25 cholesterol derivatives, including cholesterol oleate, cholesterol linoleate, cholesterol myristilate, etc.; liposomes; and the like.

In some embodiments, the present invention is directed to a medical device coated with or containing a cationic construct according to any aspect of the present invention. Such medical devices include, but are not limited to, prosthesis, implants, depots, catheters,

30 syringes, sutures, pacemakers, infusion pumps, thermometers, tracheal tubes, nebulizers, insulin pumps, scalpels and surgical equipment, gauze, feeding tubes, speculums, airways, bands/gastric bands, vaporizers, respirators, swabs, similar devices to any of those listed, and combinations thereof.

As used herein, a “patient” or “subject” may refer to a biological system to which a treatment can be administered. A biological system can include, for example, an individual cell, a set of cells (*e.g.*, a cell culture), an organ, a tissue, or a multi-cellular organism. For example, a “patient” or “subject” may refer to human and non-human animals. Examples of
5 a non-human animal include all vertebrates, *e.g.*, mammals, such as non-human mammals, non-human primates (particularly higher primates), dog, rodent (*e.g.*, mouse or rat), guinea pig, cat, and rabbit, and non-mammals, such as birds, amphibians, reptiles, etc. In one embodiment, the subject is a human. In another embodiment, the subject is an experimental, non-human animal or animal suitable as a disease model. The term “animal” includes all
10 vertebrate animals including humans. In particular, the term “vertebrate animal” includes, but not limited to, humans, canines (*e.g.*, dogs), felines (*e.g.*, cats); equines (*e.g.*, horses), bovines (*e.g.*, cattle), porcine (*e.g.*, pigs), as well as in avians. The subject may or may not be an immunocompromised subject, *e.g.* a subject infected with human immunodeficiency virus (HIV) or suffering from another condition or pathology that results in reduced ability to fight
15 infection.

As used herein, the terms “treating” or “treatment” of a disease refers to executing a protocol, which may include administering one or more drugs to a patient (human or otherwise), in an effort to alleviate signs or symptoms of the disease. Alleviation can occur prior to signs or symptoms of the disease appearing as well as after their appearance. In
20 addition, “treating” or “treatment” does not require complete alleviation of signs or symptoms, does not require a cure, and specifically includes protocols that have only a marginal effect on the patient. Thus, “treating” or “treatment” additionally includes “preventing” or “prevention” of disease. The terms “prevent” or “preventing” or “prevention” refer to prophylactic and/or preventative measures, wherein the object is to
25 prevent or slow down the targeted pathologic condition or disorder. These terms are not limited solely to a situation in which the patient experiences no aspect of the condition whatsoever. For example, a treatment will be said to have “prevented” the condition if it is given during exposure of a patient to a stimulus that would have been expected to produce a given manifestation of the condition, and results in the patient's experiencing fewer and/or
30 milder symptoms of the condition than otherwise expected. For example, a treatment can “prevent” bacterial infection by resulting the patient's displaying only mild overt symptoms of the infection; it does not imply that there must have been no cellular damage caused by the infecting bacterial organism.

As used herein, an “effective amount” refers to the amount of an active compound/agent that is required to confer a therapeutic effect on a treated subject. Effective doses will vary, as recognized by those skilled in the art, depending on the types of conditions treated, route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatment. A therapeutically effective amount to treat or inhibit a bacterial infection is an amount that will cause a reduction in one or more of the manifestations of bacterial infection, such as amount of fungus present in the host organism and mortality as compared to untreated control animals.

The terms “co-administration,” “co-administered,” and “in combination with” as used herein may refer to the administration of at least two agents or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In other embodiments, a first agent/therapy is administered prior to a second agent/therapy. Those of skill in the art understand that the formulations and/or routes of administration of the various agents/therapies used may vary.

As used herein, the term “pharmaceutical composition” may refer to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*. A “pharmaceutically acceptable carrier,” after administered to or upon a subject, does not cause undesirable physiological effects. The carrier in the pharmaceutical composition must be “acceptable” also in the sense that it is compatible with the active ingredient, *e.g.* the cationic constructs of the present invention, and can be capable of stabilizing it. One or more solubilizing agents can be utilized as pharmaceutical carriers for delivery of an active agent. Examples of a pharmaceutically acceptable carrier include, but are not limited to, biocompatible vehicles, adjuvants, additives, and diluents to achieve a composition usable as a dosage form. Specific examples of physiologically acceptable carriers include, but not limited to, buffers such as phosphate, citrate, and other organic acids; antioxidants including, but not limited to, ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as, but not limited to, serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as, but not limited to, polyvinylpyrrolidone (PVP); amino acids such as, but not limited to, glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including, but not limited to, glucose, mannose, or dextrans; chelating agents such as, but not limited to, EDTA; sugar alcohols such as, but not limited to, mannitol or sorbitol; salt-forming counterions such as, but not limited to, sodium; and/or nonionic surfactants such as, but not limited to, TWEEN.; polyethylene glycol (PEG), and

PLURONICS. Examples of other carriers include colloidal silicon oxide, magnesium stearate, cellulose, and sodium lauryl sulfate. Additional suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington: The Science and Practice of Pharmacy (Remington the Science and Practice of Pharmacy)

5 Twenty-First Edition (2005), hereby incorporated by reference in its entirety.

Where a value of ranges is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller
10 ranges which may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same
15 meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

20 As used herein and in the appended claims, the singular forms “a”, “and” and “the” include plural references unless the context clearly dictates otherwise.

The term “about” refers to a range of values which would not be considered by a person of ordinary skill in the art as substantially different from the baseline values. For example, the term “about” may refer to a value that is within 20%, 15%, 10%, 9%, 8%, 7%,
25 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value, as well as values intervening such stated values. Context will dictate which value or range of value the term “about” may refer to in any given instance throughout this disclosure, and may vary in different circumstances.

Publications disclosed herein are provided solely for their disclosure prior to the filing
30 date of the present invention. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

Each of the applications and patents cited in this text, as well as each document or reference, patient or non-patient literature, cited in each of the applications and patents (including during the prosecution of each issued patent; “application cited documents”), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference in their entirety. More generally, documents or references are cited in this text, either in a Reference List before the claims; or in the text itself; and, each of these documents or references (“herein-cited references”), as well as each document or reference cited in each of the herein-cited references (including any manufacturer's specifications, instructions, etc.), is hereby expressly incorporated herein by reference.

The following non-limiting examples serve to further illustrate the present invention.

EXAMPLES

Example 1- *Synthesis of “cell poisoning” constructs*

20 mg (0.1 mmol) of 1,5-difluoro-2,4-dinitrobenzene dissolved in 0.1 ml of DMF were supplemented with 6 μ L (0.11 mmol) of propargylamine and equal amount of diisopropylethylamine. After 5 min. incubation at room temperature the mixture was supplemented with 0.4 mL of water and the product extracted by ether (2 x 0.5 mL). Organic layer was collected, dried over anhydrous Na₂SO₄ and applied on 1 mL silicagel column. The **compound II (FIG. 7)** was eluted by 3 mL hexane-ethylacetate mixture (2:1) and the solvent evaporated under reduced pressure. The resulting oil was supplemented with hexane and left at room temperature. The formed crystals were washed with hexane and dried in vacuo. Yield was approximately 16 mg (84 μ mol).

To the solution of 106 mg (53 μ mol) PEG2000 in 0.8 mL of DMF the **compound II (FIG. 7)** (10 mg, 53 μ mol) in 0.2 mL of DMF was slowly added under rigorous agitation and the mixture left for 30 min at room temperature. The TLC analysis in chloroform-ethanol (5:1) developing system revealed two yellow-colored products with R_f = 0.15 and 0.7. DMF was removed by extraction with the mixture hexane-ether (3:1) (2 x 13 mL), the residue dissolved in chloroform and applied on silicagel column (30 mL). The column was washed first with chloroform-ethanol mixture (5:1) until the higher mobility product was eluted, and then lower mobility product was recovered by elution with 2:1 mixture of the same solvents. The solvent was removed under reduced pressure. Yield of **compound III (FIG. 7)** was approximately 44 mg (20 μ mol).

0.2 mL of 43 mM **compound III (FIG. 7)** solution in DMF was supplemented with 20 μ L of 0.5 M chloroform solution of thiocarbonyldiimidazole under rigorous agitation. After 5 min. incubation at room temperature 5 μ L of TFA were added. After incubation at 50 °C for 30 min. TLC analysis in chloroform-ethanol (5:1) developing system revealed
5 complete conversion to the reaction product with R_f = 0.6. The product was precipitated by addition of 5 mL of ether, followed by washing with the same solvent (2 x 3 mL) and dried in vacuo. Yield of **compound IV** was approximately 13 mg (6 μ mol).

BODIPY-modified poly-lysine was synthesized as follows. To the solution of 3.8 mg of polylysine-hydrobromide (MW = 4,000-15,000) in 0.2 mL of aqueous DMF 4 μ L of 0.1 M
10 BODIPY-NHS solution in the same solvent were added. The mixture was supplemented with 1 μ L of 1 M KOH and left for 5 min. at room temperature followed by gel-filtration on Sephadex G100 column (0.5 x 15 cm) using eluent of the composition: 10 mM Hepes pH 8.0, 50 mM NaCl. Polymeric fractions (total 0.6 mL) were collected and combined. The concentration of the resulting solution containing **compound V (FIG. 7)** was 0.6 mM (for
15 poly-lysine with average MW equal to 10,000).

Click-reactive derivative of BODIPY-labeled polylysine was synthesized as follows. Fifty microliters of **compound V (FIG. 7)** were mixed with 10 μ L of 40 mM **compound IV (FIG. 7)** dissolved in DMF) and 1 μ L of triethylamine. After 1 h incubation at 50° 1 μ L of acetic acid and 0.2 mL of water were added and the sample was subjected to gel-filtration on
20 0.5 x 12 cm G-100 Sephadex column equilibrated with 10 mM Hepes pH 8.0, 50 mM NaCl buffer. Size exclusion fractions were collected and combined yielding **compound VI (FIG. 7)**.

The synthesis of click-reactive rifampicin-derivative **compound VIII (FIG. 7)** was achieved according to two different mechanisms. The first was based on a cystamine
25 derivative of rifamycin B. Ten milligrams (13.5 μ mol) of rifamycin B were suspended in 0.1 mL of THF and supplemented with 7.5 mg (36 μ mol) of DCC. After 10 min incubation at 20 °C a solution of 10 mg (47 μ mol) of cystamine dihydrochloride and 10 μ L of triethylamine in 0.2 mL of DMF was added. After 5 min. incubation TLC analysis on silicagel plate in chloroform-ethanol (5:1) developing system revealed ~60-70% conversion of rifamycin B (
30 R_f = 0.85) to the reaction product (R_f = 0.3). The product was purified by preparative TLC in chloroform-ethanol (4:1) developing system. Yield was around 5 μ mol. The second was based on an azido derivative of rifamycin B. To the solution of 5 μ mol of the rifamycin B-cystamine adduct in 0.2 mL DMF 20 μ L of 0.4 M 4-nitrophenyl 6-azidohexanoate in THF and 3 μ L of triethylamine were added. After 10 min. incubation at 37 °C TLC analysis in

chloroform-ethanol (4:1) developing system revealed complete conversion to the product with $R_f = 0.8$. The reaction mixture was applied on a silicagel plate, and subsequently developed in hexane-ethylacetate (2:1) and chloroform-ethanol (3:1) solvent systems. The product was eluted with methanol and the solvent removed under reduced pressure on rotary evaporator. The residue was dissolved in 0.175 mL of DMF affording 20 mM solution. Yield was approximately 3.5 μmol .

Attachment of Rifampicin-PEG2000 derivative to click-reactive BODIPY-polylysine construct **compound VII (FIG. 7)**, rifampicin “cargo” designated as “P”) was achieved as follows. To 80 μL of 0.25 mM (concentration for the attached alkyne groups) **compound VI (FIG. 7)** an equal volume of 0.25 mM RifamycinB-azidoderivative in DMF was added. The mixture was supplemented with 10 μL of 2 M triethylammonium acetate pH 7.0 buffer, 2 μL of 0.1 M ascorbic acid and 5 μL of 10 mM Cu-TBTA complex. After 5 min. incubation the mixture was extracted by ethylacetate (2 x 0.2 mL), and the product was purified by gel filtration on Sephadex G-100 column (0.5 x 12 cm).

Example 2- Synthesis of “cell entrapment” constructs

The synthesis of the 4-nitrophenyl derivative of DBCO was as follows. To the solution of 5.5 mg (15.5 μmol) of dibenzocyclooctyne-acid in 50 μL of dioxane 3.5 mg (25 μmol) of 4-nitrophenol, 17 mg (82 μmol) of DCC and 1.1 mg (9 μmol) of pyridine hydrochloride were subsequently added. After 10 min. incubation at room temperature TLC analysis in hexane-ethylacetate (1:1) developing system revealed nearly quantitative conversion of the original DBCO compound to the reaction product ($R_f = 0.5$), which was purified by preparative TLC in the same solvent system. Yield of **compound IX (FIG. 9)** was approximately 15 μmol .

The synthesis of the DBCO - diamino-PEG2000 conjugate was as follows. To the solution of 10 mg (5 μmol) of diamino-PEG2000 and 1 μL of glacial acetic acid in 0.1 mL of DMF 50 μL of 0.1 M 4-nitrophenyl derivative of DBCO and 2 μL of triethylamine were added. The mixture was left for 10 min. at room temperature. TLC analysis in chloroform-ethanol (6:1) revealed two products with $R_f = 0.4$ (mono-substituted diamino-PEG) and $R_f = 0.6$ (disubstituted diamino-PEG) in nearly equal ratio (judging by UV absorption). The mono-derivative was purified by preparative TLC in the same system. Yield of **compound X (FIG. 9)** was approximately 4 mg (1.7 μmol).

The synthesis of the isothiocyano-PEG2000-DBCO derivative was as follows. To the solution of 4 mg DBCO-PEG2000 derivative in DMF (0.1 mL) 10 μL of 0.2 M solution of

thiocarbonyldiimidazole in chloroform and 0.3 μ L of triethylamine and 0.5 μ L of trifluoroacetic acid were subsequently added. After incubation for 20 min. at 50 °C the product was precipitated with ether, washed with the same solvent and dried *in vacuo*. Yield of **compound XI (FIG. 9)** was approximately 3.5 mg.

5 A BODIPY-Polylysine-PEG2000-DBCO construct was synthesized as follows. Sixty microliters of **compound V (FIG. 7)** (*see* Example 1) were mixed with 20 μ L of 20 mM DMF solution of isothiocyano-PEG2000-DBCO and 2 μ L of triethylamine. After 60 min. incubation at 50 °C, 1 μ L of acetic acid and 0.2 mL of water were added and the sample was subjected to gel-filtration on 0.5 x 12 cm G-100 Sephadex column equilibrated with 10 mM
10 Heppes pH 8.0, 50 mM NaCl buffer. Size exclusion fractions were collected and combined. Attachment stoichiometry was approximately 5 DBCO residues per BODIPY-polylysine as determined by relative absorption of BODIPY and DBCO chromophores.

A conjugate of p-azidotoluic acid with diamino-PEG2000 was synthesized as follows. 4-nitrophenyl ester of p-azidotoluic acid was synthesized as follows. To the solution of 34
15 mg (0.2 mmol) p-azidotoluic acid and 34 mg (0.24 mmol) 4-nitrophenol in 0.3 mL of dioxane 83 mg (0.4 mmol) of DCC were added and the mixture left for 10 min. at room temperature. The residue was separated by centrifugation, washed with dioxane, and the wash combined with the original solution. After evaporation under reduced pressure the residue was washed with 2 mL of ethylacetate-ether (1:1 mixture) followed by centrifugation
20 and extraction of the supernatant with 0.1 M sodium carbonate (2 x 2 mL). Organic layer was dried with anhydrous sodium sulfate, evaporated under reduced pressure and the residue washed with acetonitrile. Acetonitrile was removed under reduced pressure and the residue crystallized in the presence of hexane. Yield of **compound XII (FIG. 9)** was approximately 30 mg (0.1 mmol).

25 A conjugate of p-azidotoluic acid with diamino-PEG2000 was synthesized as follows. To the solution of 40 mg (20 μ mol) diamino-PEG2000 in 0.1 mL of DMF 3 μ L of acetic acid (48 μ mol), 3.3 mg (11 μ mol) of **compound XII (FIG. 9)** and 7 μ L (50 μ mol) of triethylamine were subsequently added. After 10 min. incubation at room temperature TLC analysis in chloroform-ethanol (4:1) developing system revealed one major product with R_f =
30 0.25. The product was purified by preparative TLC in Chloroform-ethanol (6:1) developing system. Yield of **compound XIII (FIG. 9)** was approximately 13 mg (6 μ mol).

An isothiocyano-PEG2000-p-azidotoluic acid derivative (**compound XIV (FIG. 9)**) was synthesized using the procedure described for synthesis of the isothiocyano-PEG2000-DBCO derivative above. Attachment of isothiocyano-PEG2000-DBCO derivative and of

isothiocyano-PEG2000-p-azidotoluic acid derivative to BODIPY-labeled polylysine was achieved as described for synthesis of click-reactive derivative of PODIPY-labeled polylysine as described in Example 1 *supra*.

5 **Example 3- Testing of the synthesized constructs**

The constructs generated in Example 1 and Example 2 were subject to a variety of tests indicating their efficacy. The cell binding properties of the synthesized constructs were tested. A suspension of 10^9 bacterial cells in 0.2 mL of LB broth was supplemented with increasing amount of fluorescently labeled construct. After incubation for 5 min. at room temperature the cells were precipitated by centrifugation and the fluorescence of the supernatant was measured. The same measurements were performed with the control mixture lacking the cells. The addition of the fluorescent constructs was continued until the cells stopped absorbing the material. The amount of bound material was calculated by subtracting the concentration of the construct, as determined by fluorescence measurement, in the supernatant in the mixture with cells from the construct concentration in the control mixture. These experiments were performed with Gram-negative and Gram-positive bacterial species. The incubation rendered the cells highly fluorescent (**FIG. 10A**). Subsequent washing of the cells did not reduce the cell's fluorescence, indicating tight binding of the constructs. The calculated binding capacity (approximately 10^6 probes per cell) was nearly equal for all kinds of cells. The calculated density of the bound probes was consistent with a continuous monolayer on the cellular surface, of particular relevance to the "cell entrapment" constructs, but still relevant to the "cell poisoning" constructs.

The binding rate of the cationic constructs to bacteria was determined. A suspension of 1 mg (ca. 10^9 cells) in 0.05 mL of BPS buffer pH 7.0 was mixed with 0.25 mL of fluorescently labeled polylysine. At time intervals of 0, 5, 10, and 15 minutes, the aliquots were centrifuged and their fluorescence was determined. As a reference the same mixture lacking cells was used. The observed binding rate (**FIG. 10C**) was beyond the capability of the measuring approach (<20 s), a surprising result showing that the cationic constructs can bind to bacteria near instantly, again of particular relevance to the "cellular entrapment" constructs. Furthermore, control experiments were performed with analogous incubations with human blood cells. The results displayed only background fluorescence that was 2-3 orders of magnitude lower than for bacterial cells, indicating that the cationic constructs have high targeting specificity for a bacterial cell surface, indicating a particular utility for *in vivo* use in humans or other animals.

The “cell poisoning” cationic construct was further validated by measuring the bacterial growth inhibitory activity on *B. subtilis*. Both full and partially assembled “cell poisoning” constructs completely blocked the growth of *B. subtilis* at 2 μ M concentration (**FIG. 11A**), indicating that these compounds retain tight targeting affinity. Remarkably, charging with rifampicin further increased growth inhibitory activity of the construct (**FIG. 11B**), indicating that the drug was released into cells even though the length of the poly(ethylene) glycol “arm” connecting to the “body” was less than the membrane thickness of *B. subtilis*. “Cell poisoning” constructs loaded with Rifamycin B strongly increased inhibitory activity of *B. subtilis* (Microbial Inhibition Concentration (MIC) 30 – 50 nM) compared to 72.9 nM MIC of rifampicin alone, *see Bandow et al., Bacillus subtilis Tolerance of Moderate Concentrations of Rifampin Involves the ζ B-Dependent General and Multiple Stress Response*, J Bacteriol. 2002 Jan; 184(2): 459–467, hereby incorporated by reference in its entirety.

This is highly surprising because the cleavable disulfide linker connecting the rifampicin to the poly(ethylene) glycol “arm” relies on the reductive intracellular environment to cleave the disulfide bridge, but because the arm could not have penetrated intracellularly, the release of rifampicin must have occurred by some other means than cleavage of the disulfide bridge. Without wishing to be bound by theory, the rifampicin release likely occurred via an “oxidative” mechanism (**FIG. 12**) in which oxidation of the rifampicin moiety generates unstable intermediate that after hydrolysis affords free oxidized drug form active in bacterial transcription inhibition. Without wishing to be bound by theory, this reaction is expected to be efficient only in cellular membranes, since oxygen solubility is much higher in hydrophobic media, which would preserve the linkage between the drug and the construct until the construct binds to bacteria. Thus, release of an antibiotic “payload,” *e.g.* rifampicin or some other antibiotic, may occur either intracellularly or in the cellular membrane, and likely occurs by two different mechanisms, the former by cleavage of the disulfide bridge introduced into the “cell poisoning” cationic construct and the latter by oxidation. The “cell poisoning” cationic construct was further validated by measuring the rate of disulfide linker cleavage reaction in **compound VII** (**FIG. 7**) under biologically relevant conditions. The rate of rifampicin release in the presence of 10 mM glutathione was examined. At 40 °C the half-time of release was ~2 min., thus indicating that

The “cell entrapment” cationic construct was validated by measuring the rate of click reaction under biologically relevant conditions. Without wishing to be bound by theory, the bottleneck for the “cell-entrapment” approach is the rate of click reaction between the first

linking moiety and the second linking moiety after the binding of the reactive molecular constructs to the cellular surface. Thus, for efficient capturing of the cells, the polymerization rate for the cationic constructs to one another, *i.e.* the rate at which the first linking moiety links to the second linking moiety, must be higher than the cellular growth rate. Without wishing to be bound by theory, one known effective local concentration of the cationic constructs after binding to the cell is approximately 10-100 mM, although the invention is explicitly not limited as such. Azido derivative of sulforhodamine and DBCO-O4NP (final concentration 1 mM of each) were mixed at room temperature in 60 mM triethylammoniumacetate (TEAA) buffer pH 7.0. At time intervals indicated in **FIG. 13B** aliquots were withdrawn and the products separated by TLC in chloroform-ethanol (5:1) developing system. After separation the compounds were recovered by elution with methanol and their amount determined by absorption at 568 nm. Remarkably, the half-reaction time at 20 °C was about 30 seconds at a compound concentration of 1 mM, which suggests that under field conditions (37 °C and 10-100 mM compound concentration on the cell surface) the reaction will be complete in a matter of seconds, which indicates that DBCO and an aziderivative may be particularly effective linking moieties, as 30 seconds outpaces cellular growth rate of any known bacterium.

Construct	"Head"	"Body"	"Arms"	"Hands"	Payload
"Poisoning"	BODIPY-NHS	Poly-L-lysine	Diamino-PEG	Alkyne w/ isothiocyano joint	Rifamycin B
"Entrapment"	BODIPY-NHS	Poly-L-lysine	Diamino-PEG	DBCO/Adizoderivative	N/A

TABLE 1. Description of cationic constructs used in the Examples.

Construct	Chemical Validation	<i>In Vitro</i> Validation
"Poisoning"	Rate of Rifamycin B release under reductive conditions	Binding affinity to 3 bacterial strains and growth inhibition assay
"Entrapment"	Rate of click reaction to form polymer net	Binding affinity to 3 bacterial strains

TABLE 2. Validation of cationic constructs used in the Examples.

The foregoing examples and description of the preferred embodiments should be taken as illustrating, rather than as limiting the present invention as defined by the claims. As will be readily appreciated, numerous variations and combinations of the features set forth above can be utilized without departing from the present invention as set forth in the claims.

- 5 Such variations are not regarded as a departure from the scope of the invention, and all such variations are intended to be included within the scope of the following claims. All references cited herein are incorporated by reference in their entireties.

CLAIMS

1. A cationic construct comprising: a cationic polymer core linked to a plurality of polymer linkers, each individual polymer linker in the plurality of polymer linkers being linked to a functional moiety.

5 2. The cationic construct claim 1, wherein the cationic polymer core comprises a poly-lysine oligomer.

 3. The cationic construct of claim 2, wherein the poly-lysine oligomer consists of about 10 to about 30 repeat lysines.

 4. The cationic construct of any of claims 2-3, wherein the lysines are in the L-isomer.

10 5. The cationic construct of any of claims 2-3, wherein the lysines are in the D-isomer.

 6. The cationic construct of any of claims 1-5, wherein the individual polymer linkers in the plurality of polymers comprise poly(ethylene) glycol (PEG).

 7. The cationic construct of claim 6, wherein the individual polymer linkers in the
15 plurality of polymers comprising PEG each have a molecular weight (MW) of about 1000 to about 3000.

 8. The cationic construct of claim 6, wherein the individual polymer linkers in the plurality of polymers comprising PEG each have a molecular weight (MW) of about 1500 to about 2500.

20 9. The cationic construct of claim 6, wherein the individual polymer linkers in the plurality of polymers comprising PEG each have a molecular weight (MW) of about 1800 to about 2200.

 10. The cationic construct of any of claims 1-9, further comprising a detectable label bound to the cationic polymer core.

25 11. The cationic construct of claim 10, wherein the detectable label comprises BODIPY.

 12. The cationic construct of any of claims 1-11, wherein the functional moiety comprises a delivery agent.

 13. The cationic construct of claim 12, wherein the delivery agent is linked to the
30 individual polymer linker by a cleavable linker.

 14. The cationic construct of claim 13, wherein the cleavable linker comprises a disulfide linker.

15. The cationic construct of any of claims 12-14, wherein the delivery agent comprises an antibacterial agent.

16. The cationic construct of any of claims 1-11, wherein the functional moiety comprises a first linking moiety.

5 17. The cationic construct of claim 9, wherein the first linking moiety comprises one of N_3 and DBCO.

18. A system comprising a first cationic construct comprising the cationic construct of claim any of claims 16-17 and further comprising a second cationic construct comprising a cationic polymer core linked to a plurality of polymer linkers, each individual polymer linker
10 in the plurality of polymer linkers being linked to a second linking moiety,

the first cationic construct being covalently linked to the second cationic construct through the first linking moiety linked to the second linking moiety through click chemistry.

19. The system of claim 18, wherein the first cationic construct is covalently linked to the second cationic construct through a triazole moiety formed through click chemistry.

15 20. The system of claim 18, wherein the triazole moiety is formed from a reaction between DBCO and N_3 .

21. The system of any of claims 18-20, wherein the cationic polymer core of the second cationic construct comprises a poly-lysine oligomer.

22. The system of claim 21, wherein the poly-lysine oligomer of the second cationic
20 construct consists of about 10 to about 30 repeat lysines.

23. The system of any of claims 21-22, wherein the lysines of the second cationic construct are in the L-isomer.

24. The system of any of claims 21-22, wherein the lysines of the second cationic construct are in the D-isomer.

25 25. The system of any of claims 18-24, wherein the individual polymer linkers in the plurality of polymer linkers of the second cationic construct comprise poly(ethylene) glycol (PEG).

26. The system of claim 25, wherein the individual polymer linkers in the plurality of polymers in the second cationic construct comprising PEG each have a molecular weight
30 (MW) of about 1000 to about 3000.

27. The system of claim 25, wherein the individual polymer linkers in the plurality of polymers in the second cationic construct comprising PEG each have a molecular weight (MW) of about 1500 to about 2500.

28. The system of claim 25, wherein the individual polymer linkers in the plurality of polymers in the second cationic construct comprising PEG each have a molecular weight (MW) of about 1800 to about 2200.

29. The system of any of claims 18-28, wherein the second cationic construct further comprises a detectable label bound to the cationic polymer core.

30. The system of claim 29, wherein the detectable label of the second cationic construct comprises BODIPY.

31. A method of delivering a delivery agent to a target cell comprising:

i. providing a target cell; and
ii. contacting the target cell with the cationic construct of any of claims 12-15 under conditions allowing for the delivery agent to

1) internalize in the host cell or the host cell membrane and

2) release the delivery agent from the cationic construct.

32. The method of claim 31, wherein the target cell is a bacterial cell.

33. A method of entrapping a target cell comprising:

i. providing a target cell; and
ii. contacting the target cell with a plurality of first cationic constructs of any of claims 16-17 and a plurality of second cationic constructs comprising a cationic polymer core linked to a plurality of polymer linkers, each individual polymer linker in the plurality of polymer linkers being linked to a second linking moiety under conditions allowing individual first cationic constructs to covalently link to individual second cationic constructs through covalently linking the first linking moieties to the second linking moieties, and in an amount sufficient to entrap the target cell,

the first cationic construct being covalently linked to the second cationic construct through the first linking moiety linked to the second linking moiety through click chemistry.

34. The method of claim 33, wherein the cationic polymer cores of the plurality of second cationic constructs comprise poly-lysine oligomers.

35. The method of claim 34, wherein the poly-lysine oligomer of the second cationic construct consists of about 10 to about 30 repeat lysines.

36. The method of any of claims 34-36, wherein the lysines of the second cationic construct are in the L-isomer.

37. The method of any of claims 34-36, wherein the lysines of the second cationic construct are in the D-isomer.

38. The method of any of claims 33-37, wherein the individual polymer linkers in the plurality of polymer linkers of the plurality of second cationic constructs comprise poly(ethylene) glycol (PEG).

39. The method of claim 38, wherein the individual polymer linkers in the plurality of polymers in the second cationic construct comprising PEG each have a molecular weight (MW) of about 1000 to about 3000.

40. The method of claim 38, wherein the individual polymer linkers in the plurality of polymers in the second cationic construct comprising PEG each have a molecular weight (MW) of about 1500 to about 2500.

41. The method of claim 38, wherein the individual polymer linkers in the plurality of polymers in the second cationic construct comprising PEG each have a molecular weight (MW) of about 1800 to about 2200.

42. The method of any of claims 33-41, wherein individual second cationic constructs in the plurality of second cationic constructs further comprise detectable labels bound to the cationic polymer cores.

43. The method of claim 42, wherein the detectable labels bound to the cationic polymer cores of the second cationic constructs comprise BODIPY.

44. The method of any of claims 33-43, wherein the target cell is a bacterial cell.

45. A method of treating a bacterial infection in a subject in need thereof comprising administering to said subject a composition comprising the cationic construct of any of claims 1-17 and a pharmaceutically acceptable carrier or diluent.

46. The method of claim 45, wherein the subject in need thereof is immunocompromised.

47. The method of any of claims 45-46, wherein the bacterial infection is a multi-drug resistant (MDR) bacterial infection.

48. The method of any of claims 45-46, wherein the bacterial infection is an extensively-drug resistant (XDR) bacterial infection.

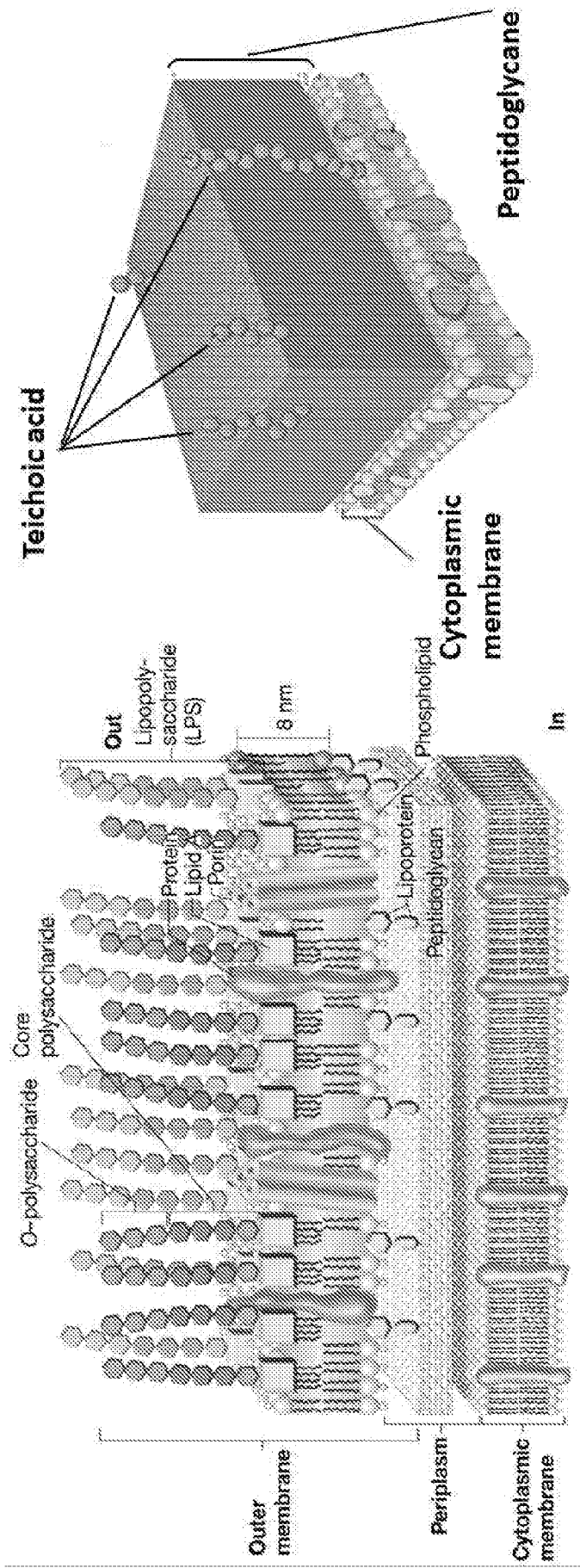
49. A medical device coated with a composition comprising the cationic construct of any of claims 1-17.

50. The medical device of claim 49 wherein the device comprises an implant or prosthesis.

51. A method of disinfecting a surface comprising contacting said surface with a composition comprising the cationic construct of any of claims 1-17.

52. The method of claim 51, wherein the surface is an environmental surface.

FIG. 1



Gram-positive

Gram-negative

FIG. 2

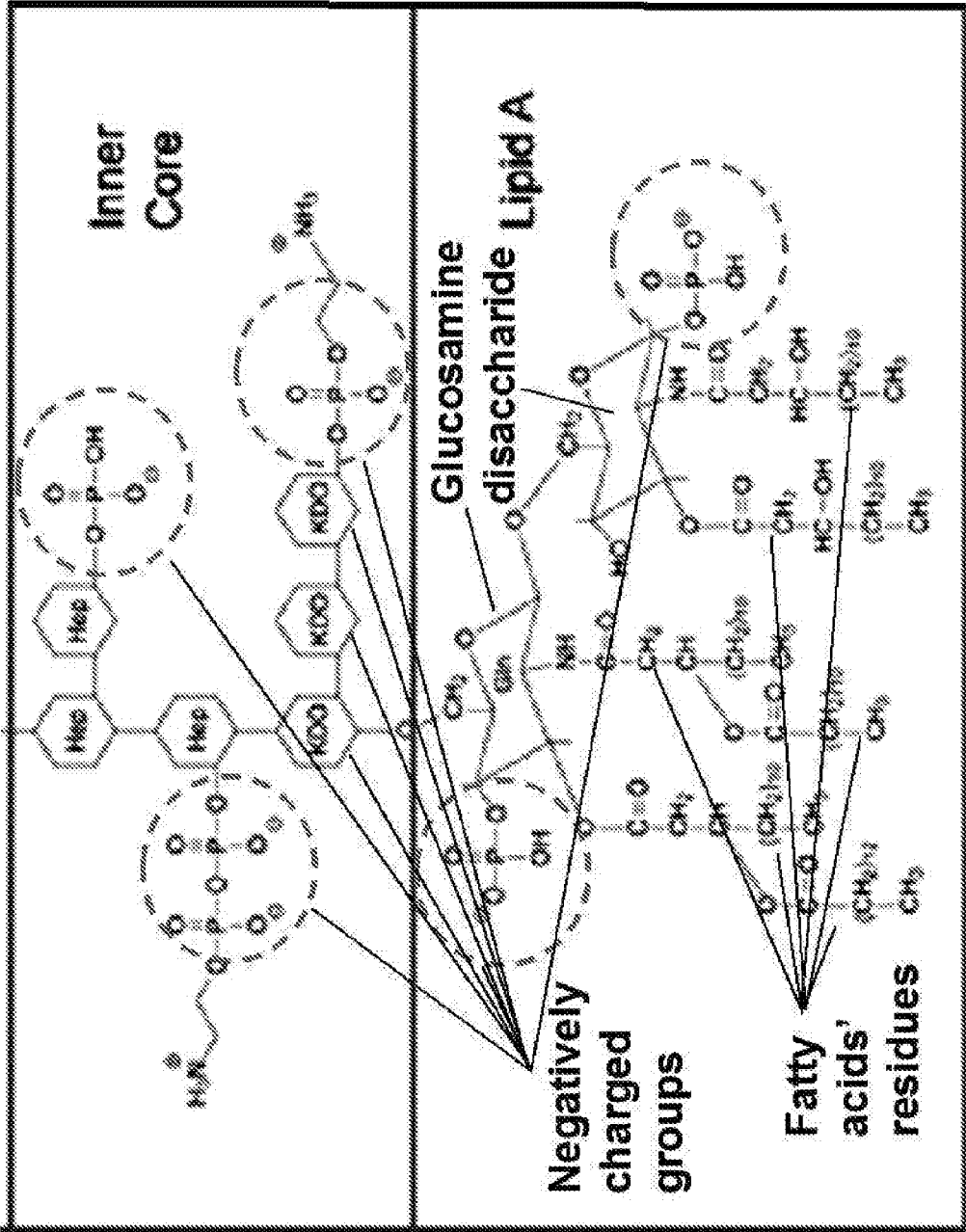


FIG. 3

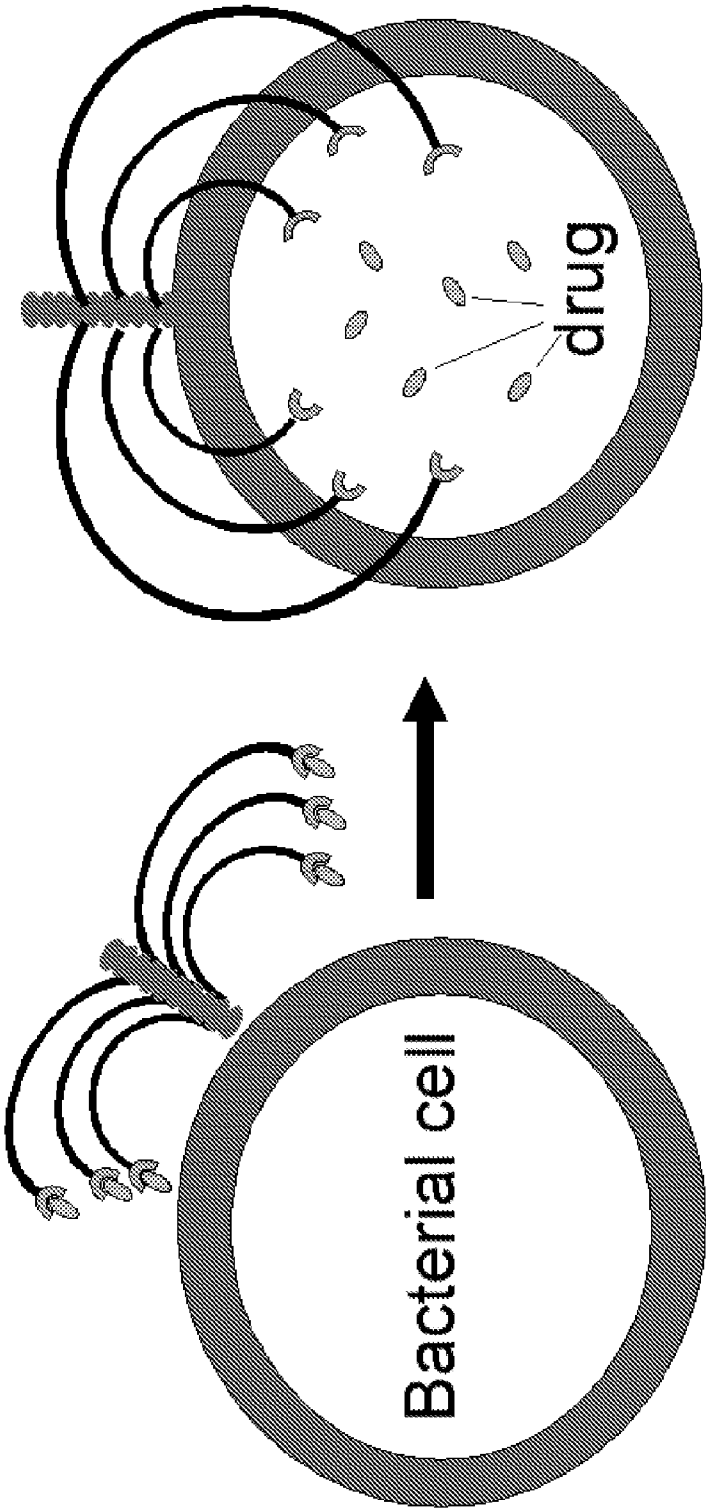
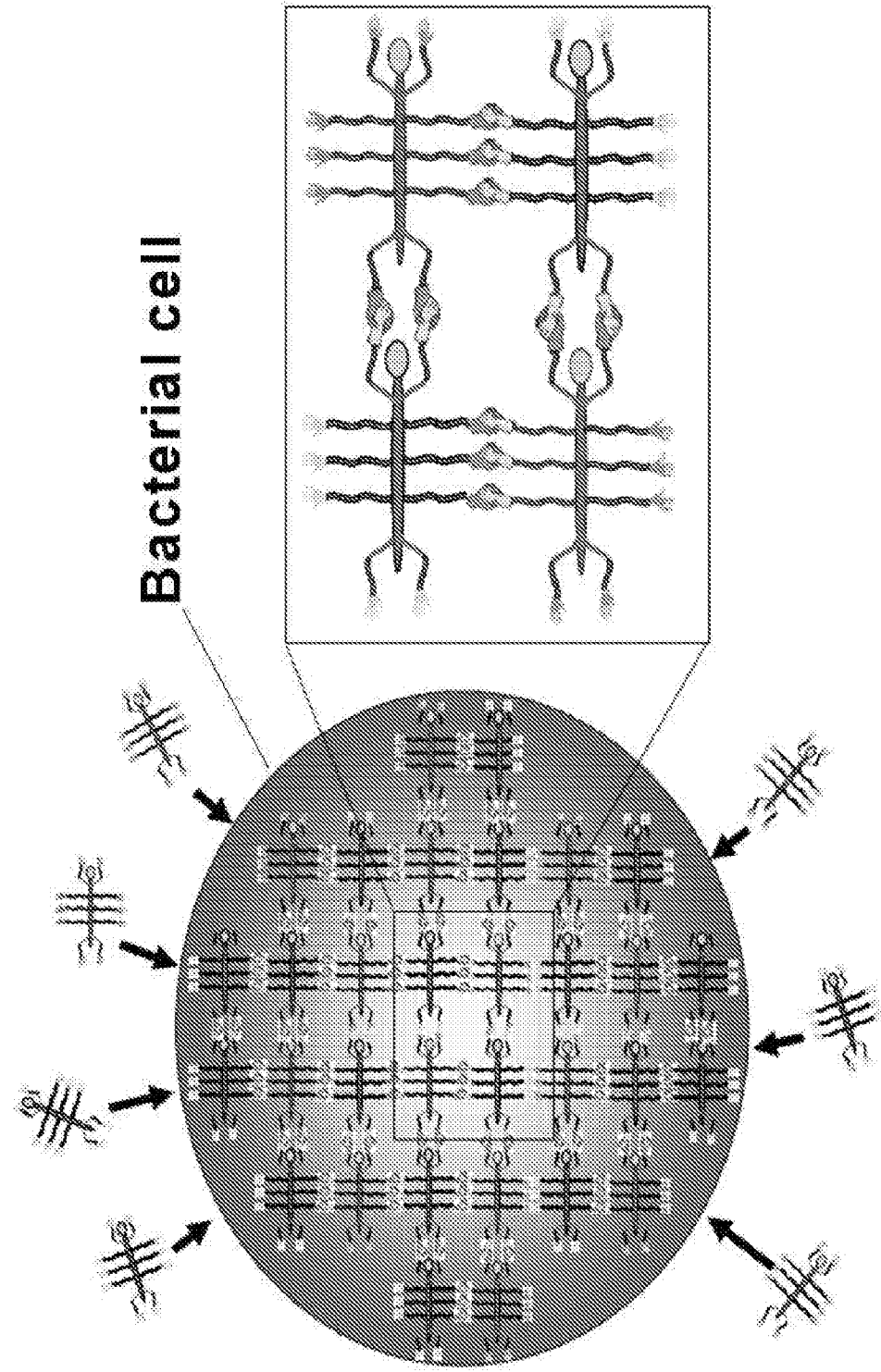


FIG. 4



FIGs. 5A and 5B

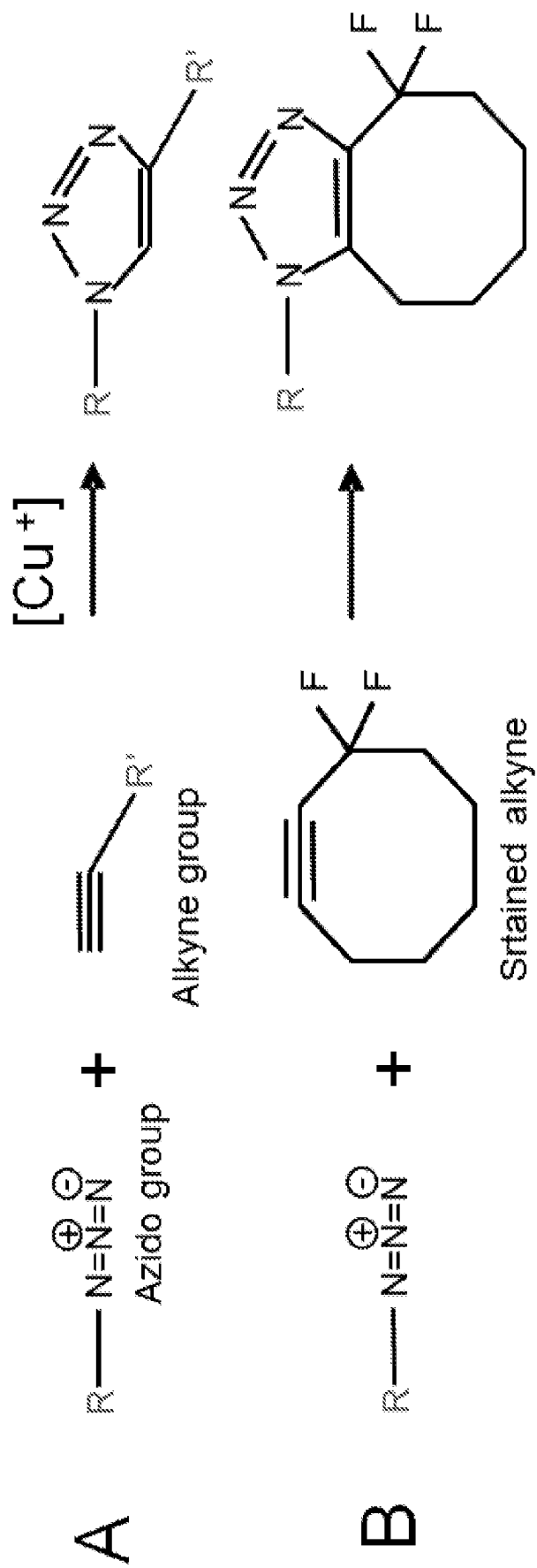


FIG. 6A

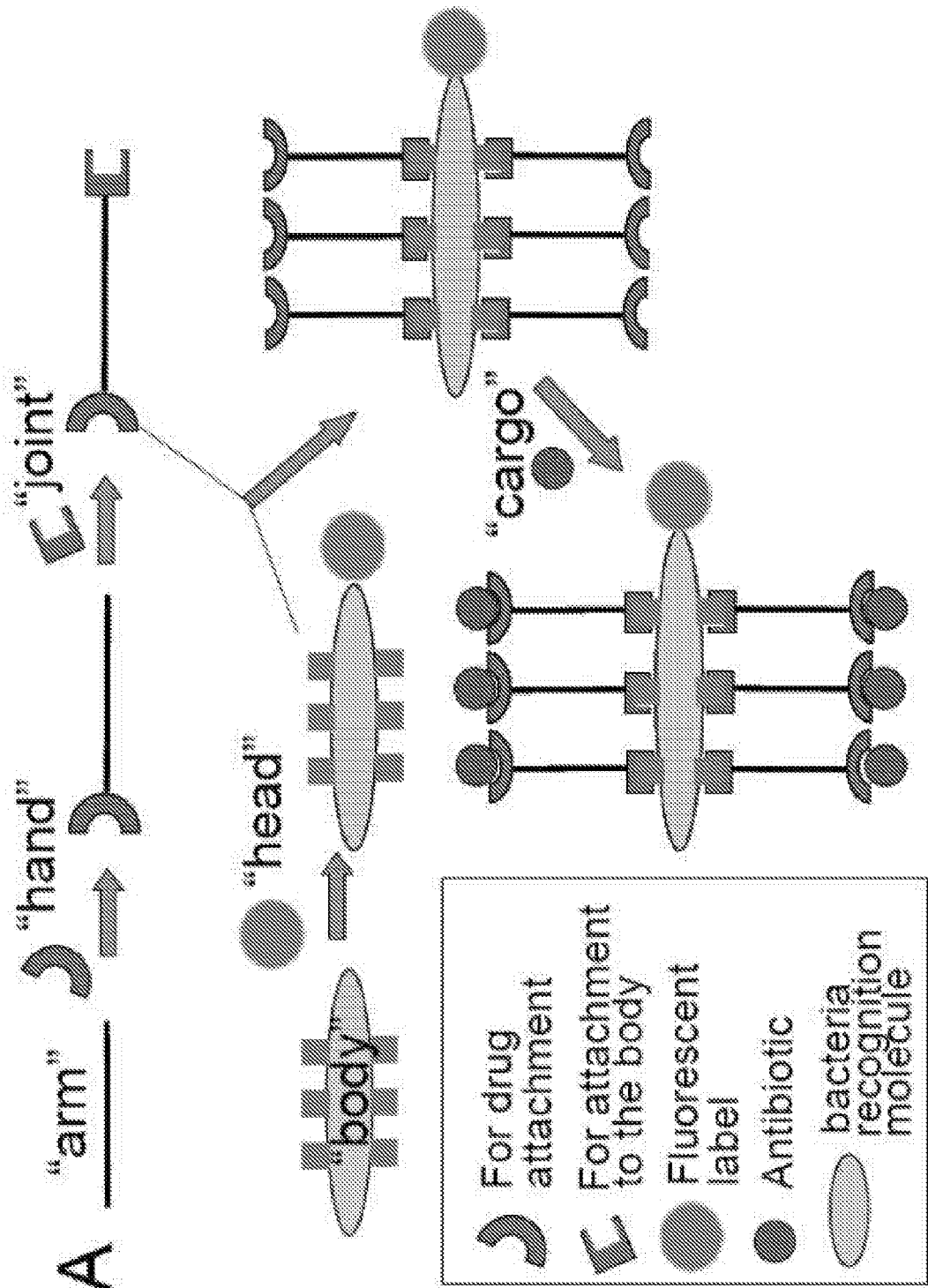


FIG. 6B

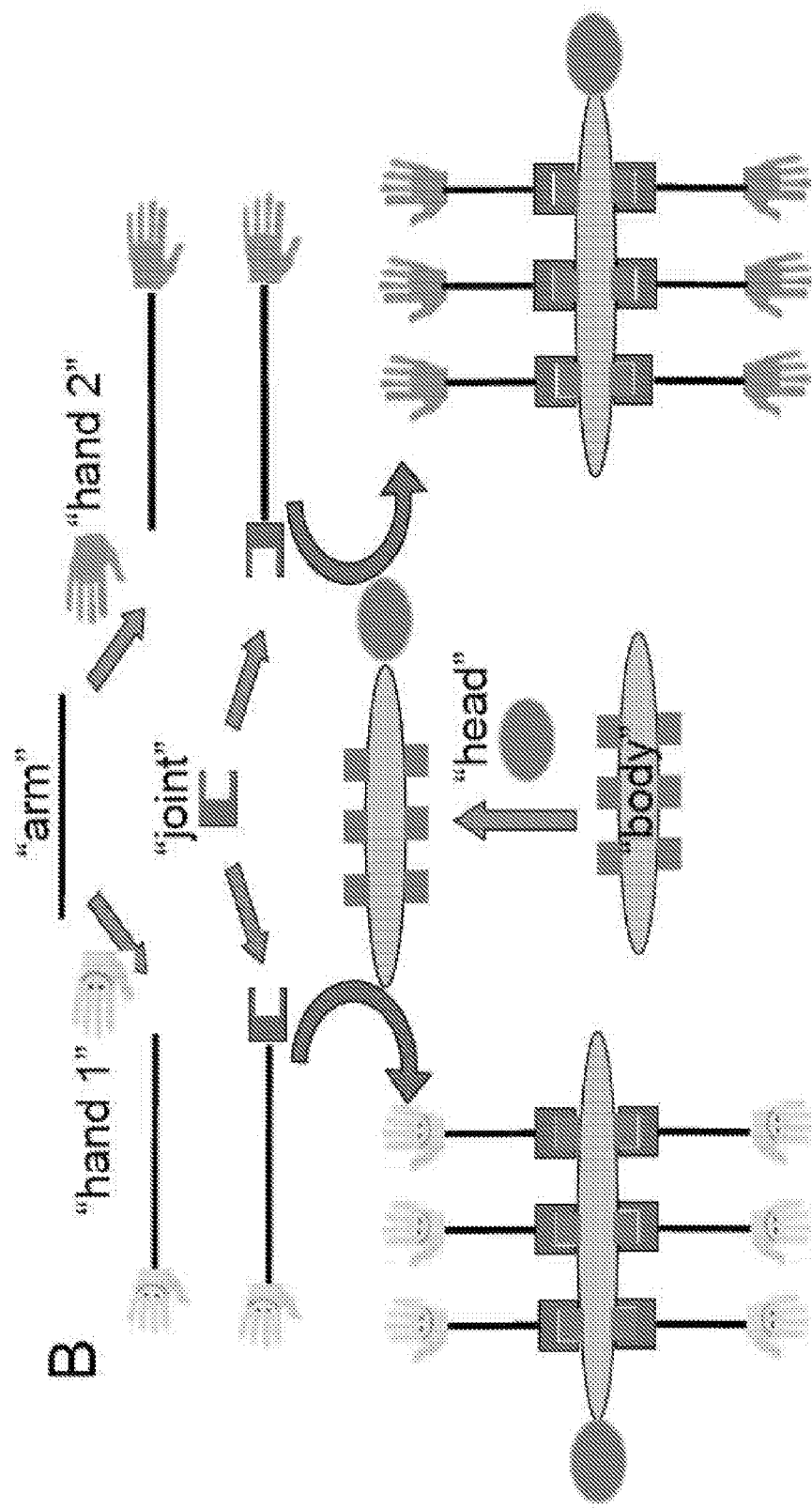


FIG. 7

The synthesis of “cell poisoning” constructs

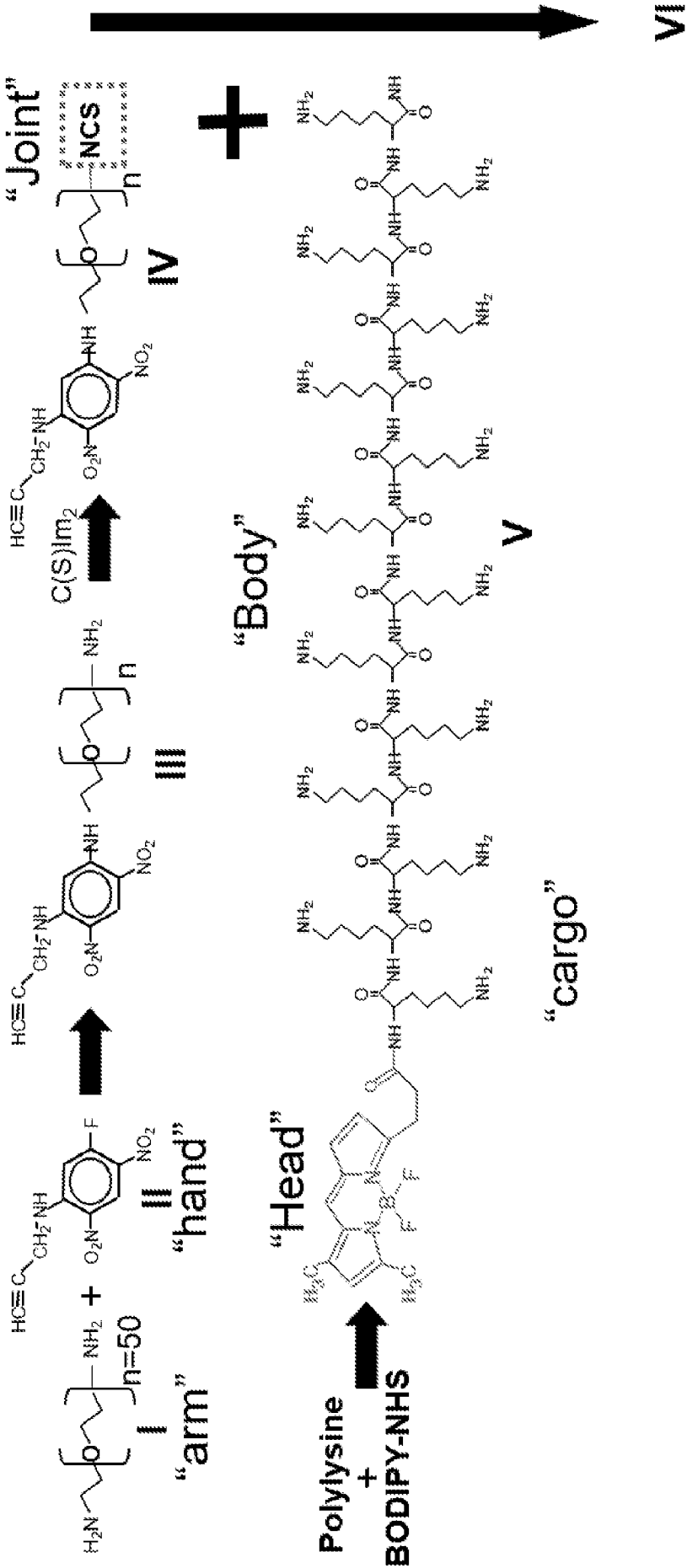


FIG. 7 (Cont'd)

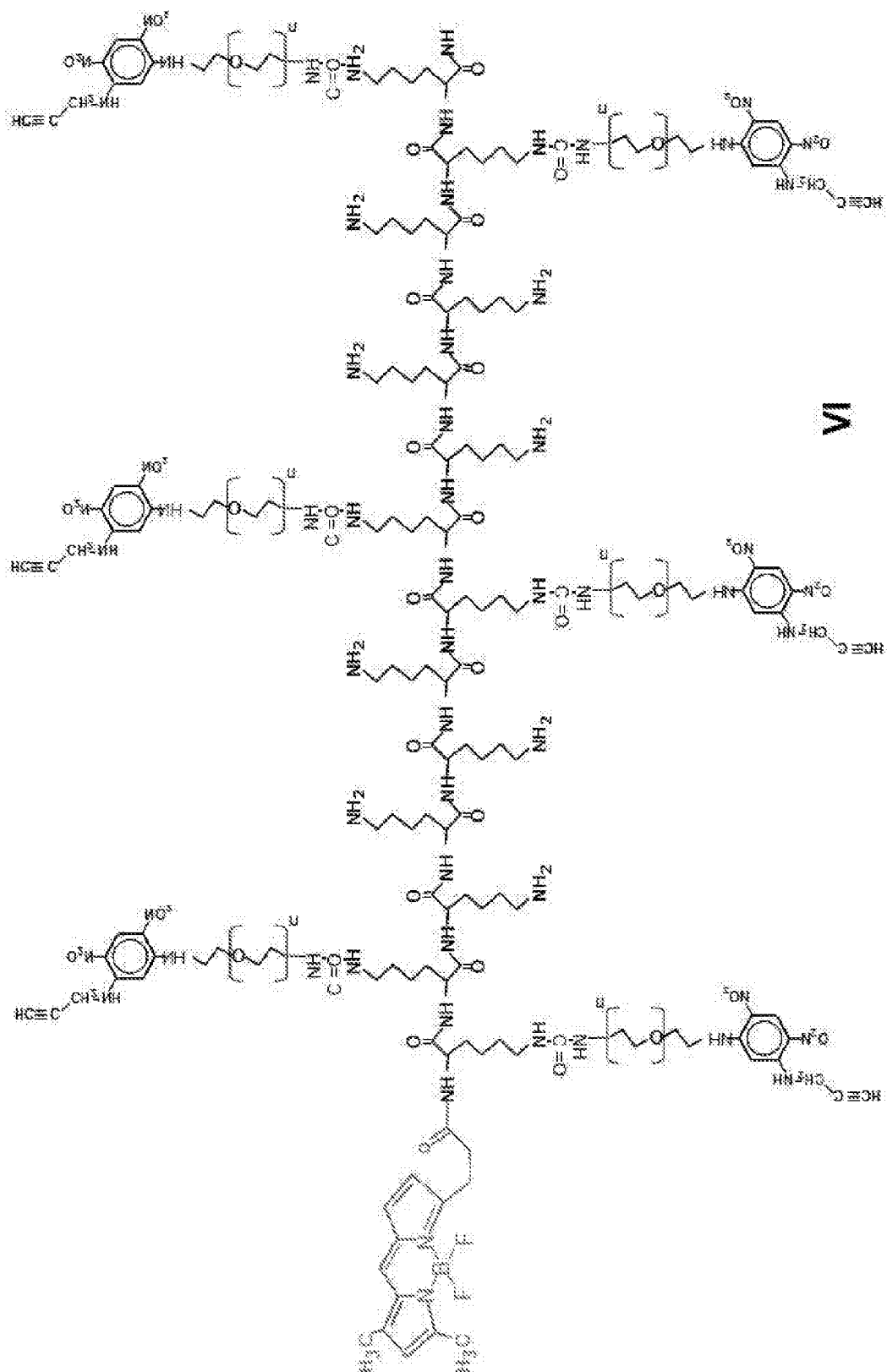


FIG. 7 (Cont'd)

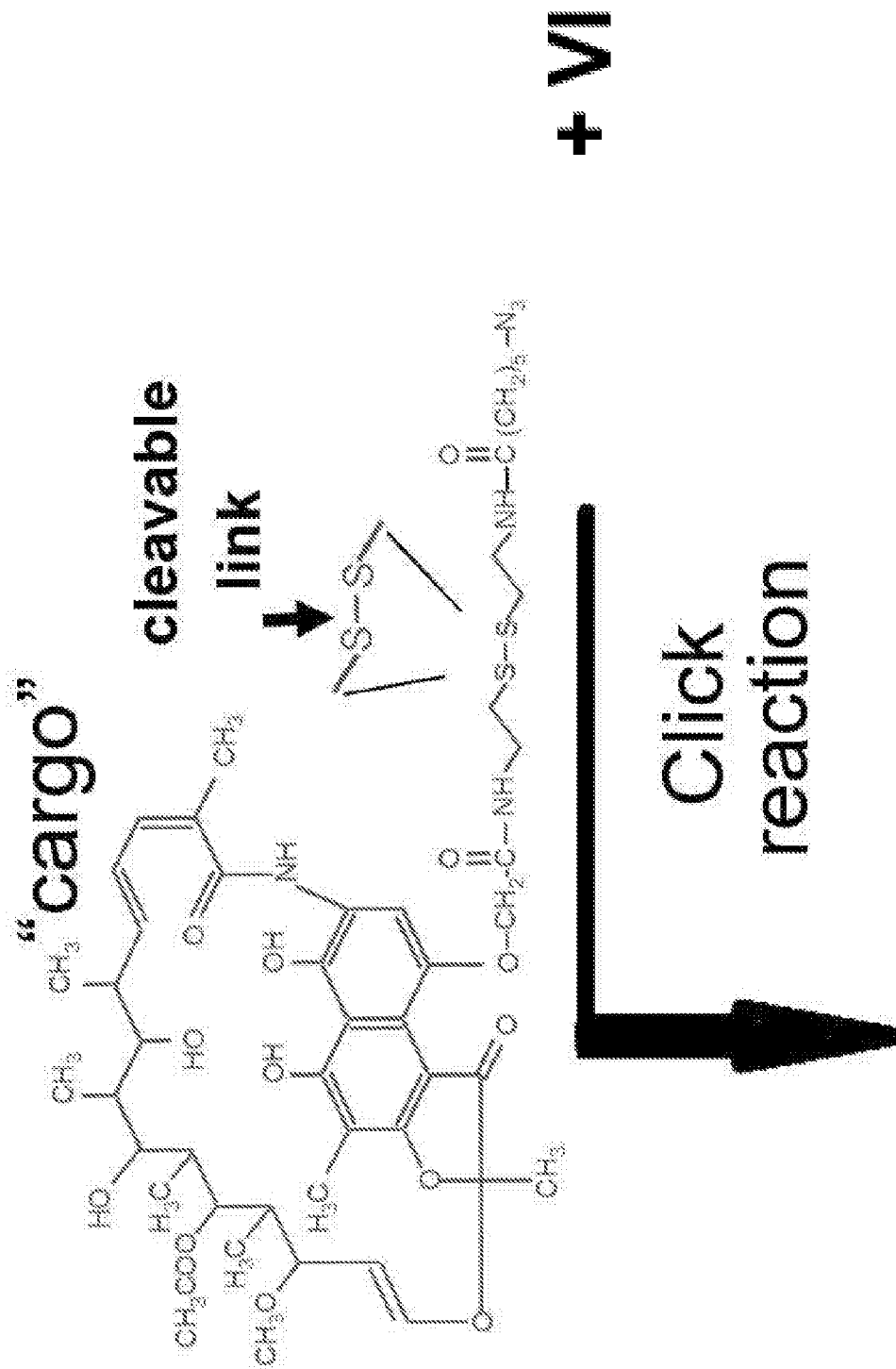


FIG. 7 (Cont'd)

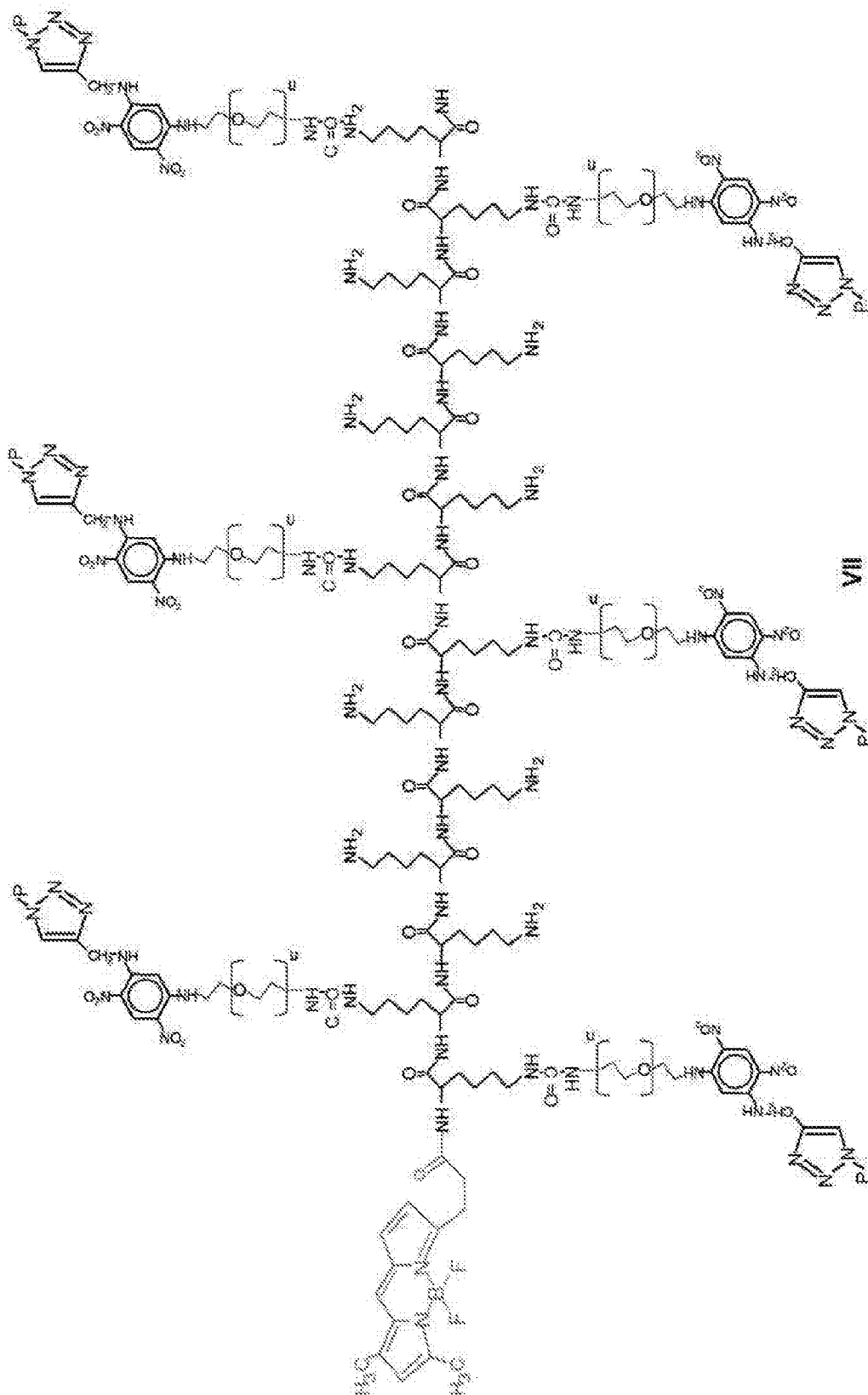


FIG. 7 (Cont'd)

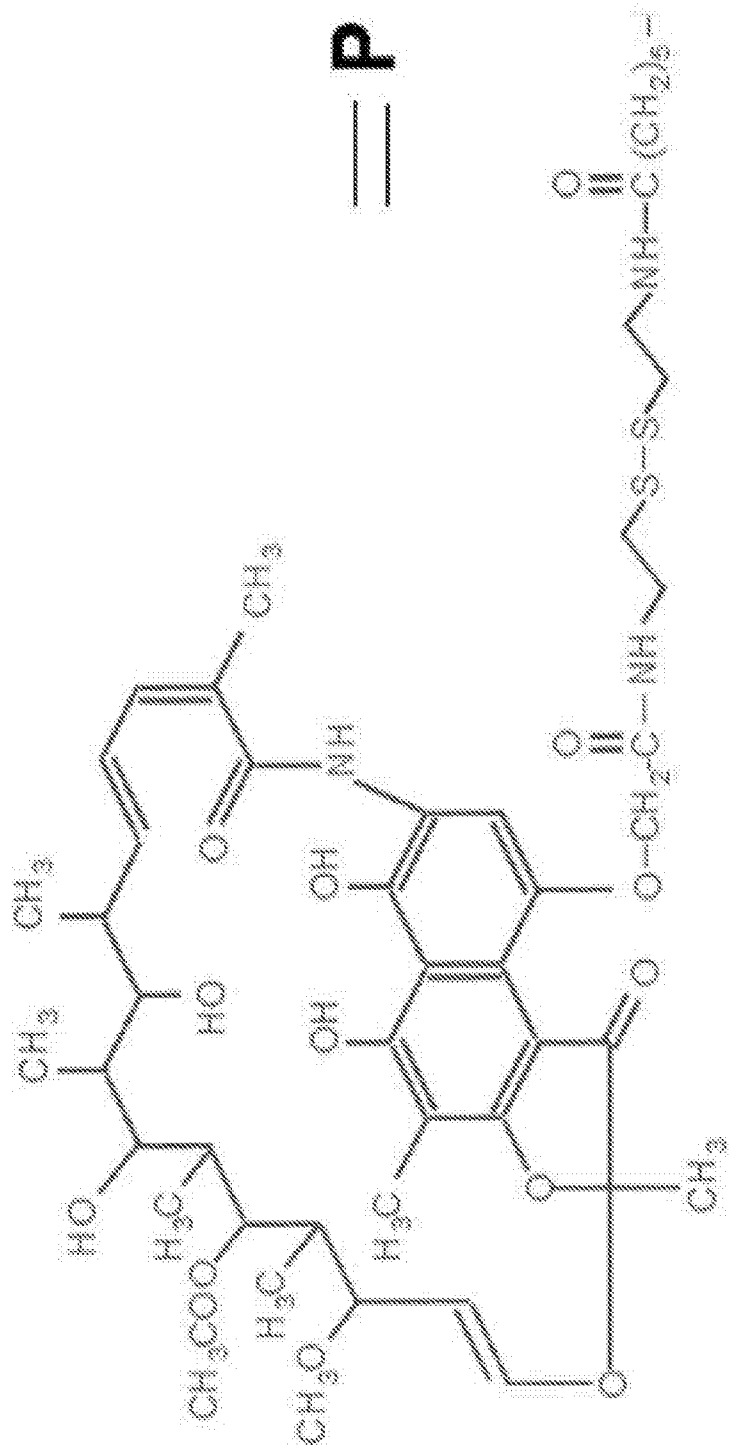


FIG. 8

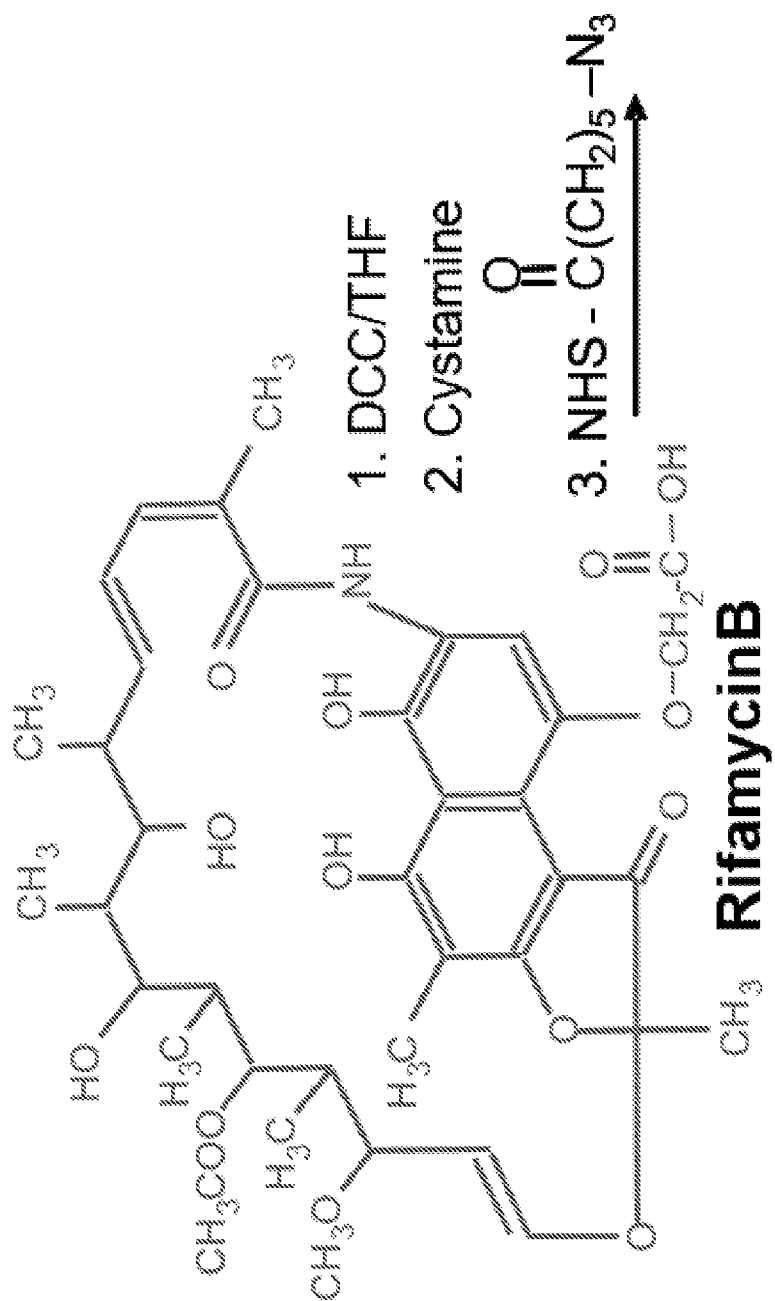
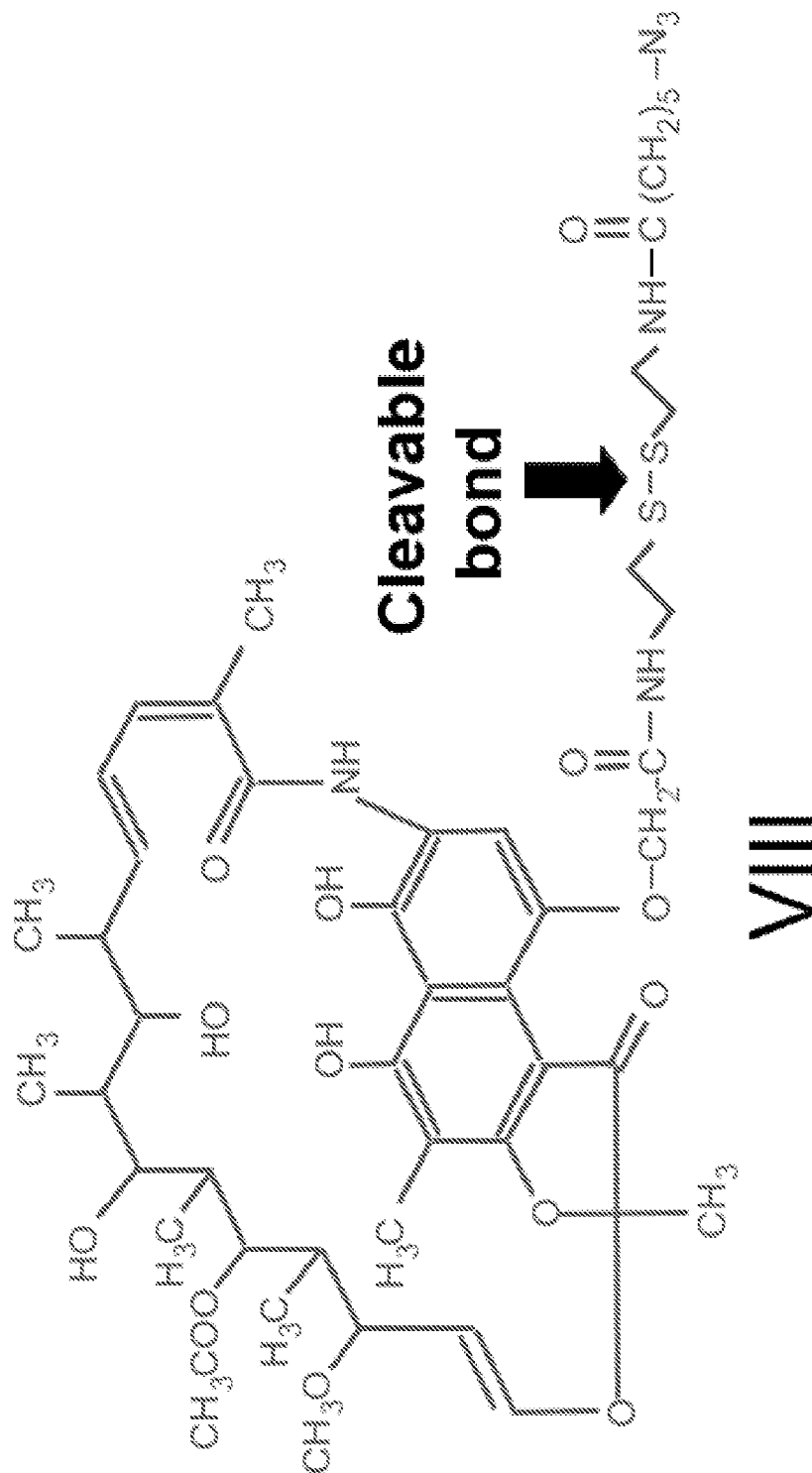


FIG. 8 (Cont'd)



FIGs. 10A and 10B

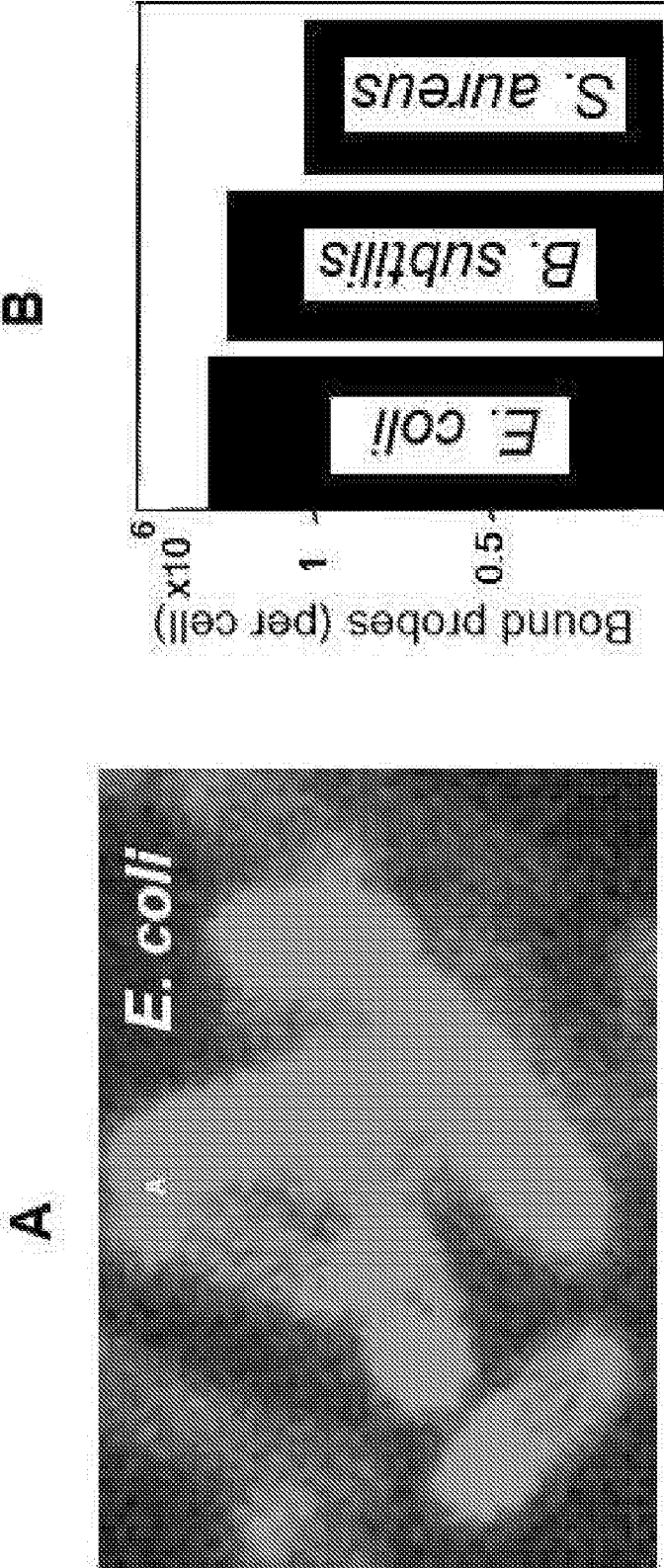
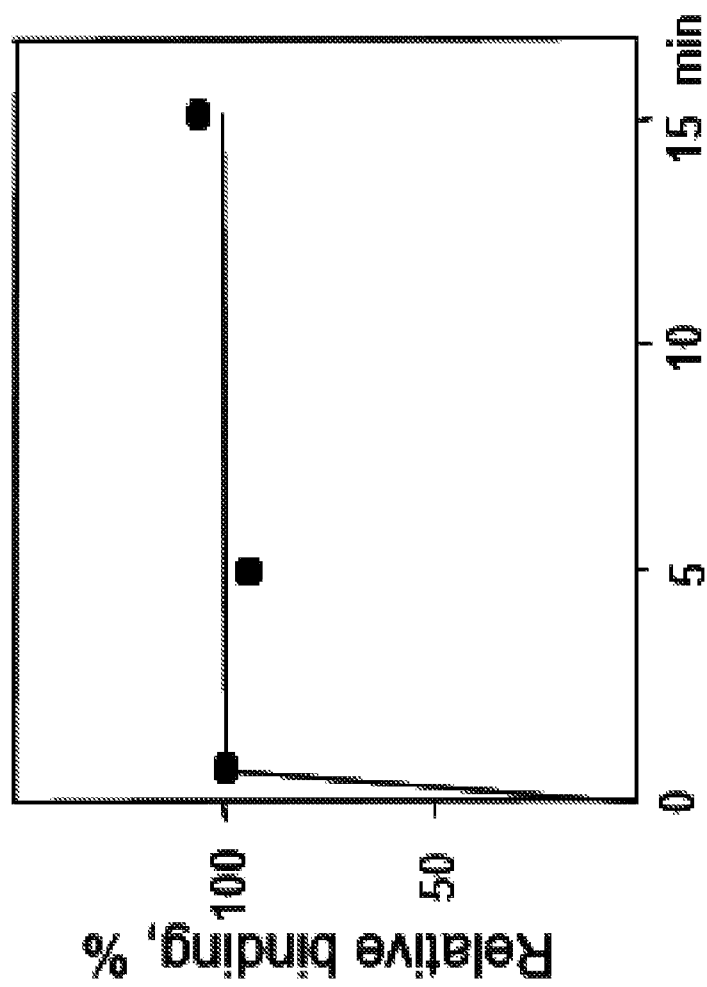


FIG. 10C



FIGs. 11A and 11B

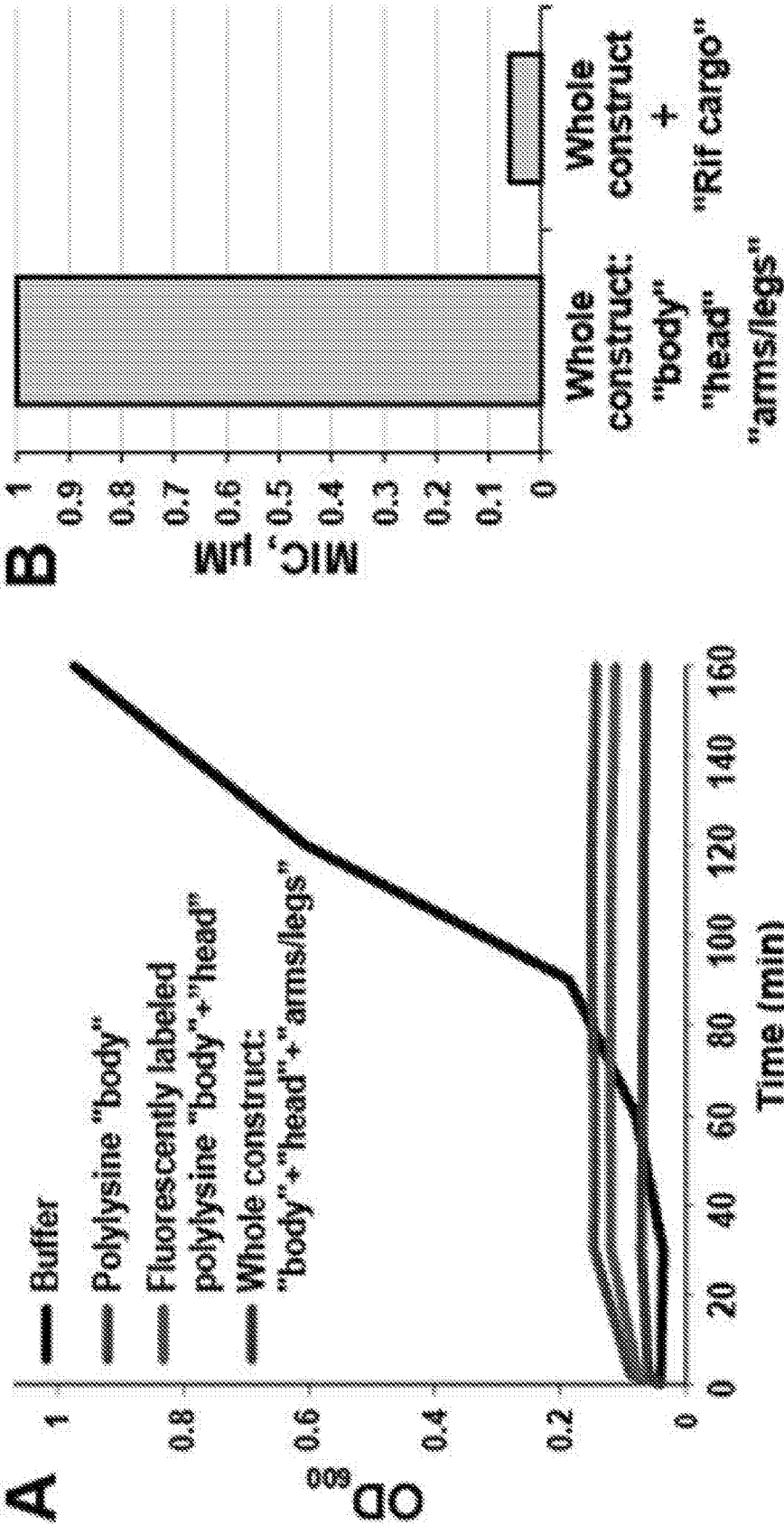


FIG. 12

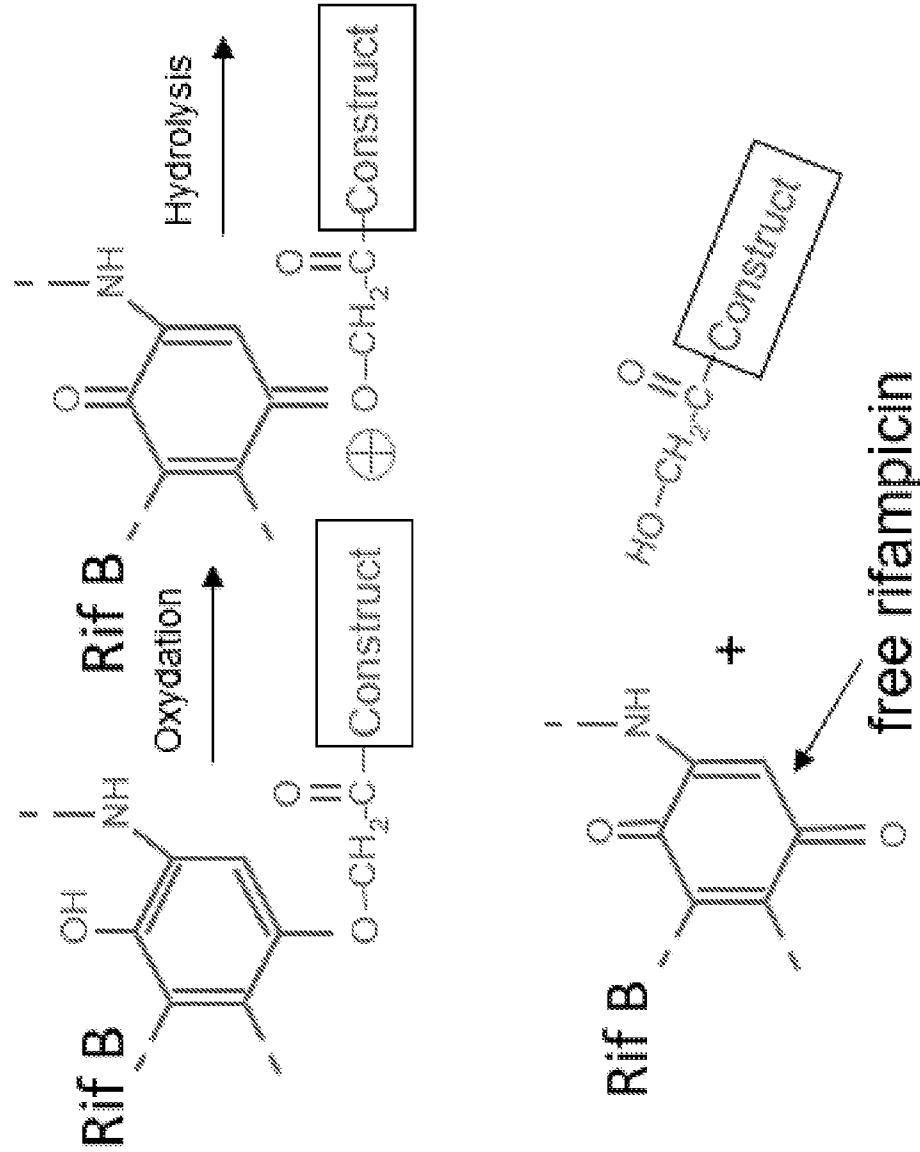


FIG. 13A

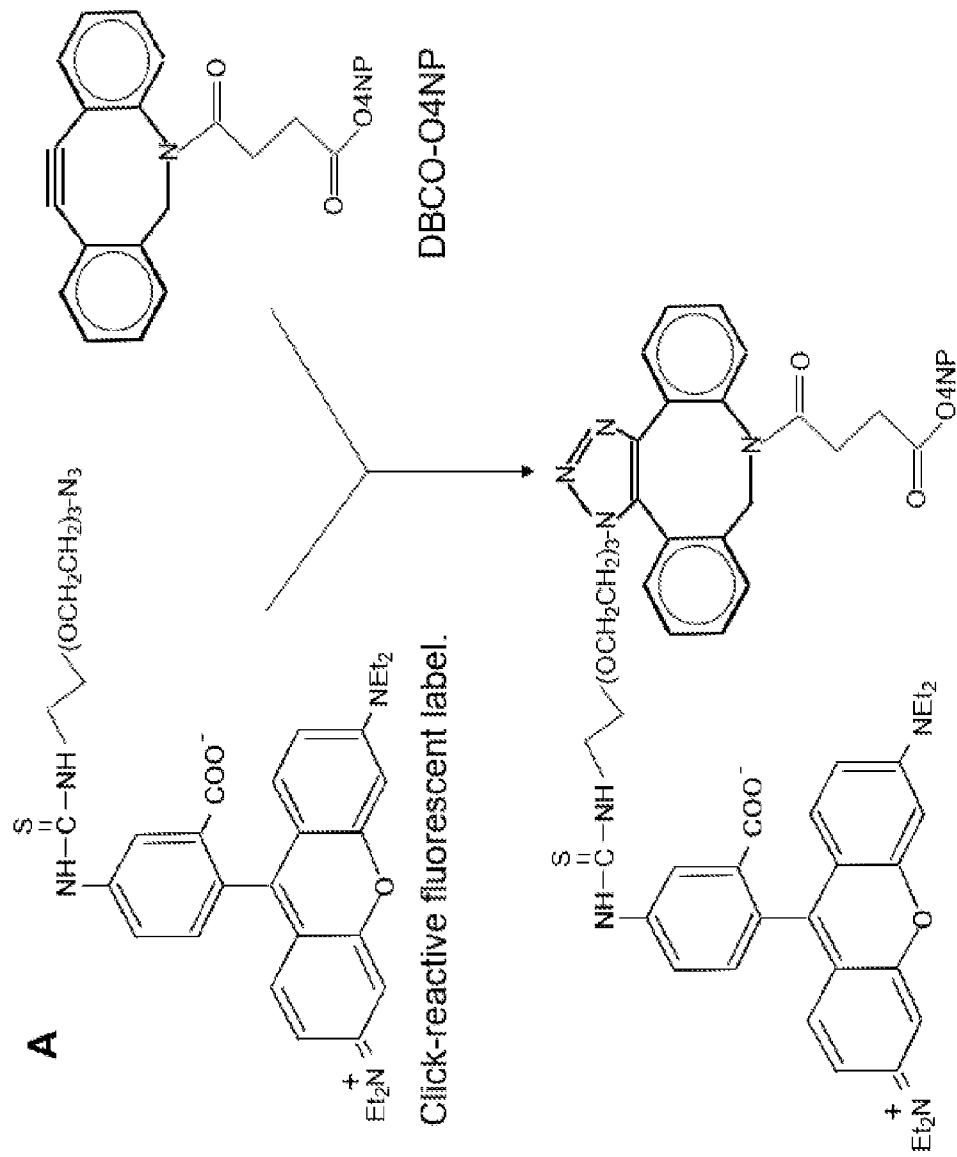
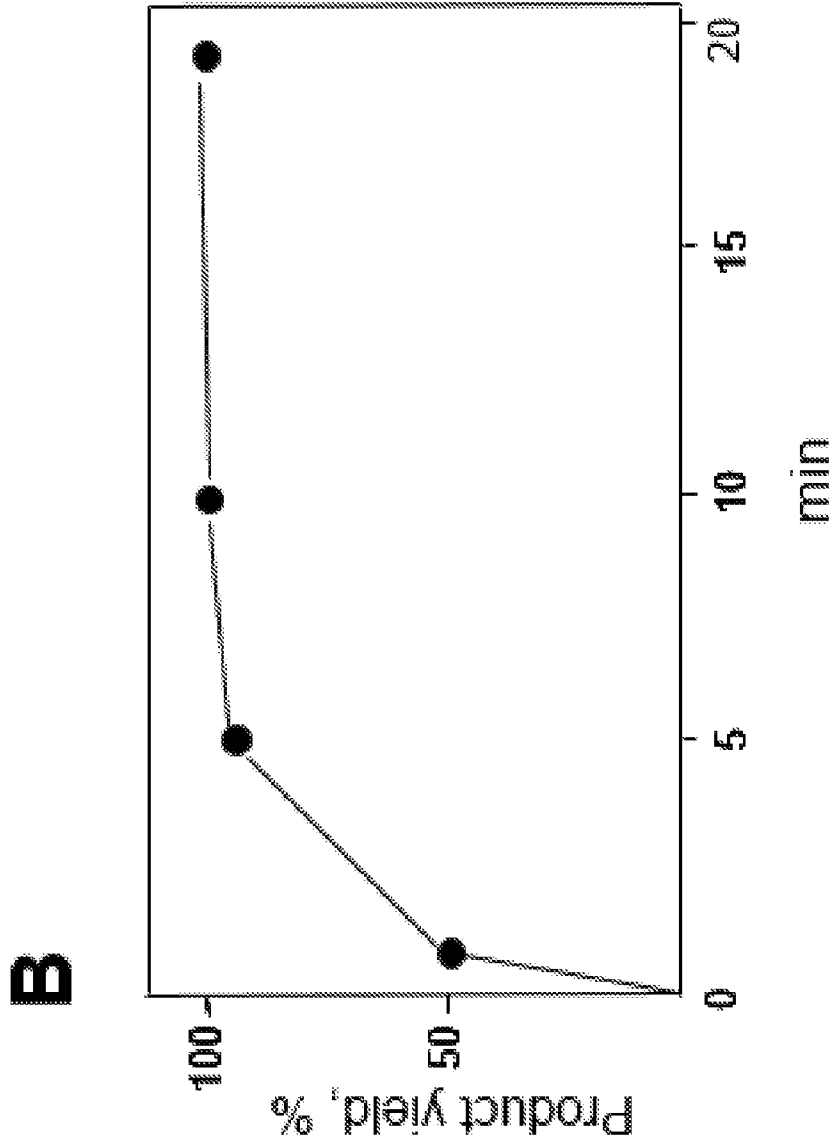


FIG. 13B



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/43288

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C07C 233/47; A61K 38/00 (2017.01)
 CPC - C07C233/47; C09D5/14; A61K 8/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2013/0302257 A1 (MINKO et al.) 14 November 2013 (14.11.2013) Fig 24; para [0033], [0045]-[0046]	1 ----- 2-5
Y	US 2015/0284476 A1 (PHARMAIN CORPORATION) 08 October 2015 (08.10.2015) Fig 1; para [0051]-[0052], [0056]-[0057], [0059]	2-5
A	KAMINSKAS et al. 'Pharmacokinetics and tumour disposition of PEGylated, methotrexate conjugated poly-L-lysine dendrimers', Molecular Pharmaceutics, 2009, Vol.6(4), pp. 1190-1204. Entire Document	1-5
A	US 2007/0274953 A1 (BERG et al.) 29 November 2007 (29.11.2007) Entire Document	1-5
A	WO 90/11778 A1 (THE ROCKEFELLER UNIVERSITY) 18 October 1990 (18.10.1990) Entire Document	1-5

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

07 September 2017 (07.09.2017)

Date of mailing of the international search report

05 OCT 2017

Name and mailing address of the ISA/US

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 Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/43288

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-52
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.