increasing the sensitivity, precision and specificity of detection of infected wounds; providing for the ability of qualitative and quantitative measurements; and, increasing the speed of detection of infected wounds *in situ* and in real-time. The assays and methods described herein are partly based on the use of specific reagents that detect biomarkers and/or probes which are present in infected or chronic wounds. The detection process may involve use of reagents that are specific to the markers present in infected wounds but not non-infected or non-chronic wounds and the detection step may involve qualitative or quantitative measurements of the signal(s) that are generated when the probe is acted upon by the marker. In examples wherein the detection method involves detection of enzymes present in wounds, the probes comprise modified enzyme substrates that are specific to the enzyme, which generate signals that may be optionally amplified. This greatly improves efficiency and specificity of detection. Moreover, a plurality of detection probes, each specific for one or more targets, *e.g.*, enzymes that are specific to the wounds, may be employed. This greatly helps to maximize both efficiency and accuracy of diagnostic assays while minimizing the incidence of false positives (*e.g.*, due non-specific interactions and/or target redundancy). Furthermore, the experimental results disclosed herein confirm that the novel probes and the assay techniques based thereon are capable of detecting and characterizing various types of wounds. Finally, the reagents of the disclosed technology may be used together with therapeutic molecules such as antibiotics, antifungal agents, etc. to monitor and evaluate treatment and management of chronic wounds.

[0009] Examples described herein are based, in part, on the discovery that cells of the immune system, including enzymes generated thereby, may serve as markers in the early diagnosis of wounds. These cells, e.g., neutrophils, are recruited at the wound situs to combat infection, do so by engulfing bacteria (and other pathogens) and/or neutralizing them with enzymes. Some enzymes are specific towards proteins (e.g., elastase, cathepsin G, lipase), others are specific towards cell wall components (e.g., lysozyme) and yet others mediate protein denaturation (e.g., NADPH oxidase, xanthine oxidase, myeloperoxidase (MPO) and other peroxidases). These cells, e.g., neutrophils, are generally only short-lived and when they lyse in the area of the infection, they release the contents of their lysosomes including the enzymes, which can then be detected to provide a reliable measurement of the status of the wound.

[0010] Accordingly, various examples described herein utilize the detection of enzyme markers, which are indicative of the presence of myeloid cells, and neutrophils in particular, in a biological sample of interest, for example, wound tissue. Increased level or activity of such enzymes in the wound fluid, therefore, corresponds to a heightened bacterial challenge and a manifestation of disturbed host/bacteria equilibrium in favor of the invasive bacteria.

[0011] In one example, provided herein is a chemical entity capable of detecting enzyme activity from a body fluid, the chemical entity comprising one or more of an anchor region, an enzyme recognition region, an enzyme-labile or enzyme-reactive region , and an indicator region.

[0012] In some examples, the chemical entity comprises at least three of an anchor region, an enzyme recognition region, an enzyme-labile or enzyme-reactive region and an indicator region. In some examples, the chemical entity comprises one of an anchor region, an enzyme-labile or enzyme-reactive region and an indicator region. In some examples, the chemical entity comprises one of an anchor region, one of an enzyme recognition region, one of an enzyme-labile or enzyme-reactive region and one of an indicator region. In some examples, the chemical entity binds to a support material via the anchor region. In some embodiments, the chemical entity comprises at least two indicator regions. In some examples, the enzyme recognition region partially or fully overlaps with the enzyme-labile or enzyme-reactive region. In some examples, the anchor region partially or fully overlaps with the enzyme-labile or enzyme-reactive region. In some examples, the anchor region partially or fully overlaps with the indicator region. In some examples, the indicator region partially or fully overlaps with the enzyme-labile or enzyme-reactive region. In some examples, the indicator region, once separated from the chemical entity by target enzyme activity, interacts with one or more accessory enzymes selected from a lipase, esterase, peroxidase, oxidase, glycosidase, glucuronidase, glucosidase, galactosidase, and a combination thereof. In some examples, the enzyme-labile or enzyme-reactive region interacts with one or more target enzymes selected from Napsin (aspartyl protease), Glucosylceramidase glucuronidase, palmitoyl protein thioesterase, Cathepsins A, B, D, G, L, S, Z, Acid ceramidase, lactoferrin (LF), lysozyme, myeloperoxidase (MPO), elastase, cathepsins, and proteinase-3 elastase, lysozyme, esterase, lipase and, and a combination thereof.

[0013] In some examples, the enzyme-labile or enzyme-reactive region comprises a moiety capable of producing a visible color or detectable electronic change upon interaction of the enzyme-labile or enzyme-reactive region with one or more target enzymes, the moiety being selected from a peroxidase substrate, arylamine, an amino phenol, a phenol, a quinone, a neutral dye, a charged dye, a nanoparticle, a quantum dot, a colloidal gold particle, or an analog thereof. In some examples, the peroxidase substrate is selected from *p*-aminophenol, ABTS **(**2,2inophenol, ABTS (rate is selected from gold acid) diammonium salt)**,** 3,3'-diaminobenzidine, DCPIP, *N*,*N*-dimethyl-*p*-phenylenediamine, *o*-dianisidine, *p*-phenylenediamine, 4-chloro-1-naphthol, *o*-phenylenediamine *N*-(4-aminobutyl)-*N*-ethylisoluminol, 3-amino-9-ethylcarbazole, 4-aminophthalhydrazide, 5-aminosalicylic acid,

2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), indoxyl, indigo, Fast Blue RR, and 4-chloro-7-nitrobenzofurazan, and an analog thereof. In some examples, the peroxidase substrate an aniline analog. In some examples, the peroxidase substrate is an N-alkyl derivative of Fast Blue RR with more than 6 carbon units. In some examples, the indicator region comprises a moiety capable of producing a visible color or detectable electronic change upon interaction of the enzyme-labile or enzyme-reactive region with one or more target enzymes, the moiety being selected from an indoxyl analog, a neutral dye, a charged dye, a nanoparticle, and a colloidal gold particle. In some examples, the moiety capable of producing a visible color or detectable electronic change is a charged dye or a luminol derivative. In some examples, the charged dye is selected from toluidine blue, reactive black 5, remazol brilliant blue, reactive violet 5, and reactive orange 16. In some examples, the charged dye is selected from reactive blue 4, reactive red 120, reactive blue 2, reactive green 19, and reactive brown 10. In some examples, the enzyme-labile or enzyme-reactive region is labile to or reactive with lysozyme, and the enzyme-labile or enzyme reactive region comprises a polysaccharide, glucosamine, or peptidoglycan, and the polysaccharide, glucosamine, or peptidoglycan. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptidoglycan, and peptidoglycan is labile to or reactive with lysozyme. In some examples, the enzyme-labile or enzyme-reactive region comprises a phenol, a napthol, an indoxyl, or a quinone, and the phenol, carboxyaminophenyl, indoxyl, or quinone is labile to or reactive with myeloperoxidase and not reactive to heme. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide, peptidomimetic, or protein, and the peptide, peptidomimetic, or protein is labile to or reactive with elastase. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of X_yAAPX_y-L-Z, wherein each X is independently any amino acid, each y is independently a number selected from 1 to 50, L is a linking moiety, and Z is a moiety capable of causing a visible color change or a detectable electronic change. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of X_yAAPVX_y-L-Z, wherein each X is independently any amino acid, each y is independently a number selected from 0 to 50, L is a linking moiety such as an ester or amide, and Z is a moiety capable of causing a visible color change or a detectable electronic change. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide, peptidomimetic, or protein, and the peptide, peptidomimetic, or protein is labile to or reactive with cathepsin G. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of $X_yN^4N^3N^2N^1X_y$-L-Z, wherein each X is independently any amino acid; each y is independently a number selected from 0 to 6; N^4 is

selected from alanine, glycine, valine, and glutamine; N^3 is selected from alanine, glycine, proline, lysine, and serine; N^2 is selected from proline, alanine, and glycine; N^1 is selected from serine, lysine, phenylalanine, arginine, leucine, and methionine; L is a linking moiety, and Z is a moiety capable of causing a visible color change or a detectable electronic change. In some examples, the anchor region is selected from a polystyrene bead, silica gel bead, polysaccharide bead, polyacrylamide bead, cellulose bead, polysaccharide, derivatized cellulose, polyacrylate, polyethyleneimine, polyacrylamide, UV-activatable group, phenolic azide, epoxide, peptidoglycan, an aliphatic chain, an aliphatic alcohol chain, a multi-cyclic or multi-aromatic ring system, a lipophilic group, and a combination thereof. In some examples, the anchor region binds to a support material after a short period of UV irradiation. In some examples, the anchor region comprises an ionic chemical group for binding to a support material. In some examples, the anchor region comprises a reactive moiety for covalent attachment to the support material. In some examples, the anchor region and the enzyme labile region are polypeptides and the anchor region comprises a polymer binding domain. In some examples, the enzyme labile region is labile to a protease and the polymer binding domains are selected from hydrophobic binding domains. In some examples, the enzyme labile region is labile to cathepsin or elastase. In some examples, the chemical entity is selected from a small molecule entity or a modified polymer.

[0014] In one example, provided herein is a chemical entity for the detection of infection, the chemical entity comprising an indicator region comprising a pH-sensitive moiety that presents a visible color change. In some examples, the chemical entity further comprises an reactive group that allows the reaction to a solid phase. In some examples, the pH-sensitive moiety is bromothymol blue, phenol red, bromophenol red, chlorophenol red, thymol blue, bromocresol green, bromocresol purple; or other sulfophthalein dyes.

[0015] In some examples, the anchor region binds to a support material after a short period of UV irradiation. In some examples, the anchor region comprises an ionic chemical group for binding to a support material. In some examples, the anchor region comprises a reactive moiety for covalent attachment to the support material. In some examples, the anchor region comprises a hydrophobic moiety that causes little or no solubility in aqueous systems thus allowing the material to stay associated with a solid phase.

[0016] In some examples, the reactive region interacts with the bacterial enzyme β-lactamase. This is a bacterial enzyme that is capable of degrading common antibacterial drugs and its presence is of interest to treating physicians. A chromogenic β-lactamase substrate is generally useful in addition to reagents that report on other biomarkers.

[0017] In some examples, the reactive region is a substrate for viral proteases or the host furin protease. In some examples, the detection of the cleaved product requires a polycationic trap. In certain examples, these can be cross-linked. Depending on the degree and type of cross-linking they can also be superabsorbers. In some examples, the enzyme substrates are able to give rise to a redox active species that can be detected electronically. In other examples, the electronic detection of enzyme products in solid phase is made by means of reflected light. Disclosed herein are chemical entities, which can be of monomeric, oligomeric or polymeric nature. These are modified to serve as medium for detection of chosen marker for infection in a wound or in body fluids, before the infections are otherwise apparent. In some examples, the chemical entity is for detecting infection in a mammal. In some examples, the chemical entity detects one or more biomarkers of infection and produces a visible change in the presence of the one or more biomarkers. In some examples, the one or more biomarkers are leukocyte enzymes. In some examples, the one or more biomarkers are selected from elastase, lysozyme, myeloperoxidase, leukocyte peroxidase, esterase, lipase, napsin (aspartyl protease), glucosylceramidase glucuronidase, palmitoyl protein thioesterase, cathepsins A, B, D, G, L, S, Z, Acid ceramidase, lactoferrin (LF), and proteinase-3, β-lactamase, and other similar enzymes, or combination thereof. In some examples, the chemical entity detects a specific pH range. In some examples, the chemical entity detects one or more leukocyte enzymes and a specific pH range; and produces a visible change in the presence of the one or more leukocyte enzymes and the specific pH range. In some examples, the visible change is a color change that is easily distinguished from colors common in wounds or body fluids (*e.g.*, red, yellow, pink, or brown). In some examples, the visible change is fluorescent, luminescent, or mediated via physical means such as refraction, gas evolution, or a change in polymer state. In some examples, the chemical entity comprises one or more components selected from the group consisting of: an anchor region, an enzyme recognition region, an enzyme-labile or enzyme-reactive region, and an indicator region. In some examples, the body fluid is blood, plasma, serum, cerebrospinal fluid, sputum, urine or wound exudate. In preferred examples, the body fluid is wound exudate.

[0018] In some examples, the chemical entity is incorporated into a wound dressing where the chemical entity reacts with wound exudates that come into contact with the wound dressing. In some examples, the chemical entity is incorporated into a wound dressing where the chemical entity reacts with wound exudates that are drawn up through the wound dressing to a reagent layer comprising the chemical entity. In some examples, the chemical entity is an indicator conjugate that gives rise to a color or other visible marker when infection biomarkers are present. In some examples, the chemical entity is used in methods to diagnose an

infected wound in a mammal. In some examples, the chemical entity is used in methods to treat a wound in a mammal. In some examples, the chemical entity is used in methods to diagnose and treat a wound in a mammal.

[0019] In some examples, the chemical entity is incorporated into a vacume wound therapy system via tubing or other components where the chemical entity reacts with vacume exudates that come into contact with the insert in the device.

[0020] In some examples, the chemical entity is incorporated into a ventilator system via tubing or other components where the chemical entity reacts with aspirates, aerosols or sputum exudates that come into contact with the chemical entities.

[0021] In some examples, the chemical entity is incorporated into a dipstick where the chemical entity reacts after the fluids to be assess are applied using an external swab or similar. In some examples, the chemical entity gives rise to a change that can be detected electronically either via reflected light, amperometry or a similar electrochemical process.

[0022] In some examples, use of the chemical entity makes it feasible to detect changes in the infection status of a mammal or patient prior to these changes being otherwise apparent. In some examples, the methods are the basis for an improved or proactive therapy, wherein a subsequent change of treatment or an application of a more detailed diagnostic is subsequently used to select a therapy to prevent a worsening of the medical condition of a mammal or patient.

[0023] Chemical Entity

[0024] In some examples, the chemical entity is a small molecule chemical entity or a modified polymer comprising one or more components selected from the group consisting of: an anchor region, an enzyme recognition region, an enzyme-labile or enzyme-reactive region, and an indicator region.

[0025] In some examples, the enzyme-labile or enzyme-reactive region is a structure that is reacted by an enzyme. In some examples, the enzyme recognition site is a structure that allows binding to an enzyme.

[0026] In certain examples, the chemical entity is a modified polymer. In certain examples, the chemical entity is a small molecule chemical entity. Disclosed herein, are chemical entities comprising one or more components selected from the group consisting of: an anchor region, an enzyme-labile or enzyme-reactive region, an enzyme recognition region, and an indicator region. In some examples, the chemical entity comprises at least one anchor region, at least one enzyme recognition region, at least one enzyme-labile or enzyme-reactive region, and at least one indicator region. In some examples, the chemical entity binds to a support material through the anchor region. In some examples, the chemical entity is an indicator conjugate. In some examples, the chemical entity comprises at least one enzyme-

labile or enzyme-reactive region, at least one enzyme recognition region and at least one indicator region. In some examples, the chemical entity comprises at least one anchor region, and at least one indicator region.

[0027] In some examples, the chemical entity comprises an anchor region, an enzyme recognition region, an enzyme-labile or enzyme-reactive region, and an indicator region. In some examples, the chemical entity comprises an enzyme recognition region, an enzyme-labile or enzyme-reactive region, and an indicator region. In some examples, the chemical entity comprises an anchor region, an enzyme recognition region, and an enzyme-labile or enzyme-reactive region. In some examples, the chemical entity comprises an enzyme recognition region, an anchor region and two enzyme-labile or enzyme-reactive regions. In some examples, the chemical entity comprises an enzyme recognition region, an anchor region, two enzyme-labile or enzyme-reactive regions, and two indicator regions. In some examples, the chemical entity comprises an enzyme recognition region, two enzyme-labile or enzyme-reactive regions, and two indicator regions.

[0028] In some examples, the one or more anchor regions and the one or more enzyme-labile or enzyme-reactive regions overlap partially with one another. In some examples, the anchor region and the enzyme recognition region or the enzyme-labile or enzyme-reactive region partially or fully overlap with one another. In some examples, the enzyme recognition region and the enzyme-labile or enzyme-reactive region partially or fully overlap with one another. In some examples, the one or more anchor regions are within the one or more enzyme-labile or enzyme-reactive regions. In some examples, the anchor region is within the enzyme-labile or enzyme-reactive region. In some examples, the one or more enzyme-labile or enzyme-reactive regions are within the one or more anchor regions. In some examples, the enzyme-labile or enzyme-reactive region is within the anchor region.

[0029] In some examples, the anchor region of the chemical entity binds the chemical entity to a support material. In some examples, the anchor region comprises an ionic chemical group. In some examples, the ionic chemical group forms an ionic bond with the support material. In some examples, the anchor region comprises a hydrophobic moiety. In some examples, the hydrophobic moiety interacts with the support material to bind the chemical entity to the support material. In some examples, the anchor region comprises a hydrophilic moiety. In some examples, the hydrophilic moiety interacts with the support material to bind the chemical entity to the support material.

[0030] In some examples, the anchor region is a bead, a polymer, a material with an ionic chemical group, a material with a hydrophilic moiety, or a material with a hydrophobic moiety. In some examples, the anchor region is a bead. In some examples, the anchor

region is a polymer. In some examples, the anchor region is a material with an ionic chemical group, wherein the ionic chemical group is positively charged. In some examples, the anchor region is a material with an ionic chemical group, wherein the ionic chemical group is negatively charged. In some examples, the anchor region is a material with a hydrophilic moiety. In some examples, the anchor region is a material with a hydrophobic moiety such as an aliphatic chain or an aliphatic alcohol. In some examples, the anchor region comprises a reactive moiety for covalent attachment to a support material such as a photoactive phenylazide or an epoxide group.

[0031] In some examples, the anchor region is a polystyrene bead, silica gel bead, polysaccharide bead, polyacrylamide bead, cellulose bead, polysaccharide, derivatized cellulose, polyacrylate, polyethyleneimine, polyacrylamide, UV-activatable reactive group or peptidoglycan derivative, or a combination thereof. In some examples, the anchor region binds to a support material after a short period of UV irradiation.

[0032] In some examples, the enzyme-labile or enzyme-reactive region reacts with one or more target enzymes selected from elastase, lysozyme, myeloperoxidase, leukocyte peroxidase, esterase, lipase, napsin (aspartyl protease), glucosylceramidase glucuronidase, palmitoyl protein thioesterase, cathepsins A, B, D, G, L, S, Z, Acid ceramidase, lactoferrin (LF), and proteinase-3, □-lactamase and other similar enzymes, or a combination thereof. In some examples, the enzyme-labile or enzyme-reactive region reacts with elastase. In some examples, the enzyme-labile or enzyme-reactive region reacts with lysozyme. In some examples, the enzyme-labile or enzyme-reactive region reacts with cathepsin G. In some examples, the enzyme-labile or enzyme-reactive region reacts with myeloperoxidase.

[0033] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide, peptidomimetic, or protein that is labile to elastase or cathepsin G, or a combination thereof. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide, peptidomimetic, or protein that is labile to elastase. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide, peptidomimetic, or protein that is labile to cathepsin G.

[0034] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide that is labile to elastase. In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of $X_yAAP(V/F/A)X_y$-L-Z, wherein each X is independently any amino acid, each y is independently an integer greater than 0, or each y is independently an integer from 1 to 50, or each y is independently an integer from 1 to 10, or each y is independently an integer from 1 to 6, L is a linking moiety, and Z is a moiety capable of causing a visible color change or a detectable electronic change; and the

peptide is labile to elastase. In some examples, one or more of the amino acids in the amino acid sequence is protected. In some examples, one or more of the amino acids in the amino acid sequence is protected with an fmoc group. In some examples, one of the amino acid in the amino acid sequence is protected with an fmoc group.

[0035] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of $X_yAAP(V/F/A)X_y$-Z, wherein each X is independently any amino acid, each y is independently an integer greater than 0, or each y is independently an integer from 1 to 50, or each y is independently an integer from 1 to 10, or each y is independently an integer from 1 to 6, and Z is a moiety capable of causing a visible color change or a detectable electronic change; and the peptide is labile to elastase. In some examples, one or more of the amino acids in the amino acid sequence is protected. In some examples, one or more of the amino acids in the amino acid sequence is protected with an fmoc group. In some examples, one of the amino acid in the amino acid sequence is protected with an fmoc group.

[0036] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of X_yAAPX_y-L-Z, wherein each X is independently any amino acid, each y is independently a number selected from 1 to 50, or each y is independently an integer from 1 to 10, or each y is independently an integer from 1 to 6, L is a linking moiety, and Z is a moiety capable of causing a visible color change or a detectable electronic change; and the peptide is labile to elastase. In some examples, one or more of the amino acids in the amino acid sequence is protected. In some examples, one or more of the amino acids in the amino acid sequence is protected with an fmoc group. In some examples, one of the amino acid in the amino acid sequence is protected with an fmoc group.

[0037] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of X_yAAPX_y-Z, wherein each X is independently any amino acid, each y is independently a number selected from 1 to 50, or each y is independently an integer from 1 to 10, or each y is independently an integer from 1 to 6, and Z is a moiety capable of causing a visible color change or a detectable electronic change; and the peptide is labile to elastase. In some examples, one or more of the amino acids in the amino acid sequence is protected. In some examples, one or more of the amino acids in the amino acid sequence is protected with an fmoc group. In some examples, one of the amino acid in the amino acid sequence is protected with an fmoc group.

[0038] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of X_yUUUU_y-Z, wherein each X is independently any amino acid, each y is independently a number selected from 1 to 50, or each y is

independently an integer from 1 to 10, or each y is independently an integer from 1 to 6, U represents an amino acid selected from arginine, lysine, glycine or alanine, and Z is a moiety capable of causing a visible color change or a detectable electronic change

[0039] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of $X_yUUUUUU_y$-Z, wherein each X is independently any amino acid, each y is independently a number selected from 1 to 50, or each y is independently an integer from 1 to 10, or each y is independently an integer from 1 to 6, U represents an amino acid selected from LEVLFQ, and Z is a moiety capable of causing a visible color change or a detectable electronic change

[0040] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide that is labile to cathepsin G.

[0041] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of $X_yN^4N^3N^2N^1X_y$-L-Z, wherein each X is independently any amino acid; each y is independently a number selected from 0 to 6; N^4 is selected from alanine, glycine, valine, and glutamine; N^3 is selected from alanine, glycine, proline, lysine, and serine; N^2 is selected from proline, alanine, and glycine; N^1 is selected from serine, lysine, phenylalanine, arginine, leucine, and methionine; L is a linking moiety, and Z is a moiety capable of causing a visible color change or a detectable electronic change; and the peptide is labile to cathepsin G. In some examples, one or more of the amino acids in the amino acid sequence is protected. In some examples, one or more of the amino acids in the amino acid sequence is protected with an fmoc group. In some examples, one of the amino acid in the amino acid sequence is protected with an fmoc group.

[0042] In some examples, the enzyme-labile or enzyme-reactive region comprises a peptide comprising an amino acid sequence of $X_yN^4N^3N^2N^1X_y$-Z, wherein each X is independently any amino acid; each y is independently a number selected from 0 to 6; N^4 is selected from alanine, glycine, valine, and glutamine; N^3 is selected from alanine, glycine, proline, lysine, and serine; N^2 is selected from proline, alanine, and glycine; N^1 is selected from serine, lysine, phenylalanine, arginine, leucine, and methionine; and Z is a moiety capable of causing a visible color change or a detectable electronic change; and the peptide is labile to cathepsin G. In some examples, one or more of the amino acids in the amino acid sequence is protected. In some examples, one or more of the amino acids in the amino acid sequence is protected with an fmoc group. In some examples, one of the amino acid in the amino acid sequence is protected with an fmoc group.

[0043] In some examples, Z is a peroxidase substrate, an arylamine, an amino phenol, an aminophenyl ether, an indoxyl, a neutral dye, a charged dye, a nanoparticle, or a colloidal gold

particle. In some examples, Z is a peroxidase substrate. In some examples, the peroxidase substrate is selected from *p*-aminophenol, ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt**),** 3,3'-diaminobenzidine, DCPIP, *N*,*N*-dimethyl-*p*-phenylenediamine, *o*-dianisidine, *p*-phenylenediamine, 4-chloro-1-naphthol, *o*-phenylenediamine *N*-(4-aminobutyl)-*N*-ethylisoluminol, 3-amino-9-ethylcarbazole, 4-aminophthalhydrazide, 5-aminosalicylic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), indoxyl, indigo, Fast Blue RR, 4-chloro-7-nitrobenzofurazan. In some examples, Z is an arylamine. In some examples, Z is an amino phenol. In some examples, Z is an aminophenol ether. In some examples, Z is an indoxyl. In some examples, Z is a neutral dye. In some examples, Z is a charged dye. In some examples, the charged dye is selected from remazole brilliant blue, toluidine blue, reactive black 5, remazol brilliant blue, reactive violet 5, and reactive orange 16, or a hydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is remazole brilliant blue, or a hydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is toluidine blue. In some examples, the charged dye is reactive black 5, or ahydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is reactive violet 5, or hydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is reactive orange 16, or hydrolytic or ammonolytic derivatives thereof.

[0044] In some examples, Z is a dichlorotriazine-based reactive dye such as reactive blue 4, reactive red 120, reactive blue 2, reactive green 19 and reactive brown 10. In some examples, the dichlorotriazine-based reactive dye appears black.

[0045] In some examples, Z is a reactive dye containing a sulfonylethyl-hydrogensulphate-reactive-group. In some examples, the reactive dye is reactive black 5, remazol brilliant blue, reactive violet 5 or reactive orange 16. In some examples, the reactive dye is reactive black 5. In some examples, the reactive dye is remazol brilliant blue. In some examples, the reactive dye is reactive violet 5. In some examples, the reactive dye is reactive orange 16. In some examples, the reactive dye is reactive black 5, remazol brilliant blue, or reactive violet 5. In some examples, the reactive dye is reactive black 5 or remazol brilliant blue.

[0046] In some examples, Z is a nanoparticle. In some examples, Z is a colloidal gold particle.

[0047] In some examples, Z is a charged dye, an indole derivative, or a luminol derivative. In some examples, Z is an indole derivative. In some embodiments, Z is a luminol derivative.

[0048] In some examples, the enzyme-labile or enzyme-reactive region reacts with lysozyme. In some examples, the enzyme-labile or enzyme-reactive region comprises peptidoglycan.

[0049] In some examples, the enzyme-labile or enzyme-reactive region is a beta lactam.

[0050] In some examples, the enzyme-labile or enzyme-reactive region comprises a phenol, an amino phenol, an aminophenyl ether, an indoxyl, or a quinone. In some examples, the enzyme-labile or enzyme-reactive region comprises a phenol. In some examples, the enzyme-labile or enzyme-reactive region comprises an amino phenol. In some examples, the enzyme-labile or enzyme-reactive region comprises an amino phenol ether. In some examples, the enzyme-label or enzyme-reactive region comprises an indoxyl. In some examples, the enzyme-labile or enzyme-reactive region comprises a quinone. In some examples, the enzyme-labile or enzyme-reactive region reacts with myeloperoxidase but does not react with heme.

[0051] In some examples, the enzyme-labile or enzyme-reactive region comprises a peroxidase substrate, an arylamine, an amino phenol, a neutral dye, a charged dye, a nanoparticle, or a colloidal gold particle. In some examples, the enzyme-labile or enzyme-reactive region comprises a peroxidase substrate. In some examples, the peroxidase substrate is selected from *p*-aminophenol, ABTS (2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), 3,3'-diaminobenzidine, , DCPIP, *N*,*N*-dimethyl-*p*-phenylenediamine, *o*-dianisidine, *p*-phenylenediamine, 4-chloro-1-naphthol, *o*-phenylenediamine *N*-(4-aminobutyl)-*N*-ethylisoluminol, 3-amino-9-ethylcarbazole, 4-aminophthalhydrazide, 5-aminosalicylic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and 4-chloro-7-nitrobenzofurazan, Fast Blue RR, N-(2-hydroxy)tetradecyl-Fast Blue RR. In some examples, the enzyme-labile or enzyme-reactive region comprises an arylamine. In some examples, the enzyme-labile or enzyme-reactive region comprises an amino phenol. In some examples, the enzyme-labile or enzyme-reactive region comprises a neutral dye. In some examples, the enzyme-labile or enzyme-reactive region comprises a charged dye. In some examples, the charged dye is selected from remazole brilliant blue, toluidine blue, reactive black 5, remazol brilliant blue, reactive violet 5, and reactive orange 16, or hydrolytic or ammonolytic derivatives of each of these. In some examples, the charged dye is remazole brilliant blue, or hydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is toluidine blue. In some examples, the charged dye is reactive black 5, or hydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is reactive violet 5, or hydrolytic or ammonolytic derivatives thereof. In some examples, the charged dye is reactive orange 16, or hydrolytic or ammonolytic derivatives thereof.

[0052] In some examples, the enzyme-labile or enzyme-reactive region comprises a dichlorotriazine-based reactive dye such as reactive blue 4, reactive red 120, reactive blue 2, reactive green 19 and reactive brown 10.

[0053] In some examples, the enzyme-labile or enzyme-reactive region comprises a nanoparticle. In some examples, Z is a colloidal gold particle.

[0054] In some examples, the enzyme-labile or enzyme-reactive region comprises a charged dye, an indole derivative, or a luminol derivative. In some examples, the enzyme-labile or enzyme-reactive region comprises an indole derivative. In some examples, the enzyme-labile or enzyme-reactive region comprises a luminol derivative.

[0055] In some examples, the indicator region comprises a dye that presents a visible color change in normal ambient lighting. In some examples, the dye has a contrasting color to wound products, which are commonly red, yellow, or brown. In further examples, the dye is violet, blue or dark green. In some examples, the dye is violet. In some examples, the dye is blue. In some examples, the dye is dark green. In some examples, the dye has low molecular weight, is charged, contains reactive or linkable groups, is stable to gamma irradiation, and is deeply colored. In some examples, the dye is selected from cibracron series dyes, azo dyes, and remazol dyes, or hydrolytic or ammonolytic derivatives thereof. In some examples, the dye is selected from cibracron series dyes. In some examples, the dye is selected from azo dyes. In some examples, the dye is selected from remazol dyes, or hydrolytic or ammonolytic derivatives thereof. In some examples, the dye is selected from rhodamine, coumarin, cyanine, xanthene, polymethine, pyrene, dipyrromethene borondifluoride, napthalimide, a phycobiliprotein, peridinium chlorophyll proteins, fluorescein, 6-FAM, rhodamine, Texas Red, California Red, iFluor594, tetramethylrhodamine, a carboxyrhodamine, carboxyrhodamine 6F, carboxyrhodol, carboxyrhodamine 110, Cascade Blue, Cascade Yellow, coumarin, Cy2®, Cy3®, Cy3.5®, Cy5®, Cy5.5®, Cy7®, Cy-Chrome, DyLight 350, DyLight 405, DyLight 488, DyLight 549, DyLight 594, DyLight 633, DyLight 649, DyLight 680, DyLight 750, DyLight 800, phycoerythrin, PerCP (peridinin chlorophyll-a Protein), PerCP-Cy5.5, JOE (6-carboxy-4’,5’-dichloro-2’,7’-dimethoxyfluorescein), NED, ROX (5-(and-6-)-carboxy-X-rhodamine), HEX, Lucifer Yellow, Marina Blue, Oregon Green 488, Oregon Green 500, Oregon Green 514, Alexa Fluor® 350, Alex Fluor® 430, Alexa Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 633, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, 7-amino-4-methylcoumarin-3-acetic acid, BODIPY® FL, BODIPY® FL-Br_2, BODIPY® 530/550, BODIPY® 558/568, BODIPY® 630/650, BODIPY® 650/665, BODIPY® R6G, BODIPY® TMR, BODIPY® TR, and dimethylaminoazobenzenesulfonic acid (dabsyl), or conjugates thereof, or combinations thereof.

[0056] In some examples, the indicator region comprises a dichlorotriazine-based reactive dye such as reactive blue 4, reactive red 120, reactive blue 2, reactive green 19 and reactive brown 10. In some examples, the dichlorotriazine-based reactive dye appears black.

[0057] In some examples, the indicator region comprises the reaction product of a reactive dye containing a sulfonylethyl-hydrogensulphate-reactive-group. In some examples, the reactive dye is reactive black 5, remazol brilliant blue, reactive violet 5 or reactive orange 16. In some examples, the reactive dye is reactive black 5. In some examples, the reactive dye is remazol brilliant blue. In some embodiments, the reactive dye is reactive violet 5. In some examples, the reactive dye is reactive orange 16. In some examples, the reactive dye is reactive black 5, remazol brilliant blue, or reactive violet 5. In some examples, the reactive dye is reactive black 5 or remazol brilliant blue.

[0058] In some examples, the indicator region comprises a particle (*e.g.*, colloidal metal or quantum dots) that present color changes in normal ambient lighting. In some examples, the indicator region comprises a nanoparticle. In some examples, the indicator region comprises a colloidal gold particle.

[0059] In some examples, the indicator region comprises a dye that presents a visible color change under UV light. In some examples, the indicator region comprises a dye that is fluorescent. In some examples, the indicator region comprises a dye that is luminescent.

[0060] In some examples, the indicator region comprises an enzyme-reactive moiety. In some examples, the enzyme-reactive moiety interacts with an accessory enzyme to produce a product that is visible to the naked eye or detectable by electronic means. In some examples, the enzyme-reactive moiety interacts with an accessory enzyme to produce a product that is visible to the naked eye. In some examples, the enzyme-reactive moiety interacts with an accessory enzyme to produce a product that is detectable by electronic means. In some examples, the indicator region comprises an indoxyl glycoside or galactoside that is cleaved by glucuronidase, glucosidase or galactosidase depending on the terminal sugar used, to produce indigo. In some examples, the indicator region comprises a phenol or napthol that is oxidized by an accessory enzyme to produce a visible product. In some examples, the indicator region comprises a phenol that is oxidized by peroxidase to produce a visible product. In some examples, the indicator region comprises a metallo motif that is detectable by electronic means. In some examples, the indicator region comprises a ferrocene or ferrocene analog that is detectable by electronic means. In some examples, the accessory enzyme is selected from lipase, esterase, peroxidase, oxidase, glycosidase, and glucosidase. In some examples, the accessory enzyme is not present in the wound fluid. In some examples,

the accessory enzyme is present in the wound fluid. In some examples, the enzyme-reactive moiety interacts with an accessory enzyme to produce a product that is visible under UV light.

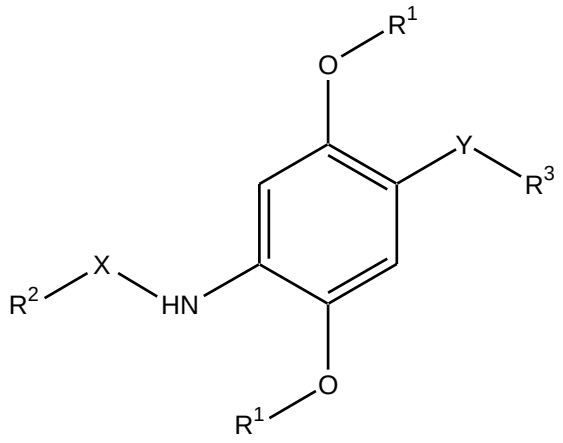
[0061] In some examples, the chemical entity consists essentially of at least one anchor region, at least one enzyme-labile or enzyme-reactive region, and at least one indicator region. In some examples, the chemical entity consists essentially of at least one enzyme-labile or enzyme-reactive region, and at least one indicator region. In some examples, the chemical entity consists essentially of at least one anchor region and at least one enzyme-labile or enzyme-reactive region. In some examples, the chemical entity is capable of being bound to a support material without an anchor region.

[0062] In some examples, the chemical entity is printed on or in a support material such as filter paper or a woven or non-woven material that is capable of being wet by a wound fluid and which displays capillary action. In some examples, the reporting entity or chemical entity is chemically bonded onto or into a support material such as filter paper or a woven or non-woven material that is capable of being wet by a wound fluid and which displays capillary action that is similar in all dimensions. In some examples, the chemical entity is ionically bound onto or into a support material such as filter paper or a woven or non-woven material that is capable of being wet by a wound fluid and which displays capillary action. In some examples, the chemical entity is covalently bound onto or into a support material such as filter paper or a woven or non-woven material that is capable of being wet by a wound fluid and which displays capillary action. Support material includes, but is not limited to, cellulose, polyamide, polyester, polyacrylate and other similar polymers that are useful as fibers. In some examples, the support material is cellulose. In some examples, the support material is polyamide. In some examples, the support material is polyester. In some examples, the support material is polyacrylate.

[0063] In some instances, the pH of a wound can influence many factors of wound healing, such as angiogenesis, protease activity, oxygen release, and bacterial toxicity. Chronic non-healing wounds may have an elevated alkaline environment. As the wound progresses towards healing, the pH of the wound moves to neutral and then becomes acidic. Monitoring of the pH of the wound may provide a method to assess the condition of the wound (*e.g.*, infection or no infection) and aid in determining a wound's response to treatment.

[0064] In some examples, the pH-sensitive moiety is **bromothymol blue**, phenol red, bromophenol red, **chlorophenol red,** thymol blue, **bromocresol green**, **bromocresol purple;** or other sulfophthalein dyes.

[0065] In some examples, the MPO responsive indicator incorporates an alkyl aniline amide with a molecular weight greater than 459 Da. In preferred examples, this indicator has the following structure:



[0066] Wherein,

[0067] R^1 = $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_2CH_3$

[0068] X = $-C(=O)-$, $-S(=O)_2-$

[0069] R^2 = can be but is not limited to:

[0070] $-C_1$-C_{10} alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl

[0071] wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl and alkylheteroaryl groups are optionally substituted by one to five substituents selected independently from: ferrocene, halogen (as can be F, Cl, Br, I), (C_1-C_4)alkyl, (C_1-C_4)alkenyl, (C_1-C_4)alkynyl, (C_3-C_7)cycloalkyl, (C_1-C_6)heterocycloalkyl, (C_6-C_{10})aryl, (C_1-C_9)heteroaryl, (C_1-C_4)alkoxy, $-NR^4R^5$, $R^4C(=O)-$, $R^4C(=O)O-$, $R^4OC(=O)O-$, $R^4NHC(=O)-$, $R^4C(=O)NH-$, $R^4R^5NC(=O)-$, $R^4OC(=O)-$

[0072] R^4 and R^5 can independently be, but are not limited to:

[0073] $-(C_1$-$C_{12})$alkyl

[0074] $-(C_1$-$C_{12})$alkenyl

[0075] $-(C_1$-$C_{12})$akynyl

[0076] $-(C_1$-$C_8)[(C_1$-$C_4)$alkoxy]alkyl

[0077] $-(C_1$-$C_8)[(C_1$-$C_4)$alkoxy]alkenyl

[0078] $-(C_6$-$C_{10})$aryl-$(C_1$-$C_5)$alkyl

[0079] $-(C_2$-$C_9)$heteroaryl-$(C_1$-$C_5)$alkyl

[0080] wherein alkyl, alkenyl, alkynyl, aryl and heteroaryl are optionally substituted by one to five substituents selected independently from halogen (as can be F, Cl, Br, I), (C_1-C_4)alkyl, (C_1-C_4)alkenyl, (C_1-C_4)alkynyl, (C_3-C_7)cycloalkyl, (C_1-C_6)heterocycloalkyl, (C_6-C_{10})aryl, (C_1-C_9)heteroaryl, (C_1-C_4)alkoxy, ,

[0081] or $N(R^4R^5)$ is an aziridine, azetidine, pyrrolidine, piperidine, azepane or azocane, 1-substituted piperazine, or morpholine moiety

[0082] Y =-NH-CH2-CH(OH)- ,-NH-, -NH-C(=O)-NH-, -NH-C(=S)-NH-

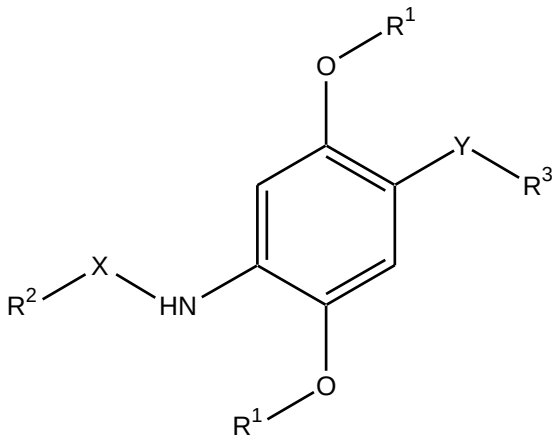
[0083] R^3 can be but is not limited to:

[0084] -C_6-C_{30} alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl

[0085] wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl and alkylheteroaryl groups are optionally substituted by one to five substituents selected independently from: ferrocene, halogen (as can be F, Cl, Br, I), (C_1-C_4)alkyl, (C_1-C_4)alkenyl, (C_1-C_4)alkynyl, (C_3-C_7)cycloalkyl, (C_1-C_6)heterocycloalkyl, (C_6-C_{10})aryl, (C_1-C_9)heteroaryl, (C_1-C_4)alkoxy, cyano (-CN), azido (-N_3), -NR^4R^5, $R^4C(=O)$-, $R^4C(=O)O$-, $R^4OC(=O)O$-, $R^4NHC(=O)$-, $R^4C(=O)NH$-, $R^4R^5NC(=O)$-, $R^4OC(=O)$-

[0086] In some examples, it is desired that the indicator substrates stay in place and react in place. Thus, limited solubility in aqueous systems is preferred. The ability to stay in place on a solid phase is defined as the water resistance and the means by which it is measured is recorded in example 114. In preferred examples, the water resistance of a substrate is greater than one, and in still more preferred examples, it is greater than 2.

[0087] In some examples, disclosed herein are compounds of the following formula (Formula D):



(Formula D)

[0088] wherein,

[0089] R^1 = -CH_3, -CH_2CH_3, -$CH_2CH_2CH_2CH_3$

[0090] X = -C(=O)-, -$S(=O)_2$-

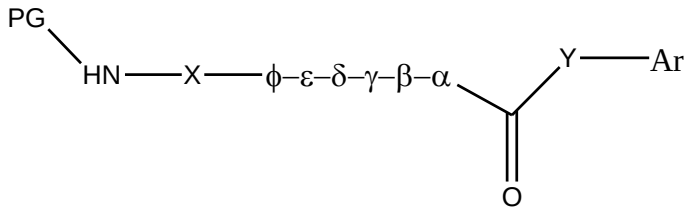
[0091] R^2 = can be but is not limited to: -C_1-C_{10} alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl and alkylheteroaryl groups are optionally substituted by one to five substituents selected

independently from: ferrocene, halogen (as can be F, Cl, Br, I), (C_1-C_4)alkyl, (C_1-C_4)alkenyl, (C_1-C_4)alkynyl, (C_3-C_7)cycloalkyl, (C_1-C_6)heterocycloalkyl, (C_6-C_{10})aryl, (C_1-C_9)heteroaryl, (C_1-C_4)alkoxy, -NR^4R^5, $R^4C(=O)$-, $R^4C(=O)O$-, $R^4OC(=O)O$-, $R^4NHC(=O)$-, $R^4C(=O)NH$-, $R^4R^5NC(=O)$-, $R^4OC(=O)$-

[0092] R^4 and R^5 can independently be, but are not limited to: -(C_1-C_{12})alkyl -(C_1-C_{12})alkenyl -(C_1-C_{12})akynyl -(C_1-C_8)[(C_1-C_4)alkoxy]alkyl -(C_1-C_8)[(C_1-C_4)alkoxy]alkenyl -(C_6-C_{10})aryl-(C_1-C_5)alkyl -(C_2-C_9)heteroaryl-(C_1-C_5)alkyl; wherein alkyl, alkenyl, alkynyl, aryl and heteroaryl are optionally substituted by one to five substituents selected independently from halogen (as can be F, Cl, Br, I), (C_1-C_4)alkyl, (C_1-C_4)alkenyl, (C_1-C_4)alkynyl, (C_3-C_7)cycloalkyl, (C_1-C_6)heterocycloalkyl, (C_6-C_{10})aryl, (C_1-C_9)heteroaryl, (C_1-C_4)alkoxy, or N(R^4R^5) is an aziridine, azetidine, pyrrolidine, piperidine, azepane or azocane, 1-substituted piperazine, or morpholine moiety; Y =-NH-CH2-CH(OH)- ,-NH-, -NH-C(=O)-NH-, -NH-C(=S)-NH-

[0093] R^3 can be but is not limited to: -C_6-C_{30} alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl and alkylheteroaryl groups are optionally substituted by one to five substituents selected independently from: ferrocene, halogen (as can be F, Cl, Br, I), (C_1-C_4)alkyl, (C_1-C_4)alkenyl, (C_1-C_4)alkynyl, (C_3-C_7)cycloalkyl, (C_1-C_6)heterocycloalkyl, (C_6-C_{10})aryl, (C_1-C_9)heteroaryl, (C_1-C_4)alkoxy, cyano (-CN), azido (-N_3), -NR^4R^5, $R^4C(=O)$-, $R^4C(=O)O$-, $R^4OC(=O)O$-, $R^4NHC(=O)$-, $R^4C(=O)NH$-, $R^4R^5NC(=O)$-, $R^4OC(=O)$-.

[0094] In some examples, disclosed herein are compounds of the following formula (Formula A)



(Formula A)

[0095] wherein

[0096] Y is O, or N

[0097] Ar is

[00103] δ is a bond, or independently, P, A, R, L, G, or V;

[00104] ε is a bond, or independently, P, A, R, L, G, V, or E;

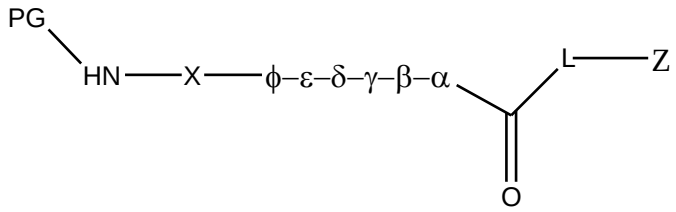
[00105] ϕ is a bond, or independently, P, A, R, L, or G;

[00106] wherein, if β, γ, δ, ε, and ϕ, are each bonds, then the N-terminal of α is bonded to X;

[00107] X is a peptide chain comprising from 0 to14 amino acids;

[00108] PG is -C(=O)-O-R^1 wherein R^1 is C_1-C_{30} alkyl, t-butyl, or methylfluorenyl, or -C(=O)-R^2 wherein R^2 is -C_1-C_{30} alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, or alkylheteroaryl.

[00109] In some examples, disclosed herein are compounds of the following formula (Formula B)



(Formula B)

[00110] wherein

[00111] L is A, AA, V, or triazolyl,

[00112] Z is ferrocene, crystal violet, malachite green, toluidine blue, reactive black 5, remazol brilliant blue, reactive violet 5, and reactive orange 16, reactive blue 4, reactive red 120, reactive blue 2, reactive green 19, or reactive brown 10

[00113] α is selected from: A, V, F, G, M, R, or L

[00114] β is a bond, or independently, P, F, A, R, L, or G

[00115] γ is a bond, or independently, P, A, R, L, or G

[00116] δ is a bond, or independently, P, A, R, L, G, or V

[00117] ε is a bond, or independently, P, A, R, L, G, V, or E

[00118] ϕ is a bond, or independently, P, A, R, L, or G,

[00119] wherein, if β, γ, δ, ε, and ϕ, are each bonds, then the N-terminal of α is bonded to X;

[00120] X is a peptide chain comprising from 0 to14 AA, wherein AA is an amino acid, a polyamine, or a polyoxyalkylene;

[00121] PG is polystyrene bead, silica gel bead, polysaccharide bead, polyacrylamide bead, cellulose bead, polysaccharide, derivatized cellulose, polyacrylate, polyethyleneimine, polyacrylamide, UV-activatable group, phenolic azide, epoxide, peptidoglycan, an aliphatic chain, an aliphatic alcohol chain, an aliphatic amine, mercaptoethyl, a multi-cyclic or multi-

aromatic ring system, a lipophilic group, or a combination thereof, -C(=O)-O-R^1 wherein R^1 is C_1-C_{30} alkyl, t-butyl, methylfluorenyl, -C(=O)-R^2 wherein R^2 is -C_1-C_{30} alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylaryl, or alkylheteroaryl.

[00122] In general, the elastase substrates have the formula a-b-c-d-e-f, wherein

[00123] In one example,

[00124] a is selected from: A, V, F ,G ,M, R, L

[00125] b is selected from: no amino acid, or independently, P, F, A, R, L, G

[00126] g is selected from: no amino acid, or independently, P, A, R, L, G

[00127] d is selected from: no amino acid, or independently, P, A, R, L, G, V

[00128] e is selected from: no amino acid, or independently, P, A, R, L, G, V, E

[00129] f is selected from: no amino acid, or independently, P, A, R, L, G.

[00130] In another example, elastase substrates have the formula a-b-c-d-e-f, wherein

[00131] a is selected from: A, V, F ,G ,M, R, L

[00132] b is selected from: no amino acid, or independently, P, F, A, G

[00133] g is selected from: no amino acid, or independently, P, A, R, L, G

[00134] d is selected from: no amino acid, or independently, P, A, R, L, G, V

[00135] e is selected from: no amino acid, or independently, P, A, G, V, E

[00136] f is selected from: no amino acid, or independently, P, A, G.

[00137] In another example, elastase substrates have the formula a-b-c-d-e-f, wherein

[00138] a is selected from: A, V, F ,G ,M, R, L

[00139] b is selected from: no amino acid, or independently, P, F, A, G

[00140] g is selected from: no amino acid, or independently, P, A, L, G

[00141] d is selected from: no amino acid, or independently, P, A, L, G, V

[00142] e is selected from: no amino acid, or independently, P, A, G, V, E

[00143] f is selected from: no amino acid, or independently, P, A, G.

[00144] In another example, elastase substrates have the formula a-b-c-d-e-f, wherein

[00145] a is selected from: A, V, F ,G

[00146] b is selected from: no amino acid, or independently, P, F, A, G

[00147] g is selected from: no amino acid, or independently, P, A, G

[00148] d is selected from: no amino acid, or independently, P, A, G,

[00149] e is selected from: no amino acid, or independently, A, G, V,

[00150] f is selected from: no amino acid, or independently, A, G.

[00151] Especially, in another example, elastase substrates have the formula a-b-c-d-e-f, wherein

[00152] a is selected from: A, V, F

[00153] b is selected from: no amino acid, or independently, P, F, A,

[00154] g is selected from: no amino acid, or independently, A, G

[00155] d is selected from: no amino acid, or independently, A, G,

[00156] e is selected from: no amino acid, or independently, A, G,

[00157] f is selected from: no amino acid, or independently, A, G.

[00158] Preferably, elastase substrates have the formula a-b-c-d-e-f, wherein

[00159] a is selected from: A, V, F

[00160] b is selected from: no amino acid, or independently, P, F, A,

[00161] g is selected from: no amino acid, or independently, A,

[00162] d is selected from: no amino acid, or independently, A,

[00163] e is selected from: no amino acid, or independently, A,

[00164] f is selected from: no amino acid, or independently, A.

[00164a] In one aspect, provided herein is a chemical entity comprising a compound of Formula I:

A–R–I

Formula I

wherein

A is an anchor selected from the group of a polysaccharide, a cellulose, a polyacrylate, a polyethyleneimine, a polyacrylamide, a peptidoglycan, or a chitosan, or a monomer thereof, an oligomer thereof, a mixture thereof, or a combination thereof;

R is an enzyme recognition site;

I is an indicator region; and

the R–I is N-(2-hydroxy)tetradecyl-Fast Blue RR.

DESCRIPTION OF THE FIGURES

[00165] **FIG. 1**: Exemplary embodiment of compounds disclosed herein.

[00166] **FIG. 2**: Exemplary embodiment of compounds disclosed herein with multiple reaction requirement toward MPO and elastase (e.g. example 9, Methyl-3-Fmoc-AAPV-amide-4-aminophenol or Methyl-3-amino-4-Fmoc-AAPV-amidebenzoate).

[00167] **FIG. 3A**: Exemplary embodiment of compounds disclosed herein with multiple reaction requirement toward elastase.

[00168] **FIG**. **3B**: Exemplary embodiment of compounds disclosed herein with multiple reaction requirement toward elastase.

[00169] **FIG. 4A:** Exemplary embodiment of compounds disclosed herein with reaction toward elastase in which overlapping function for the anchor is shown for an elastase substrate.

[00170] **FIG. 4B**: Exemplary embodiment of compounds disclosed herein with reaction toward MPO in which overlapping function for for an indicator and reactive region is shown for an MPO substrate.

[00171] **FIG. 5**: Synthetic routes toward β-lactamase substrates containing an indoxyl ether.

[00172] **FIG. 6**: Esterase and lipase substrates containing an indoxyl chromophore.

[00173] **FIG. 7A**: X-Gal, X-Gluc peptidoglycan and chitosan adducts formed via the methods of examples 103 and 104.

[00174] **FIG. 7B**: X-Man, peptidoglycan and chitosan adducts formed via the methods of examples 103 and 104.

[00175] **FIG. 7C**: Esterase substrates containing an indoxyl chromophore coupled to Peptidoglycan, chitosan or a peptide via the Suzuki product of example 103.

CLAIMS

We claim:

1. A chemical entity comprising a compound of Formula I:

A–R–I

Formula I

wherein

A is an anchor selected from the group of a polysaccharide, a cellulose, a polyacrylate, a polyethyleneimine, a polyacrylamide, a peptidoglycan, or a chitosan, or a monomer thereof, an oligomer thereof, a mixture thereof, or a combination thereof;

R is an enzyme recognition site;

I is an indicator region; and

the R–I is N-(2-hydroxy)tetradecyl-Fast Blue RR.

2. The chemical entity of claim 1, wherein the anchor A is covalently or non-covalently associated with the indicator region I.

3. The chemical entity of claim 2, wherein the enzyme recognition site R is specific for a wound-specific hydrolase.

4. The chemical entity of claim 1, wherein the anchor A comprises a polysaccharide selected from hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose, hydroxymethyl cellulose, or D-galactopyranoside.

5. The chemical entity of claim 1, wherein the anchor A comprises peptidoglycan or a monomer thereof, an oligomer thereof, a mixture or a combination thereof.

6. The chemical entity of claim 6, wherein the monomer of peptidoglycan comprises sugars and amino acids, an oligomer thereof, or a combination thereof.

7. The chemical entity of claim 6, wherein the peptidoglycan comprises at least two units of alternating amino sugars selected from N-acetylglucosamine (GlcNAc or NAG) and N-acetylmuramic acid (MurNAc or NAM) or a combination thereof.

8. The chemical entity of claim 8, wherein the peptidoglycan comprises carboxylated peptidoglycan.

9. The chemical entity of claim 9, wherein the peptidoglycan comprises a carboxymethyl, a carboxyethyl, or a carboxypropyl group comprising one or more halogen, alcohol, ester or amide groups.

10. The chemical entity of claim 10, wherein the peptidoglycan comprises one or more carboxymethyl groups.

11. The chemical entity of claim 6, wherein the peptidoglycan is halogenated.

12. The chemical entity of claim 1, wherein the anchor A comprises a compound selected from the group consisting of hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose, hydroxymethyl cellulose, D-galactopyranoside; N-acetylglucosamine (GlcNAc or NAG) and N-acetylmuramic acid (MurNAc or NAM); or an oligomer thereof or a polymer thereof.

13. The chemical entity of claim 1, wherein the enzyme recognition site R is provided via a bond between the anchor A and the indicator region I.