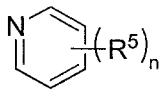
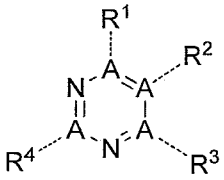
In one aspect, the present disclosure relates to a method for detecting a target in a sample by proximally depositing a marker, comprising: contacting the sample with a recognition solution, the recognition solution including a specific binding moiety specific to the target; labeling the specific binding moiety with an enzyme; contacting the sample with a detection solution, the detection solution comprising an enzymatic substrate selected from the group consisting of a chromogen and a tyramide-conjugate, so that the marker deposits proximally to the target in the presence of a deposition enhancer having a formula



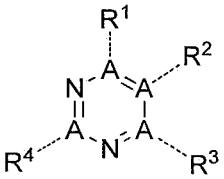
wherein R^5 is a heteroatom-containing moiety; n is 1-5; and detecting the marker.

In particular disclosed embodiments, the enzymatic substrate is selected from the group consisting of a chromogen and a tyramide-conjugate and the deposition enhancer may have a formula,



wherein R^1, R^2, R^3, and R^4 are selected from hydrogen and hydroxyl and each A is a carbon atom. In certain disclosed embodiments, R^1, R^3, and R^4 are hydrogen and R^2 is hydroxyl.

Other examples of the deposition enhancer include those having a formula,

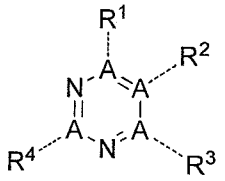


wherein R^1, R^2, and R^3 are independently selected from alkyl, alkene, alkyne, hydrogen, iodine, bromine, chlorine, fluorine, and combinations thereof.

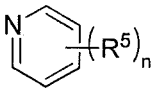
Particular disclosed embodiments concern using an enzyme, which may be an oxidoreductase or a peroxidase. Additionally, the enzyme may be selected from horseradish peroxidase, glutathione peroxidase, and microoxidase. The disclosed specific binding moiety typically comprises an antibody or a nucleic acid.

from imidazole, L-histidine, pyridine *N*-oxide, pyrimidine *N*-oxide, *N*-methyl morpholine oxide, and 2,2,6,6-tetramethylpiperidine-1-oxyl. Also, the detection solution may further comprise a non-ionic surfactant selected from a polyoxyethylene lauryl ether having a formula $(C_2H_4O)_{23}C_{12}H_{25}OH$; polyoxyethylene (20) sorbitan monoalkylate, the monoalkylate comprising between 8 and 14 carbons; a linear secondary alcohol polyoxyethylene having a formula $C_{12\text{-}14}H_{25\text{-}29}O(CH_2CH_2O]_x$, wherein x equals an integer between 2 and 12; and polyoxyethylene octyl phenyl ether. In particular disclosed embodiments, the detection solution may further comprise an antioxidant selected from sodium bisulfate, sodium stannate, sodium metabisulfate, and combinations thereof, and/or a Group I or Group II metal-containing salt having a formula MX_2 or MX where M is a Group I or Group II metal selected from lithium, sodium, potassium, cesium, calcium, magnesium, strontium, and barium; and X is selected from fluoride, chloride, bromide, iodide, carbonate, hydroxide, and phosphate.

Also contemplated in the present disclosure is a composition for detecting a target in a sample by proximally depositing a marker, comprising a deposition enhancer having a formula,

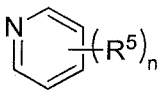


or



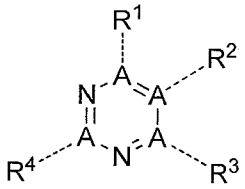
wherein R^1, R^2, R^3, and R^4 are independently selected from aliphatic, aryl, halogen, a heteroatom-containing moiety, and hydrogen; R^1 and/or R^3 can be bound to R^2 to form a fused, aromatic ring system; R^5 is a heteroatom-containing moiety; A is selected from a carbon atom, a heteroatom other than sulfur, and any combination thereof; n is 1-5; and an enzyme substrate.

In another aspect, the present disclosure relates to a composition for detecting a target in a sample by proximally depositing a marker, comprising a deposition enhancer having a formula,



wherein R^5 is a heteroatom-containing moiety; n is 1-5; and an enzymatic substrate selected from the group consisting of a chromogen and a tyramide-conjugate.

In certain disclosed embodiments, the deposition enhancer has a formula,

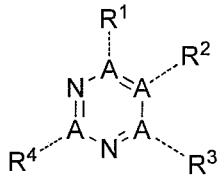


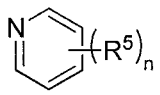
wherein R^1, R^2, R^3, and R^4 are selected from aliphatic, aryl, halogen, a heteroatom-containing moiety, and hydrogen; R^1 and/or R^3 can be bound to R^2 to form a fused,

aromatic ring system; A is selected from a heteroatom, other than sulfur, a carbon atom, and combinations thereof. Typically, the deposition enhancer may have a concentration ranging from about 5 mM to about 15 mM and the enzymatic substrate has a concentration ranging from greater than 0 mM to about 8 mM, with the enzyme substrate being selected from 1,3-diaminobenzidine, 3-amino-9-ethylcarbazole, tetramethylbenzidine, a fluorescein, a luminophore, a coumarin, a BODIPY dye, a resorufin, a rhodamine, a tyramide, or a derivative thereof.

In particular disclosed embodiments, the composition may further comprise an accelerator selected from a heteroaryl compound, a boronic acid, a phenolic compound, or a combination thereof; a non-ionic surfactant selected from Brij® 35, TWEEN®, Tergitol™, and Triton™; and an antioxidant selected from sodium bisulfate, sodium stannate, sodium metabisulfate, and combinations thereof.

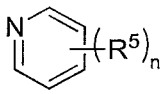
Also disclosed is a kit, comprising a detection solution, which comprises a deposition enhancer and an enzyme substrate, the deposition enhancer having a formula,



or


wherein R^1, R^2, R^3, and R^4 are independently selected from aliphatic, aryl, halogen, a heteroatom-containing moiety, and hydrogen; R^1 and/or R^3 can be bound to R^2 to form a fused, aromatic ring system; R^5 is a heteroatom-containing moiety; A is selected from a carbon atom, a heteroatom other than sulfur, and any combination thereof; n is 1-5.

In another aspect, the present disclosure relates to a kit, comprising an enzyme; a detection solution comprising a deposition enhancer and an enzymatic substrate that produces a detectable moiety after reaction with the enzyme, the deposition enhancer having a formula,



wherein R^5 is a heteroatom-containing moiety; n is 1-5 and the enzymatic substrate is selected from the group consisting of a chromogen and a tyramide-conjugate.

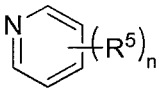
We claim:

1. A method for detecting a target in a sample by proximally depositing a marker, comprising:

contacting the sample with a recognition solution, the recognition solution including a specific binding moiety specific to the target;

labeling the specific binding moiety with an enzyme;

contacting the sample with a detection solution, the detection solution comprising an enzymatic substrate selected from the group consisting of a chromogen and a tyramide-conjugate, so that the marker deposits proximally to the target in the presence of a deposition enhancer having a formula



wherein R^5 is a heteroatom-containing moiety; n is 1-5; and

detecting the marker.

2. The method of claim 1, wherein contacting the sample with a detection solution includes enzymatically oxidizing the enzymatic substrate using an oxidizing agent to form the marker,

wherein enzymatically oxidizing the enzymatic substrate using an oxidizing agent includes reducing the solubility or stability of the enzymatic substrate so that the enzymatic substrate becomes deposited as the marker.

3. The method of claims 1 or 2, wherein the enzyme is an oxidoreductase or a peroxidase.

4. The method of any of claim 1 to 3, wherein the specific binding moiety comprises an antibody or a nucleic acid.

5. The method of any of claim 1 to 4, wherein depositing the marker proximally to the target in the presence of the deposition enhancer includes the deposition enhancer at a concentration ranging from about 5 mM to about 15 mM.

6. The method of any of claim 1 to 5, wherein the enzymatic substrate is selected from 1,3-diaminobenzidine, 3-amino-9-ethylcarbazole, tetramethylbenzidine, a fluorescein, a luminophore, a coumarin, a BODIPY dye, a resorufin, a rhodamine, or a derivative thereof.

7. The method of any of claim 1 to 5, wherein the enzymatic substrate is a tyramine derivative.

8. The method of any of claim 1 to 7, wherein the detection solution further comprises an accelerator selected from a heteroaryl compound, a boronic acid, a phenolic compound, or a combination thereof,

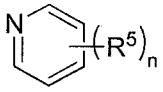
optionally wherein the heteroaryl compound is selected from imidazole, L-histidine, pyridine *N*-oxide, pyrimidine *N*-oxide, *N*-methyl morpholine oxide, and 2,2,6,6-tetramethylpiperidine-1-oxyl.

9. The method of any of claim 1 to 8, wherein the detection solution further comprises a non-ionic surfactant selected from a polyoxyethylene lauryl ether having a formula $(C_2H_4O)_{23}C_{12}H_{25}OH$; polyoxyethylene (20) sorbitan monoalkylate, the monoalkylate comprising between 8 and 14 carbons; a linear secondary alcohol polyoxyethylene having a formula $C_{12\text{-}14}H_{25\text{-}29}O(CH_2CH_2O]_x$, wherein x equals an integer between 2 and 12; and polyoxyethylene octyl phenyl ether,

or wherein the detection solution further comprises an antioxidant selected from sodium bisulfate, sodium stannate, sodium metabisulfate, and combinations thereof,

or wherein the detection solution further comprises a Group I or Group II metal-containing salt having a formula MX_2 or MX where M is a Group I or Group II metal selected from lithium, sodium, potassium, cesium, calcium, magnesium, strontium, and barium; and X is selected from fluoride, chloride, bromide, iodide, carbonate, hydroxide, and phosphate.

10. A composition for detecting a target in a sample by proximally depositing a marker, comprising a deposition enhancer having a formula,



wherein R^5 is a heteroatom-containing moiety; n is 1-5; and

an enzymatic substrate selected from the group consisting of a chromogen and a tyramide-conjugate.

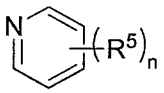
11. The composition of claim 10, wherein the enzymatic substrate is selected from 1,3-diaminobenzidine, 3-amino-9-ethylcarbazole, tetramethylbenzidine, a fluorescein, a luminophore, a coumarin, a BODIPY dye, a resorufin, a rhodamine, a tyramide, or a derivative thereof.

12. The composition of claims 10 or 11, further comprising an accelerator selected from a heteroaryl compound, a boronic acid, a phenolic compound, or a combination thereof; a non-ionic surfactant selected from a polyoxyethylene lauryl ether having a formula $(C_2H_4O)_{23}C_{12}H_{25}OH$; polyoxyethylene (20) sorbitan monoalkylate, the monoalkylate comprising between 8 and 14 carbons; a linear secondary alcohol polyoxyethylene having a formula $C_{12\text{-}14}H_{25\text{-}29}O(CH_2CH_2O]_x$, wherein x equals an integer between 2 and 12; and polyoxyethylene octyl phenyl ether and an antioxidant selected from sodium bisulfate, sodium stannate, sodium metabisulfate, and combinations thereof.

13. A kit, comprising

an enzyme;

a detection solution comprising a deposition enhancer and an enzymatic substrate that produces a detectable moiety after reaction with the enzyme, the deposition enhancer having a formula,



wherein R^5 is a heteroatom-containing moiety; n is 1-5 and the enzymatic substrate is selected from the group consisting of a chromogen and a tyramide-conjugate.

14. The kit of claim 13, further comprising a specific binding moiety, optionally wherein the specific binding moiety is an antibody or a nucleic acid that specifically binds to a target molecule

15. The kit of claim 14, wherein the specific binding moiety and the enzyme are bound together.

16. The kit of any of claims 13 to 15, wherein the enzyme is an oxide reductase or a peroxidase, optionally wherein the peroxidase is horseradish peroxidase or glutathione peroxidase.

17. The kit of any of claims 13 to 16, wherein the enzymatic substrate is selected from 1,3-diaminobenzidine, 3-amino-9-ethylcarbazole, tetramethylbenzidine, a fluorescein, a luminophore, a coumarin, a BODIPY dye, a resorufin, a rhodamine, or a derivative thereof, or wherein the enzymatic substrate is a tyramine derivative.

18. The method of any of claims 1 to 9, or the composition of any of claims 10 to 12, or the kit of any of claims 13 to 17, wherein the deposition enhancer has a concentration ranging from about 5 mM to about 15 mM and the enzymatic substrate has a concentration ranging from greater than 0 mM to about 8 mM.