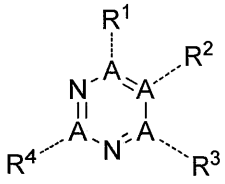
(CARD). U.S. Patent No. 6,593,100, entitled "Enhanced catalyzed reporter deposition" discloses enhancing the catalysis of an enzyme in a CARD or TSA method by reacting a labeled phenol conjugate with an enzyme, wherein the reaction is carried out in the presence of an enhancing reagent.

While methods, such as those described above, have been employed to increase the signals obtained from assays, the results from these methods indicate that signal amplification is impaired by corresponding background signal amplification. Thus, the continued need exists for signal amplification that can produce optimal results without a corresponding increase in background signals.

SUMMARY

The present disclosure concerns 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 so that the marker deposits proximally to the target in the presence of a deposition enhancer having a formula



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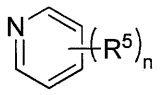


wherein R^1, R^2, R^3, and R^4 are independently selected from aliphatic, aryl, halogen, a heteroatom-containing moiety, and hydrogen; R^1 and/or R^3 can be bound to R^2 to form a fused, aromatic ring system; R^5 is a heteroatom-containing moiety; A is selected from a carbon atom, a heteroatom other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

In one aspect, there is provided 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 so that the marker deposits proximally to the target in the presence of a deposition enhancer selected from pyrimidine, 2-hydroxy-pyrimidine and pyrimidine-N-oxide; and detecting the marker.

With reference to this method, contacting the sample with a detection solution may include enzymatically oxidizing the enzyme substrate using an oxidizing agent to form the marker. In particular disclosed embodiments, enzymatically oxidizing the enzyme substrate using an oxidizing agent comprises reducing the solubility or stability of the enzymatic substrate so that the enzymatic substrate becomes deposited as the marker.

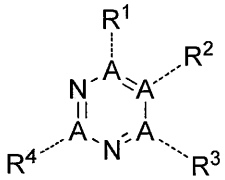
aromatic ring system; A is selected from a heteroatom, other than sulfur, a carbon atom, and combinations thereof.

In another aspect, there is provided a composition for detecting a target in a sample by proximally depositing a marker, comprising an enzyme, a deposition enhancer selected from pyrimidine, 2-hydroxy-pyrimidine and pyrimidine-N-oxide; and an enzyme substrate, wherein the enzyme substrate produces a detectable moiety after reaction with the enzyme.

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,



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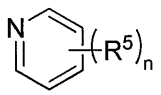
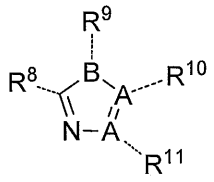
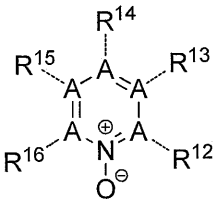


wherein R^1, R^2, R^3, and R^4 are independently selected from aliphatic, aryl, halogen, a heteroatom-containing moiety, and hydrogen; R^1 and/or R^3 can be bound to R^2 to form a fused, aromatic ring system; R^5 is a heteroatom-containing moiety; A is selected from a carbon atom, a heteroatom other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

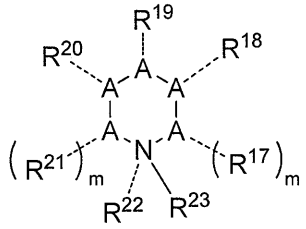
In particular embodiments, the heteroaryl compound has a formula according to



and/or a formula according to



and/or a formula according to

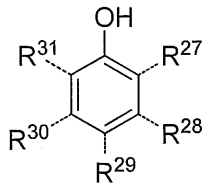


where $R^8 - R^{22}$ independently are aliphatic, aryl, halogen, a heteroatom-containing moiety, hydrogen, or any combination thereof; R^{23} is [O]˙ or [O]$^-$; A is a carbon atom, a heteroatom, other than sulfur, or any combination thereof; B is oxygen, carbon, or nitrogen; and m is 0-2. The halogen can be selected from iodine, bromine, chlorine or fluorine, and the heteroatom-containing moiety can be selected from hydroxyl, ether, silyl ether, ester, carboxylic acid, silyl, phosphonate, phosphine, amide, NR^6R^7 where R^6 and R^7 independently are hydrogen, aliphatic, aryl, heteroaliphatic, heteroaryl, or any combination thereof. In particular embodiments, R^{17} and R^{21} each comprise methyl group and m is 2. Exemplary heteroaryl compounds include, but are not limited to imidazole, L-histidine, pyridine *N*-oxide, pyrimidine *N*-oxide, *N*-methyl morpholine oxide, and 2,2,6,6-tetramethylpiperidine-1-oxyl.

In another aspect, there is provided a kit, comprising a detection solution comprising an enzyme, a deposition enhancer and an enzyme substrate, the deposition enhancer selected from pyrimidine, 2-hydroxy-pyrimidine and pyrimidine-N-oxide, wherein the enzyme substrate produces a detectable moiety after reaction with the enzyme.

In particular embodiments, the optional enhancer is a boron-containing compound, such as an organic boronic acid. Exemplary organic boronic acids include, but are not limited to boric acid.

In particular embodiments, the optional enhancer is a phenolic compound having a formula



wherein R^{27}, R^{28}, R^{29}, R^{30}, and R^{31} independently are can be selected from hydrogen, aliphatic, aryl, a heteroatom-containing moiety, or any combination thereof. The

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

7. The method of any of claims 1-5, wherein the enzyme substrate is a tyramine derivative.

8. The method of any of claims 1-7, wherein the detection solution further comprises an accelerator selected from a heteroaryl compound, a boronic acid, a phenolic compound, *N*-methyl morpholine oxide, 2,2,6,6-tetramethylpiperidine-1-oxyl or a combination thereof, 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 claim 8, wherein the heteroaryl compound is selected from imidazole, L-histidine, pyridine *N*-oxide, pyrimidine *N*-oxide.

10. The method of any of claims 1-9, wherein the detection solution further comprises a non-ionic surfactant selected from a polyoxyethylenelauryl 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.

11. The method of any of claims 1-10, wherein the detection solution further comprises an antioxidant selected from sodium bisulfate, sodium stannate, sodium metabisulfate, and combinations thereof.

14. A composition for detecting a target in a sample by proximally depositing a marker, comprising an enzyme, a deposition enhancer selected from pyrimidine, 2-hydroxy-pyrimidine and pyrimidine-N-oxide; and

an enzyme substrate, wherein the enzyme substrate produces a detectable moiety after reaction with the enzyme.

15. The composition of claim 14, wherein the enzyme 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 tyramide.

16. The composition of claim 14 or 15, further comprising an accelerator selected from a heteroaryl compound, a boronic acid, a phenolic compound, or a combination thereof, 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; a non-ionic surfactant selected from a polyoxyethylenelauryl 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 $C_{12-14}H_{25-29}O(CH_2CH_2O]_x$, 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.

17. The composition of any of claims 14-16, wherein the deposition enhancer has a concentration ranging from 5 mM to 15 mM and the enzymatic substrate has a concentration greater than 0 mM to 8 mM.