WHAT IS CLAIMED IS:

1. A method for detecting an abasic site, the method comprising:

flowing a solution over a substrate having a plurality of oligonucleotides coupled thereto,

at least one of the oligonucleotides comprising an abasic site,

the solution comprising a fluorophore coupled to a reactive group;

reacting the reactive group with the abasic site to couple the fluorophore to the abasic site; and

detecting the abasic site using fluorescence from the fluorophore.

2. The method of claim 1, wherein:

(i) the abasic site is generated by damage to the oligonucleotide~~.~~; and/or

~~3. The method of claim 1 or claim 2, wherein~~ (ii) nucleotide bases adjacent to the abasic site inhibit non-radiative energy dissipation from the respective fluorophore coupled to the abasic site.

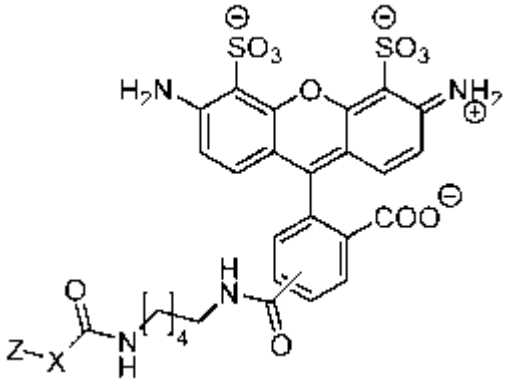
~~4~~3. The method of ~~any one of~~ claim~~s~~ 1 ~~to 3~~or claim 2, wherein the fluorophore comprises a molecular rotor dye comprising π-conjugated components separated by a rotatable C-C bond~~.~~;

~~5. The method of claim 4,~~ optionally wherein:

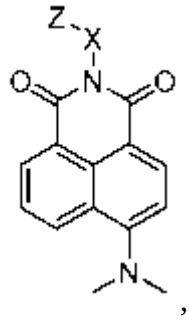
~~(~~i) nucleotide bases adjacent to the abasic site restrict rotation of the C-C bond and align the π-conjugated components with one another~~.~~; and/or

~~6. The method of claim 4 or claim 5, wherein~~ (ii) the molecular rotor dye coupled to the reactive group is selected from the group consisting of 9-(2-carboxy-2-cyanovinyl)-julolidine (CCVJ1), (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1-H-imidazol-5(4H)-one (DFHBI), and 1-methyl-4-[(3-methyl-2(3*H*)-benzothiazolylidene)methyl]quidolimium (thiazole orange).

~~7~~4. The method of ~~any one of~~ claim~~s~~ 1 ~~to 3~~ or claim 2, wherein the fluorophore coupled to the reactive group is selected from the group consisting of:



and



,

where X is a linker and Z is the reactive group.

~~8~~5. The method of any one of claims 1 to ~~7~~4, wherein:

(i) the abasic site comprises an aldehyde~~.~~; and/or

~~9. The method of any one of claims 1 to 8, wherein~~(ii) the reactive group comprises a hydroxylamine group~~.~~

~~10. The method of any one of claims 1 to 8, wherein the reactive group comprises~~ or a hydrazine group~~.~~; and/or

~~11. The method of any one of claims 1 to 10, wherein~~ (iii) reacting the reactive group with the abasic site forms an oxime linkage.

~~12~~6. A composition comprising:

a substrate having a plurality of oligonucleotides coupled thereto,

at least one of the oligonucleotides comprising an abasic site; and

a fluorophore coupled to the abasic site, the abasic site being detectable using fluorescence from the fluorophore.

~~13~~7. The composition of claim ~~12~~6, wherein:

(i) the abasic site is generated by damage to the oligonucleotide~~.~~; and/or

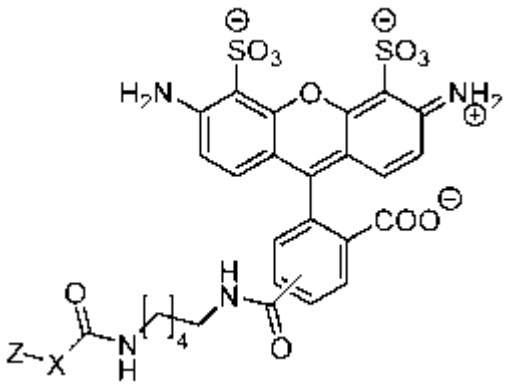
~~14. The composition of claim 12 or claim 13, wherein~~ (ii) nucleotide bases adjacent to the abasic site inhibit non-radiative energy dissipation from the respective fluorophore coupled to the abasic site.

~~15~~8. The composition of any ~~one of~~ claim~~s 12 to 14~~6 or claim 7, wherein the fluorophore comprises a molecular rotor dye comprising π-conjugated components separated by a rotatable C-C bond~~.~~; optionally wherein:

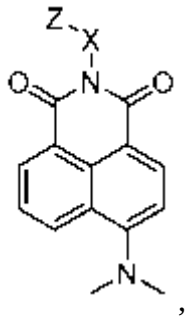
~~16. The composition of claim 15, wherein~~ (i) nucleotide bases adjacent to the abasic site restrict rotation of the C-C bond and align the π-conjugated components with one another~~.~~; and/or

~~17. The composition of claim 15 or claim 16, wherein~~ (ii) the molecular rotor dye coupled to the reactive group is selected from the group consisting of 9-(2-carboxy-2-cyanovinyl)-julolidine (CCVJ1), (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1-H-imidazol-5(4H)-one (DFHBI), and 1-methyl-4-[(3-methyl-2(3*H*)-benzothiazolylidene)methyl]quidolimium (thiazole orange).

~~18~~9. The composition of ~~any one of~~ claim 6 or claim 7~~s 12 to 14~~, wherein the fluorophore coupled to the reactive group is selected from the group consisting of:



and



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where X is a linker and Z is the reactive group.

~~19~~10. The composition of any one of claims ~~13~~ 7 to ~~18~~9, wherein:

(i) the abasic site comprises an aldehyde~~.~~; and/or

~~20. The composition of any one of claims 13 to 19, wherein~~ (ii) the reactive group comprises a hydroxylamine group or~~.~~

~~21. The composition of any one of claims 13 to 19, wherein the reactive group comprises~~ a hydrazine group~~.~~; and/or

~~22. The composition of any one of claims 13 to 21, wherein~~ (iii) reacting the reactive group with the abasic site forms an oxime linkage.

~~23~~11. A method, comprising:

preparing a solution comprising (i) glycosylases, (ii) oligonucleotides, and (iii) fluorophores coupled to reactive groups;

generating, using the glycosylases, abasic sites in the oligonucleotides in the solution;

reacting the reactive groups with the abasic sites to couple the fluorophores to the abasic sites;

measuring activity of the glycosylases using fluorescence from the fluorophores coupled to the abasic sites; and

using the glycosylases in a sequencing-by-synthesis operation.

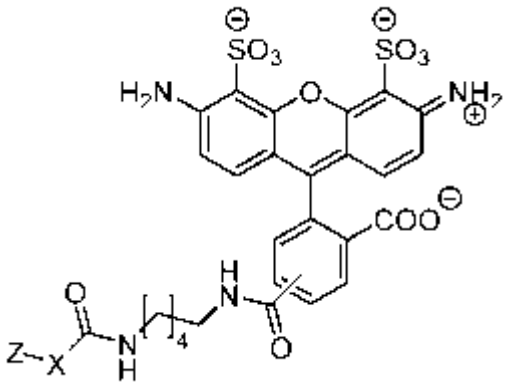
~~24~~12. The method of claim 11~~23~~, wherein nucleotide bases adjacent to the abasic site inhibit non-radiative energy dissipation from the respective fluorophore coupled to the abasic site.

~~25~~13. The method of claim ~~23~~ 11 or claim ~~24~~12, wherein the fluorophore comprises a molecular rotor dye comprising π-conjugated components separated by a rotatable C-C bond~~.~~; optionally wherein:

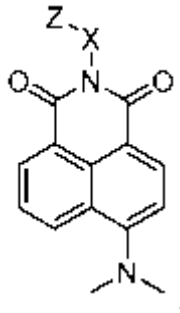
~~26. The method of claim 25, wherein~~ (i) nucleotide bases adjacent to the abasic site restrict rotation of the C-C bond and align the π-conjugated components with one another~~.~~; and/or

~~27. The method of claim 25 or claim 26, wherein~~ (ii) the molecular rotor dye coupled to the reactive group is selected from the group consisting of 9-(2-carboxy-2-cyanovinyl)-julolidine (CCVJ1), (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1-H-imidazol-5(4H)-one (DFHBI), and 1-methyl-4-[(3-methyl-2(3*H*)-benzothiazolylidene)methyl]quidolimium (thiazole orange).

~~28~~14. The method of claim ~~23~~ 11 or claim ~~24~~12, wherein the fluorophore coupled to the reactive group is selected from the group consisting of:



and



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where X is a linker and Z is the reactive group.

~~29~~15. The method of any one of claims ~~23~~ 11 to ~~28~~14, wherein:

~~(~~(i) the abasic site comprises an aldehyde~~.~~; and/or

~~30. The method of any one of claims 23 to 29, wherein~~ (ii) the reactive group comprises a hydroxylamine group~~.~~

~~31. The method of any one of claims 23 to 29, wherein the reactive group comprises~~ or a hydrazine group~~.~~; and/or

~~32. The method of any one of claims 23 to 31, wherein~~ (iii) reacting the reactive group with the abasic site forms an oxime linkage.

~~33~~16. A solution for measuring glycosylase activity, the solution comprising:

(i) glycosylases, (ii) oligonucleotides, and (iii) fluorophores coupled to reactive groups,

the oligonucleotides comprising abasic sites generated by the glycosylases in the solution,

the fluorophores being coupled to the abasic sites, and

the abasic sites being detectable using fluorescence from the fluorophores.

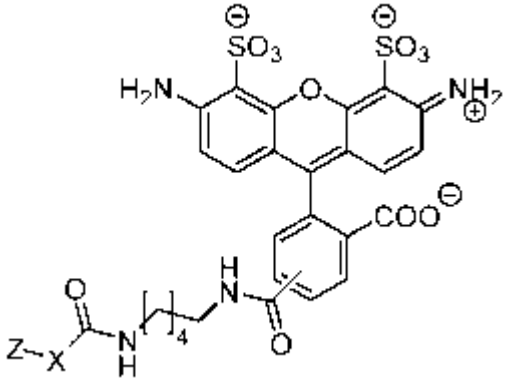
~~34~~17. The solution of claim ~~33~~16, wherein nucleotide bases adjacent to the abasic site inhibit non-radiative energy dissipation from the respective fluorophore coupled to the abasic site.

~~35~~18. The solution of claim ~~33~~ 16 or claim ~~34~~17, wherein the fluorophore comprises a molecular rotor dye comprising π-conjugated components separated by a rotatable C-C bond~~.~~; optionally wherein:

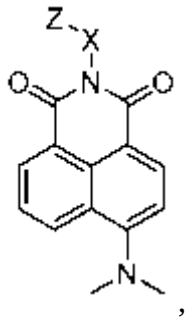
~~36. The solution of claim 35, wherein~~ (i) nucleotide bases adjacent to the abasic site restrict rotation of the C-C bond and align the π-conjugated components with one another~~.~~; and/or

~~37. The solution of claim 35 or claim 36, wherein~~ (ii) the molecular rotor dye coupled to the reactive group is selected from the group consisting of 9-(2-carboxy-2-cyanovinyl)-julolidine (CCVJ1), (Z)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1-H-imidazol-5(4H)-one (DFHBI), and 1-methyl-4-[(3-methyl-2(3*H*)-benzothiazolylidene)methyl]quidolimium (thiazole orange).

~~38~~19. The solution of claim ~~33~~ 16 or claim ~~34~~17, wherein the fluorophore coupled to the reactive group is selected from the group consisting of:



and



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where X is a linker and Z is the reactive group.

~~39~~20. The solution of any one of claims ~~33~~ 16 to ~~38~~19, wherein:

(i) the abasic site comprises an aldehyde~~.~~; and/or

~~40. The solution of any one of claims 33 to 39, wherein~~ (ii) the reactive group comprises a hydroxylamine group~~.~~

~~41. The solution of any one of claims 33 to 39, wherein the reactive group comprises~~or a hydrazine group~~.~~; and/or

~~42. The solution of any one of claims 33 to 41, wherein~~ (iii) reacting the reactive group with the abasic site forms an oxime linkage.