

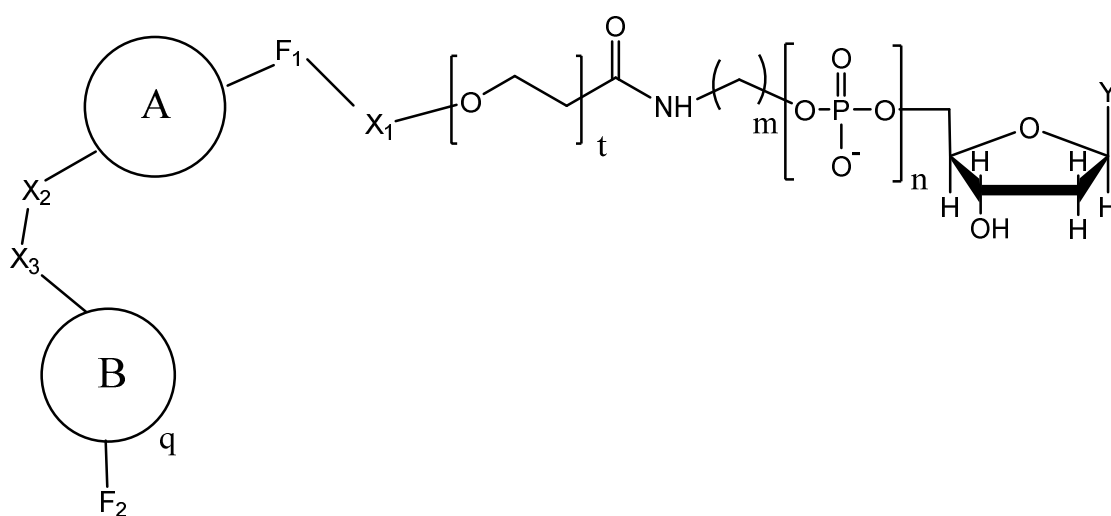
~~WHAT IS CLAIMED IS:~~ Claims

1. A method comprising:

detecting an incorporation of a labelled nucleotide into a nascent polynucleotide strand complementary to a template polynucleotide strand by a polymerase, wherein the polymerase is tethered to a solid support conductive channel by a tether,

the labelled nucleotide is a compound of Formula I

[Formula I]



wherein n is an integer from 3 to 10,

m is an integer from 1 to 10,

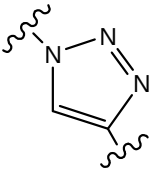
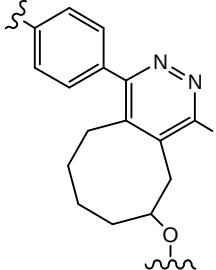
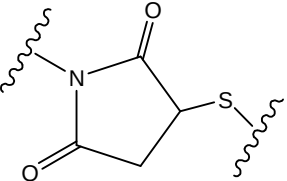
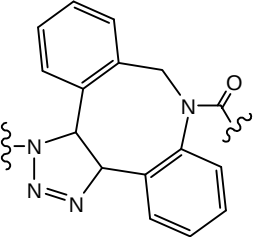
t is an integer from 0 to 50,

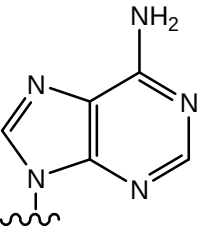
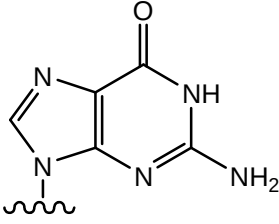
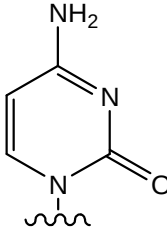
X₁ is a direct bond, a C₁-C₁₀ alkyl, a C₁-C₁₀ oxaalkyl, a C₁-C₁₀ thiaalkyl, or a C₁-C₁₀ azaalkyl,

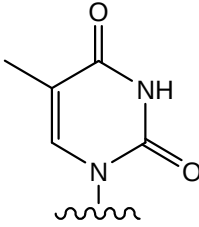
X₂ is C₁-C₂₀ alkyl wherein optionally one or more individual CH₂ residue is replaced with one or more of a peptide bond and (-O-CH₂-CH₂-)_a wherein a is an integer from 1 to 24,

X₃ is a direct bond or an oligonucleotide wherein the oligonucleotide hybridizes to an acceptor region of the tether when the label is in proximity to the conductive channel,

F₁ is selected from a fluorophore and a direct bond and F₂ is absent or a fluorophore,

A is: , , , , or an amide bond, and

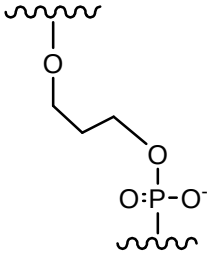
Y is selected from: , , ,

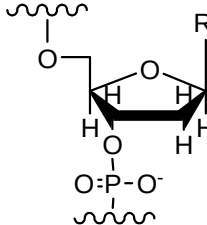
and/or ,

q is an integer from 1 to 100, and wherein

B is selected from: a) an amino acid or comprises an amino acid,

b) a nucleotide,

c) , or

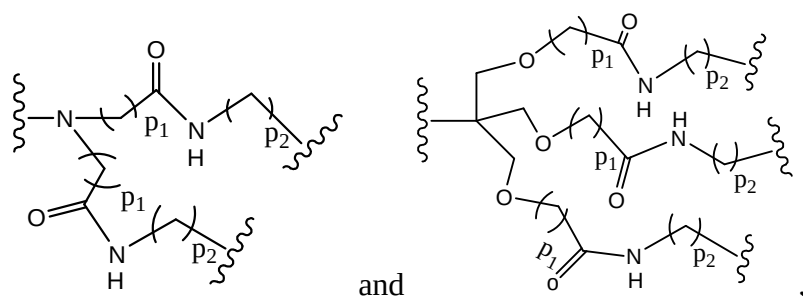
d)  wherein each R is independently selected from Y and/or hydrogen, and a dendron;

and wherein q is equal to an integer from 1 to 100 and the conductive channel is to detect the labelled nucleotide during the incorporation; optionally wherein when B is a dendron,

and the q number of B has a charge and a charge density, and;

or

B is a dendron of z generations comprising one or more constitutional repeating unit and a plurality of end units, wherein z is an integer from 1 to 6, the constitutional end units are selected from:



wherein

p₁ is an integer from 1 to 3, wherein any one or more of the p₁ -CH₂- groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂- groups,

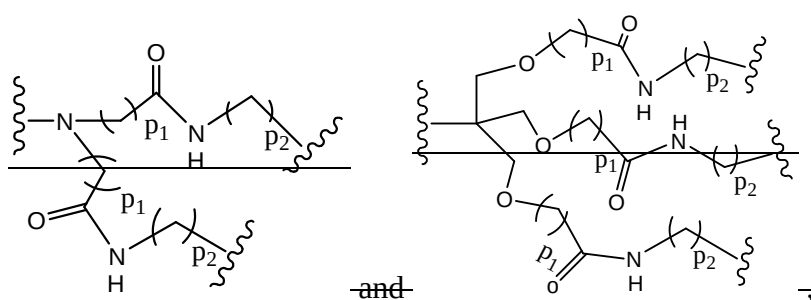
p₂ is an integer from 1 to 3, wherein any one or more of the p₂ -CH₂- groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂- groups, and

the end groups are selected from carboxylic acid, sulfonic acid, phosphonic acid, sperminyl group, amino group, and quaternary ammonium group, B has a charge and a charge density, q is 1 and the charge sensor is to detect the labelled nucleotide during the incorporation, optionally wherein

the conductive channel is to detect the labelled nucleotide during the incorporation.

2. ~~The A method of as claimed in claim 1,~~ wherein the charge is between ~~about~~-100e and ~~about~~+100e.
3. ~~The A method of as claimed in claim 2,~~ wherein the charge density is between ~~about~~-100e per cubic nanometer and ~~about~~+100e per cubic nanometer.
4. ~~The A method of as claimed in claim 1,~~ wherein the charge is between ~~about~~-200e and ~~about~~+200e.
5. ~~The A method of as claimed in claim 24,~~ wherein the charge density is between ~~about~~-200e per cubic nanometer and ~~about~~+200e per cubic nanometer.
6. ~~The A method of as claimed in claim 1,~~ wherein the q number of B comprises a polynucleotide.
7. ~~The A method of as claimed in claim 6,~~ wherein the polynucleotide is selected from a branched polynucleotide and one or more hairpin loops.
8. ~~The A method of as claimed in claim 7,~~ wherein the polynucleotide comprises between two and five hairpin loops.

9. ~~The A method of as claimed in claim 1~~, wherein the q number of B comprises a polypeptide.
10. ~~The A method of as claimed in claim 89~~, wherein the polypeptide is selected from a branched polypeptide, coiled polypeptide, and coiled-coil polypeptide.
11. ~~The A method of as claimed in claim 1~~, wherein B comprises an amino acid, and one or more of the q number of B comprise methyllysine, dimethyllysine, or trimethyllysine.
12. ~~The method of claim 1, wherein B is a dendron of z generations comprising one or more constitutional repeating unit and a plurality of end units, wherein z is an integer from 1 to 6, the constitutional end units are selected from:~~



wherein

~~p₁ is an integer from 1 to 3, wherein any one or more of the p₁-CH₂-groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂-groups,~~

~~p₂ is an integer from 1 to 3, wherein any one or more of the p₂-CH₂-groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂-groups, and~~

~~the end groups are selected from carboxylic acid, sulfonic acid, phosphonic acid, sperminyl group, amino group, and quaternary ammonium group.~~

- 13.12. ~~The A method of any one of as claimed in claims 1 through 12~~, wherein A was formed by a reaction comprising a linking reaction and the linking reaction is selecting from an azide-alkyne copper-assisted click reaction, a tetrazine-trans-cyclooctene ligation, an azide-dibenzocyclooctyne group copper-free click reaction, and a thiol-maleimide conjugation.
14. ~~The A method of as claimed in any one of claims 1 through 12~~, further comprising successively incorporating a plurality of labelled nucleotides wherein the charge of each of the plurality of labelled nucleotides differs from the charge of any other of the plurality of labelled nucleotides when the Y of the each and the Y of the any other differ from each other; optionally

~~15.13. The method of claim 14, further comprising identifying the Y of one or more labelled polynucleotide incorporated into the nascent polynucleotide strand based on the charge detected by the conductive channel.~~

~~16.14. The A method of any one of as claimed in claims 1 through 12, wherein X₂ is (-O-CH₂-CH₂-)_a wherein a is an integer from 1 to 24, optionally wherein a is 4, 8, 12, 16 or 24.~~

~~17. The method of claim 16, wherein a is 24.~~

~~18. The method of claim 16, wherein a is 16.~~

~~19. The method of claim 16, wherein a is 12.~~

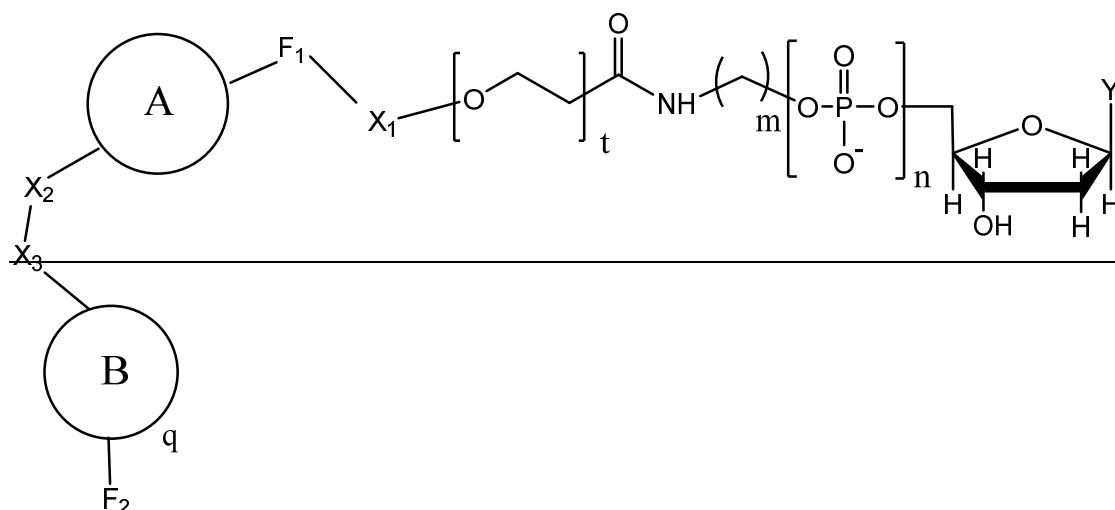
~~20. The method of claim 16, wherein a is 8.~~

~~21. The method of claim 16, wherein a is 4.~~

~~22. A method comprising:~~

~~detecting an incorporation of a labelled nucleotide into a nascent polynucleotide strand complementary to a template polynucleotide strand by a polymerase, wherein the polymerase is tethered to a solid support conductive channel by a tether,~~

~~the labelled nucleotide is a compound of Formula I~~



~~wherein n is an integer from 3 to 10,~~

~~m is an integer from 1 to 10,~~

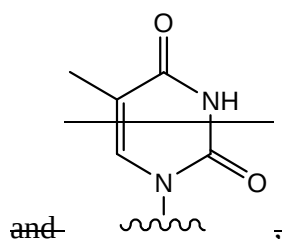
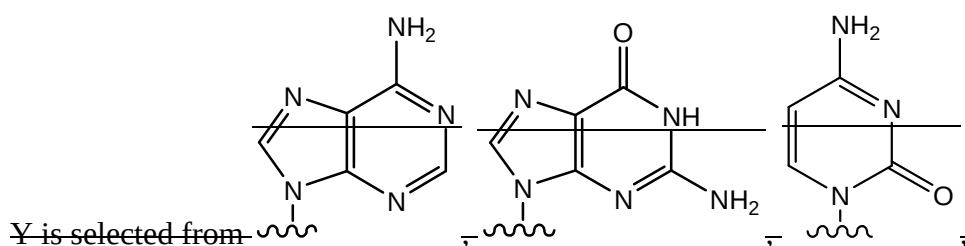
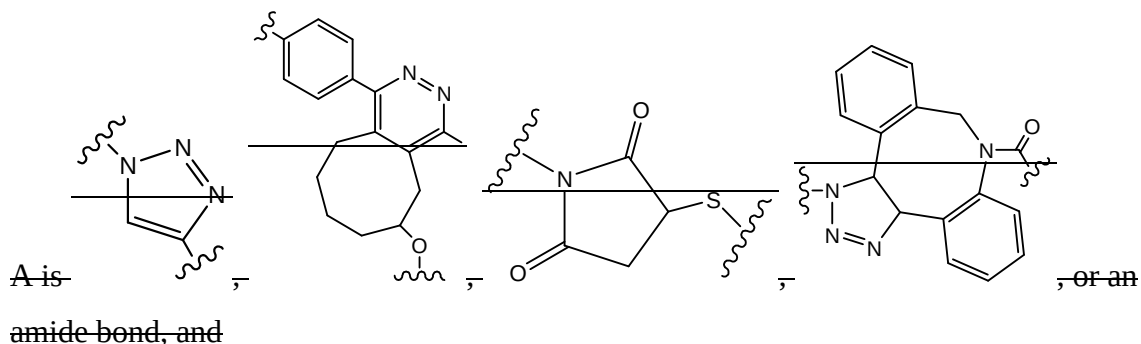
~~t is an integer from 0 to 50,~~

~~X₁ is a direct bond, a C₁-C₁₀ alkyl, a C₁-C₁₀ oxaalkyl, a C₁-C₁₀ thiaalkyl, or a C₁-C₁₀ azaalkyl,~~

X_2 is C_1 - C_{20} alkyl wherein optionally one or more individual CH_2 residue is replaced with one or more of a peptide bond and $(-O-CH_2-CH_2-)_a$ wherein a is an integer from 1 to 24,

X_3 is a direct bond or an oligonucleotide wherein the oligonucleotide hybridizes to an acceptor region of the tether when the label is in proximity to the conductive channel,

F_1 is selected from a fluorophore and a direct bond and F_2 is absent or a fluorophore,



q is an integer from 1 to 100,

B comprises an amino acid, and

the conductive channel is to detect the labelled nucleotide during the incorporation.

23. The method of claim 22, wherein the charge is between about $-100e$ and about $+100e$.

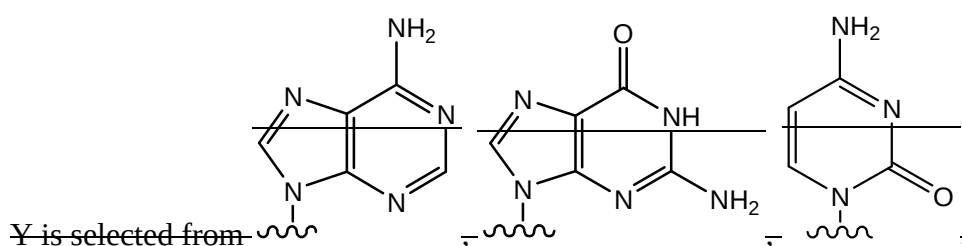
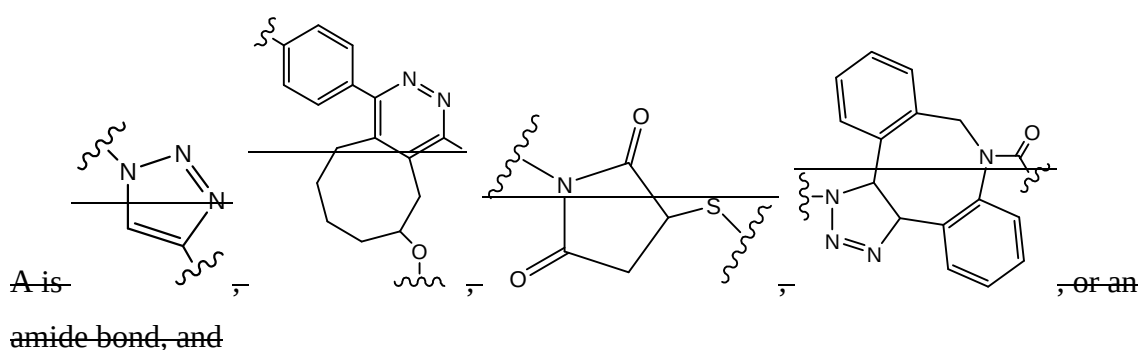
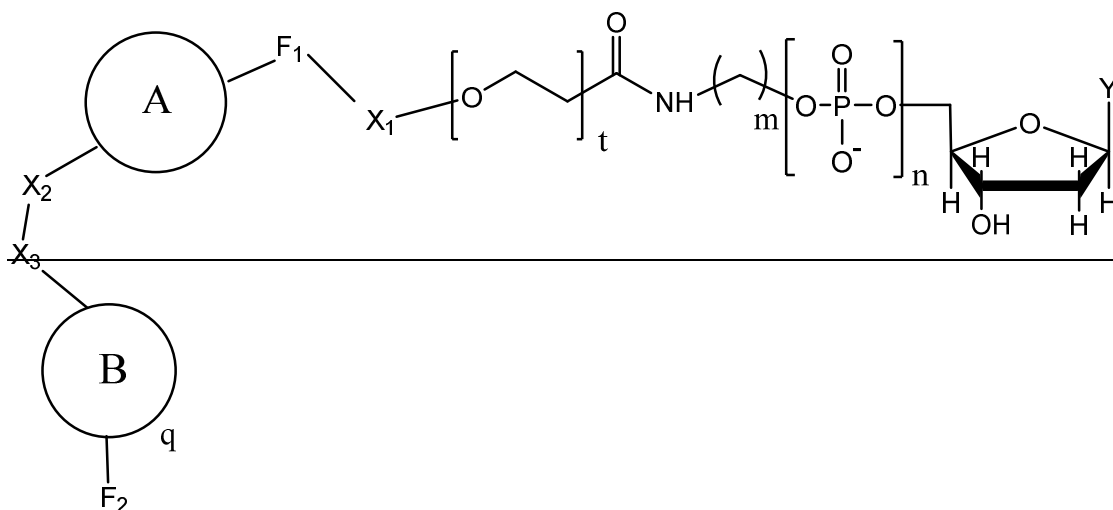
24. The method of claim 23, wherein the charge density is between about $-100e$ per cubic nanometer and about $+100e$ per cubic nanometer.

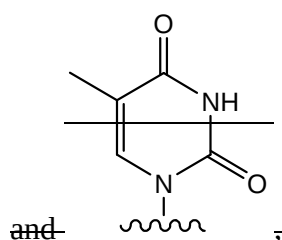
25. The method of claim 22, wherein the charge is between about $-200e$ and about $+200e$.

26. The method of claim 23, wherein the charge density is between about $-200e$ per cubic nanometer and about $+200e$ per cubic nanometer.

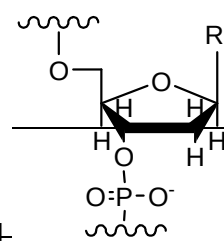
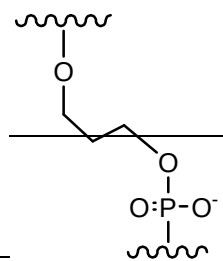
27. The method of claim 22, wherein the q number of B comprises a polypeptide.

28. ~~The method of claim 27, wherein the polypeptide is selected from a branched polypeptide, coiled polypeptide, and coiled-coil polypeptide.~~
29. ~~The method of claim 27, wherein one or more of the q number of B comprise methyllysine, dimethyllysine, or trimethyllysine.~~
30. ~~The method of any one of claims 22 through 29, wherein A was formed by a reaction comprising a linking reaction and the linking reaction is selecting from an azide-alkyne copper-assisted click reaction, a tetrazine-trans-cyclooctene ligation, an azide-dibenzocyclooctyne group copper-free click reaction, and a thiol-maleimide conjugation.~~
31. ~~The method of any one of claims 22 through 29, further comprising successively incorporating a plurality of labelled nucleotides wherein the charge of each of the plurality of labelled nucleotides differs from the charge of any other of the plurality of labelled nucleotides when the Y of the each and the Y of the any other differ from each other.~~
32. ~~The method of claim 31, further comprising identifying the Y of one or more labelled polynucleotide incorporated into the nascent polynucleotide strand based on the charge detected by the conductive channel.~~
33. ~~The method of any one of claims 22 through 29, wherein X_2 is $(-O-CH_2-CH_2-)_a$ wherein a is an integer from 1 to 24.~~
34. ~~The method of claim 33, wherein a is 24.~~
35. ~~The method of claim 33, wherein a is 16.~~
36. ~~The method of claim 33, wherein a is 12.~~
37. ~~The method of claim 33, wherein a is 8.~~
38. ~~The method of claim 33, wherein a is 4.~~
39. ~~A method comprising:~~
~~detecting an incorporation of a labelled nucleotide into a nascent polynucleotide strand complementary to a template polynucleotide strand by a polymerase, wherein the polymerase is tethered to a solid support conductive channel by a tether,~~
~~the labelled nucleotide is a compound of Formula I~~





q is an integer from 1 to 100, and



B is selected from a nucleotide,

, and

wherein each

R is independently selected from Y and hydrogen, and

the conductive channel is to detect the labelled nucleotide during the incorporation.

40. The method of claim 39, wherein the charge is between about -100e and about +100e.

41. The method of claim 40, wherein the charge density is between about -100e per cubic nanometer and about +100e per cubic nanometer.

42. The method of claim 39, wherein the charge is between about -200e and about +200e.

43. The method of claim 40, wherein the charge density is between about -200e per cubic nanometer and about +200e per cubic nanometer.

44. The method of claim 39, wherein the q number of B comprises a polynucleotide.

45. The method of claim 44, wherein the polynucleotide is selected from a branched polynucleotide and one or more hairpin loops.

46. The method of claim 44, wherein the polynucleotide comprises between two and five hairpin loops.

47. The method of any one of claims 39 through 46, wherein A was formed by a reaction comprising a linking reaction and the linking reaction is selecting from an azide-alkyne copper-assisted click reaction, a tetrazine-trans cyclooctene ligation, an azide-dibenzocyclooctyne group copper-free click reaction, and a thiol-maleimide conjugation.

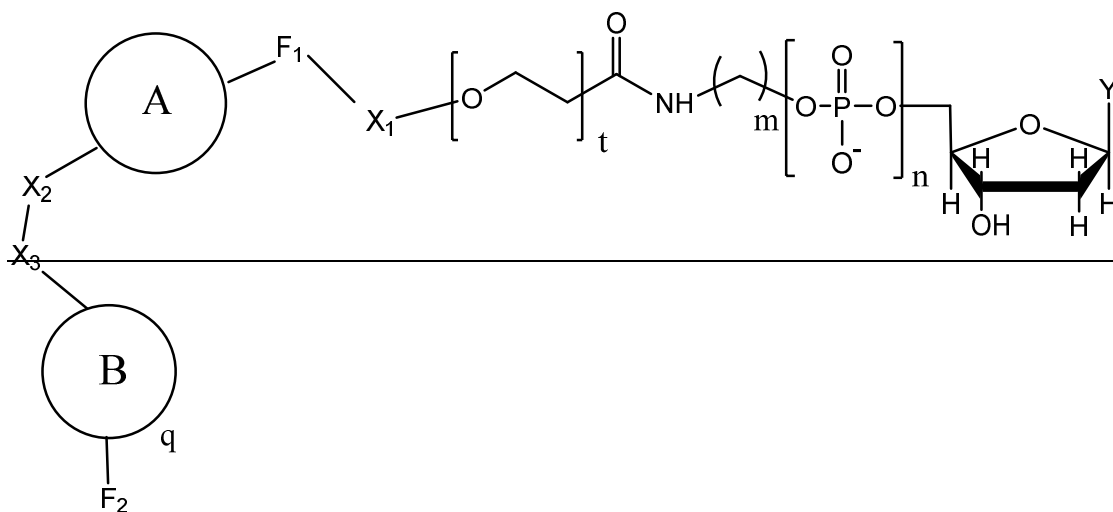
48. The method of any one of claims 39 through 46, further comprising successively incorporating a plurality of labelled nucleotides wherein the charge of each of the plurality of labelled nucleotides differs from the charge of any other of the plurality of

labelled nucleotides when the Y of the each and the Y of the any other differ from each other.

49. The method of claim 48 further comprising identifying the Y of one or more labelled polynucleotide incorporated into the nascent polynucleotide strand based on the charge detected by the conductive channel.
50. The method of any one of claims 39 through 46, wherein X_2 is $(-O-CH_2-CH_2-)_a$ wherein a is an integer from 1 to 24.
51. The method of claim 50, wherein a is 24.
52. The method of claim 50, wherein a is 16.
53. The method of claim 50, wherein a is 12.
54. The method of claim 50, wherein a is 8.
55. The method of claim 50, wherein a is 4.
56. A method comprising:

detecting an incorporation of a labelled nucleotide into a nascent polynucleotide strand complementary to a template polynucleotide strand by a polymerase, wherein the polymerase is tethered to a solid support conductive channel by a tether,

the labelled nucleotide is a compound of Formula I



wherein n is an integer from 3 to 10,

m is an integer from 1 to 10,

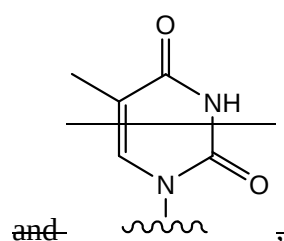
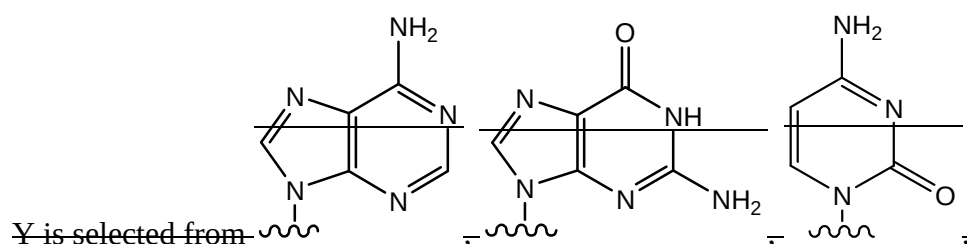
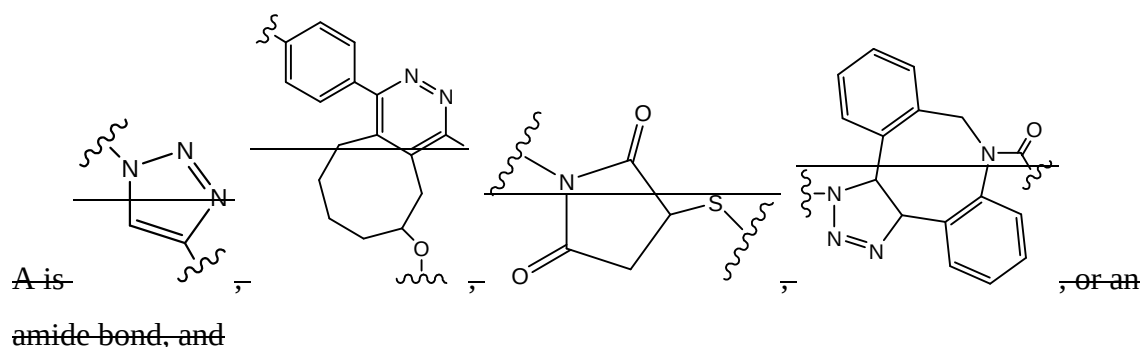
t is an integer from 0 to 50,

X_1 is a direct bond, a C_1-C_{10} alkyl, a C_1-C_{10} oxaalkyl, a C_1-C_{10} thiaalkyl, or a C_1-C_{10} azaalkyl,

~~X₂ is C₁-C₂₀ alkyl wherein optionally one or more individual CH₂ residue is replaced with one or more of a peptide bond and (-O-CH₂-CH₂-)_a wherein a is an integer from 1 to 24,~~

~~X₃ is a direct bond or an oligonucleotide wherein the oligonucleotide hybridizes to an acceptor region of the tether when the label is in proximity to the conductive channel,~~

~~F₁ is selected from a fluorophore and a direct bond and F₂ is absent or a fluorophore,~~



~~q is 1, and~~

~~B comprises a dendron, and B has a charge and a charge density, and~~

~~the charge sensor is to detect the labelled nucleotide during the incorporation.~~

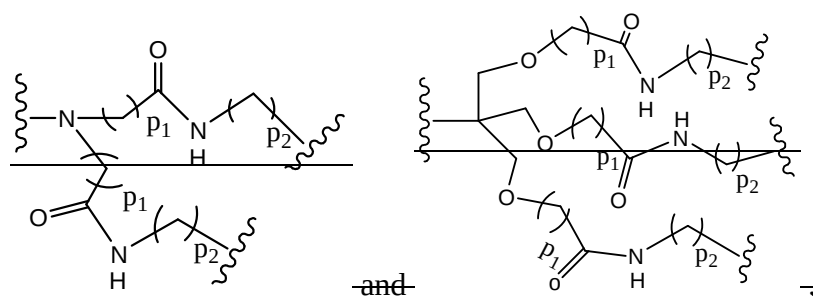
~~57. The method of claim 56, wherein the charge is between about -100e and about +100e.~~

~~58. The method of claim 57, wherein the charge density is between about -100e per cubic nanometer and about +100e per cubic nanometer.~~

~~59. The method of claim 56, wherein the charge is between about -200e and about +200e.~~

~~60. The method of claim 57, wherein the charge density is between about -200e per cubic nanometer and about +200e per cubic nanometer.~~

61. ~~The method of claim 56, wherein B is a dendron of z generations comprising one or more constitutional repeating unit and a plurality of end units, wherein z is an integer from 1 to 6, the constitutional end units are selected from:~~



~~wherein~~

~~p₁ is an integer from 1 to 3, wherein any one or more of the p₁-CH₂ groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂ groups,~~

~~p₂ is an integer from 1 to 3, wherein any one or more of the p₂-CH₂ groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂ groups, and~~

~~the end groups are selected from carboxylic acid, sulfonic acid, phosphonic acid, sperminyl group, amino group, and quaternary ammonium group.~~

62. ~~The method of any one of claims 56 through 61, wherein A was formed by a reaction comprising a linking reaction and the linking reaction is selecting from an azide-alkyne copper-assisted click reaction, a tetrazine-trans-cyclooctene ligation, an azide-dibenzocyclooctyne group copper free click reaction, and a thiol-maleimide conjugation.~~

63. ~~The method of any one of claims 56 through 61, further comprising successively incorporating a plurality of labelled nucleotides wherein the charge of each of the plurality of labelled nucleotides differs from the charge of any other of the plurality of labelled nucleotides when the Y of the each and the Y of the any other differ from each other.~~

64. ~~The method of claim 63 further comprising identifying the Y of one or more labelled polynucleotide incorporated into the nascent polynucleotide strand based on the charge detected by the conductive channel.~~

65. ~~The method of any one of claims 56 through 61, wherein X₂ is (-O-CH₂-CH₂)_a wherein a is an integer from 1 to 24.~~

66. ~~The method of claim 65, wherein a is 24.~~

67. ~~The method of claim 65, wherein a is 16.~~

68. ~~The method of claim 65, wherein a is 12.~~

69. — ~~The method of claim 65, wherein a is 8.~~

70. — ~~The method of claim 65, wherein a is 4.~~