

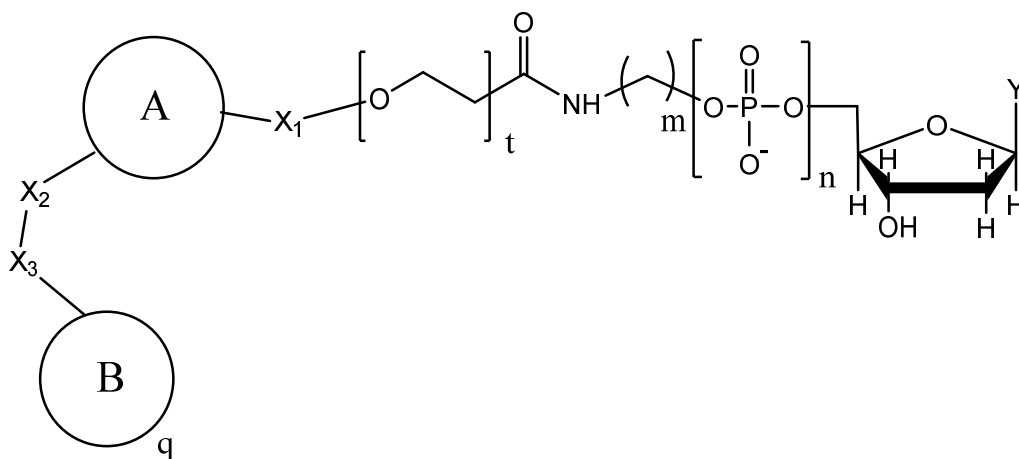
WHAT IS CLAIMED IS: CLAIMS

1. A method, comprising:
hybridizing a polynucleotide to a template, and
contacting a first template nucleotide 5-prime adjacent to a 5-prime-most nucleotide of a plurality of nucleotides that are complementary to the polynucleotide with a polymerase and a charge-tagged nucleotide, wherein the charge-tagged nucleotide is complementary to the first template nucleotide and comprises a charge tag attached to a 5-prime polyphosphate of the charge-tagged nucleotide.
2. The method of claim 1, further comprising attaching the charge-tagged nucleotide to the polynucleotide with the polymerase, wherein the attaching comprises detachment of the charge tag from the charge-tagged nucleotide.
3. The method of claim ~~1 or~~ 2, wherein the template is bound to a substrate.
4. The method of ~~any one of claims 1 through~~ claim 3, wherein the polymerase is bound to a substrate.
5. The method of ~~any one of claims 1 through~~ claim 4, wherein the template and the polymerase are bound to a substrate.
6. The method of ~~any one of claims 1 thorough~~ claim 5, wherein the polymerase is selected from a Klenow fragment and a Phi29 polymerase.
7. The method of ~~any one of claims 1 through~~ claim 6, wherein the template is attached to a substrate and comprises linking nucleotides, and wherein the linking nucleotides are between the first template nucleotide and the substrate.
- ~~8. The method of claim 7, wherein there are more than 20 linking nucleotides.~~
- ~~9. The method of claim 7, wherein there are from 1 to 20 linking nucleotides.~~
- ~~10. The method of claim 7, wherein there are from 10 to 20 linking nucleotides.~~
- ~~11. The method of claim 7, wherein there are from 1 to 10 linking nucleotides.~~
- ~~12. The method of claim 7, wherein there are from 1 to 5 linking nucleotides.~~
- ~~13. The method of claim 7, wherein there are from 5 to 10 linking nucleotides.~~
- ~~14. The method of claim 7, wherein there are from 10 to 15 linking nucleotides.~~
- ~~15. The method of claim 7, wherein there are from 15 to 20 linking nucleotides.~~
- ~~16. 9. The method of any one of claims 1 through 15~~ claim 8, wherein the substrate is a solid support conductive channel and the conductive channel is to detect the charge-

tagged nucleotide during the contacting the first template nucleotide with the charge-tagged nucleotide and attaching the charge-tagged nucleotide to the polynucleotide.

~~17.10.~~ The method of claim ~~169~~, comprising attaching the charge-tagged nucleotide in a solution, wherein the charge tag comprises a Debye length in the solution and the Debye length is between about 0.5 nm and about 10 nm.

~~18.11.~~ The method of ~~any one of claims 1 through 17~~ claim 10, wherein the charge-tagged nucleotide is a compound of Formula I



wherein n is an integer from 3 to 10,

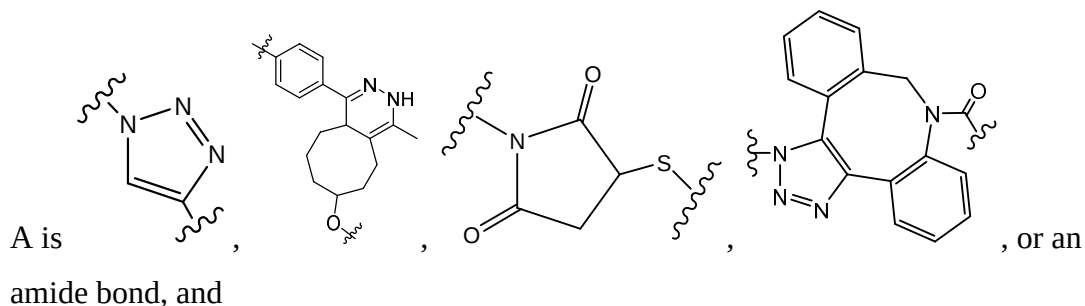
m is an integer from 1 to 10,

t is an integer from 0 to 50,

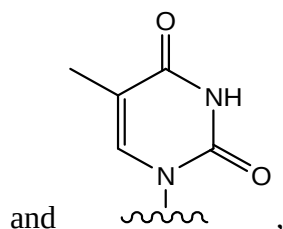
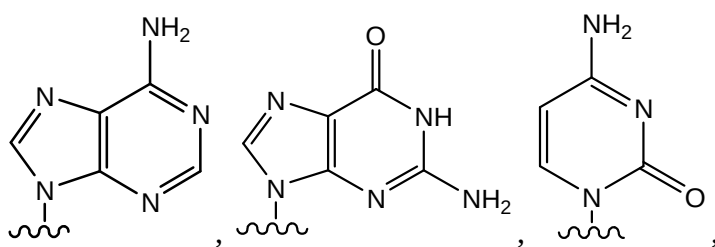
X₁ is a direct bond, a C₁-C₁₀ alkyl, a C₁-C₁₀ oxaalkyl, a C₁-C₁₀ thiaalkyl, or a C₁-C₁₀ azaalkyl,

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

X₃ is a direct bond or an oligonucleotide,



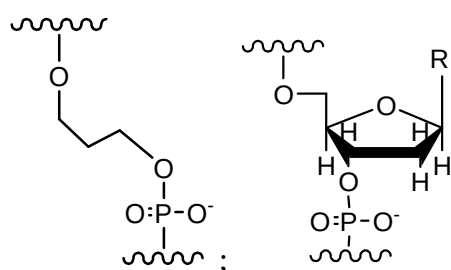
Y is selected from the group consisting of:



and ,

q is an integer from 1 to 100, and

B is selected from the group consisting of: an amino acid; a nucleotide;



, wherein each R is independently selected from Y

and hydrogen; and a dendron; and wherein q is equal to 1 when B is a dendron, and

the q number of B has a charge and a charge density.

19.12. The method of claim ~~18~~11, wherein the charge is between about -100e and about +100e.

20.13. The method of claim 1912, wherein the charge density is between about -100e per cubic nanometer and about +100e per cubic nanometer.

21.14. The method of claim ~~18~~13, wherein the charge is between about -200e and about +200e.

22.15. The method of claim ~~21~~14, wherein the charge density is between about -200e per cubic nanometer and about +200e per cubic nanometer.

23.16. The method of ~~any one of claims 18 through 22~~ claim 15, wherein the B comprises a q number of nucleotides and q is more than 1.

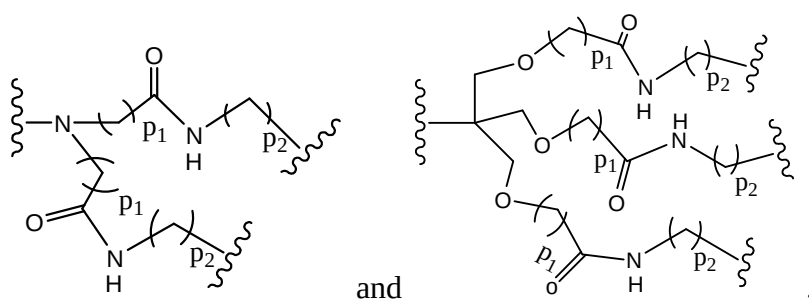
24.17. The method of claim ~~23~~16, wherein the polynucleotide is selected from a branched polynucleotide and one or more hairpin loops.

~~25.18.~~ The method of ~~any one of claims 18 through 22~~ claim 17, wherein the q number of B comprises a polypeptide.

~~26.19.~~ The method of claim ~~24~~18, wherein the polypeptide is selected from a branched polypeptide, coiled polypeptide, and coiled-coil polypeptide.

~~27.20.~~ The method of ~~any one of claims 18 through 22~~ claim 19, wherein B comprises an amino acid, and one or more of the q number of B comprise methyllysine, dimethyllysine, or trimethyllysine.

~~28.21.~~ The method of ~~any one of claims 18 through 22~~ claim 20, 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.

~~29.—The method of any one of claims 18 through 28, 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.~~

~~30.—The method of any one of claims 18 through 28, wherein X₂ is (-O-CH₂-CH₂-)_a wherein a is an integer from 1 to 24.~~

~~31.—The method of claim 30, wherein a is 24.~~

~~32.—The method of claim 30, wherein a is 16.~~

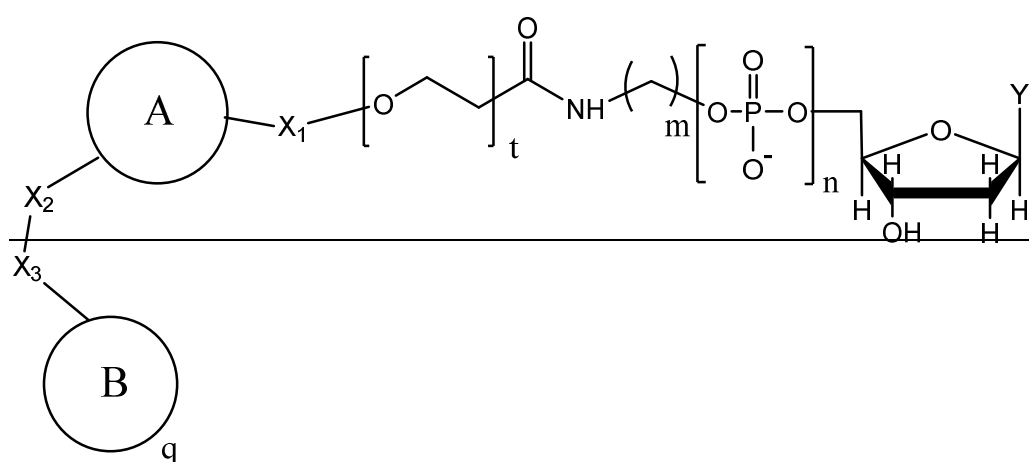
33. The method of claim 30, wherein a is 12.

34. The method of claim 30, wherein a is 8.

35. The method of claim 30, wherein a is 4.

36. The method of any one of claims 18 through 35, wherein the template is attached to a substrate and comprises linking nucleotides wherein the linking nucleotides are between the second template nucleotide and the substrate, and X_3 comprises an oligonucleotide, wherein the oligonucleotide hybridizes to a plurality of the linking nucleotides when the charge tag is in proximity to the substrate.

37. The method of any one of claims 1 through 17, wherein the charge-tagged nucleotide is a compound of Formula I



wherein n is an integer from 3 to 10,

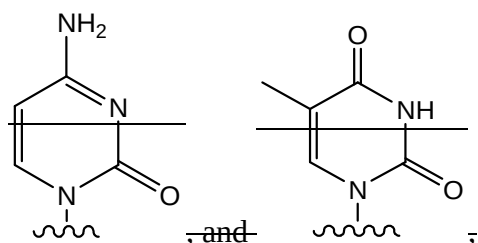
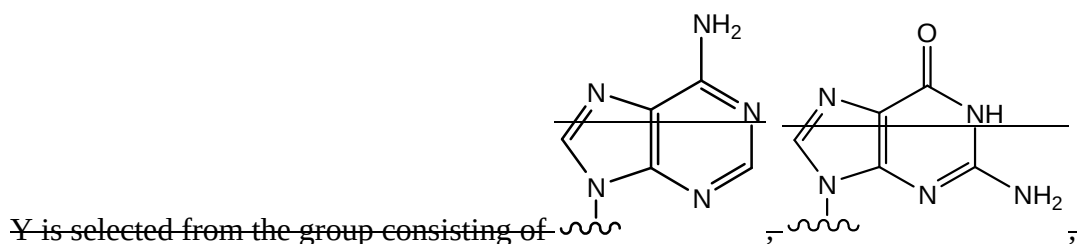
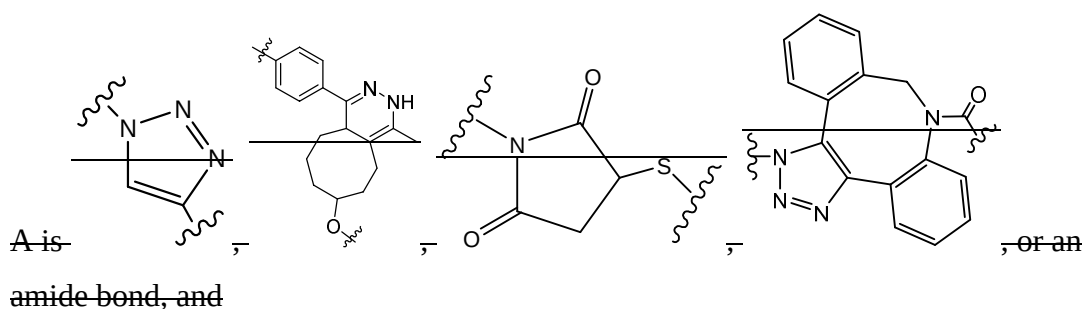
m is an integer from 1 to 10,

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,

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

X_3 is a direct bond or an oligonucleotide,



q is an integer from 1 to 100,

B comprises an amino acid, and

the q number of B has a charge and a charge density.

38. The method of claim 37, wherein the charge is between about $-100e$ and about $+100e$.

39. The method of claim 38, wherein the charge density is between about $-100e$ per cubic nanometer and about $+100e$ per cubic nanometer.

40. The method of claim 37, wherein the charge is between about $-200e$ and about $+200e$.

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

42. The method any one of claims 37 through 41, wherein the q number of B comprises a polypeptide.

43. The method of claim 42, wherein the polypeptide is selected from a branched polypeptide, coiled polypeptide, and coiled-coil polypeptide.

44.—The method of claim 42 or 43, wherein one or more of the q number of B comprises methyllysine, dimethyllysine, or trimethyllysine.

45.—The method of any one of claims 42 through 44, wherein the polypeptide is a branched polypeptide comprising one or more forks each of the one or more forks comprising a plurality of branches, and one or more of the plurality of branches each independently comprises a number of amino acids.

46.—The method of claim 45, wherein the polypeptide comprises three or more forks and the number of amino acids of one or more of the plurality of branches is at least four.

47.—The method of claim 45 or 46, wherein the polypeptide comprises seven or more forks.

48.—The method of any one of claims 45 to 47, wherein the number of amino acids of one or more of the plurality of branches is at least six.

49.—The method of any one of claims 37 through 48, 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.

50.—The method of any one of claims 37 through 49, 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.

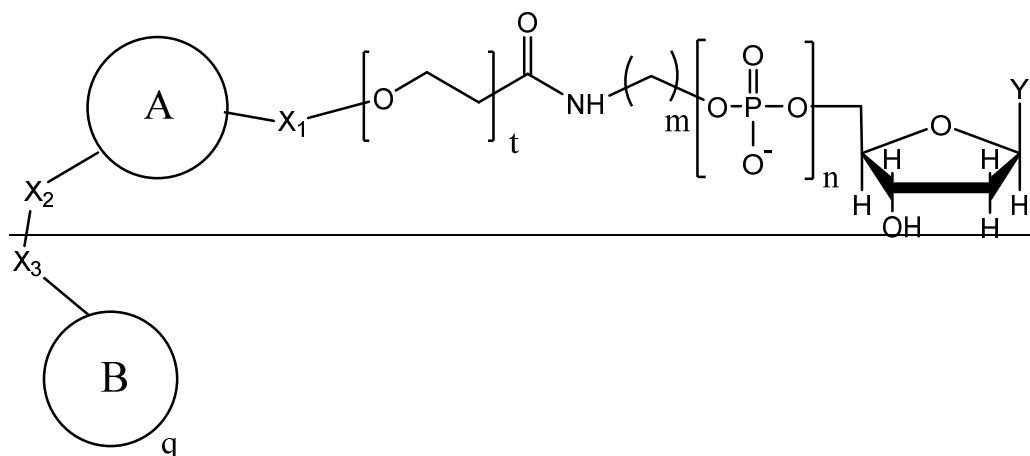
53.—The method of claim 50, wherein a is 12.

54.—The method of claim 50, wherein a is 8.

55.—The method of claim 50, wherein a is 4.

56.—The method of any one of claims 37 through 62, wherein the template is attached to a substrate and comprises linking nucleotides wherein the linking nucleotides are between the second template nucleotide and the substrate, and X_3 comprises an oligonucleotide, wherein the oligonucleotide hybridizes to a plurality of the linking nucleotides when the charge tag is in proximity to the substrate.

57.—The method of any one of claims 1 through 17, wherein the charge-tagged nucleotide is a compound of Formula I



wherein n is an integer from 3 to 10,

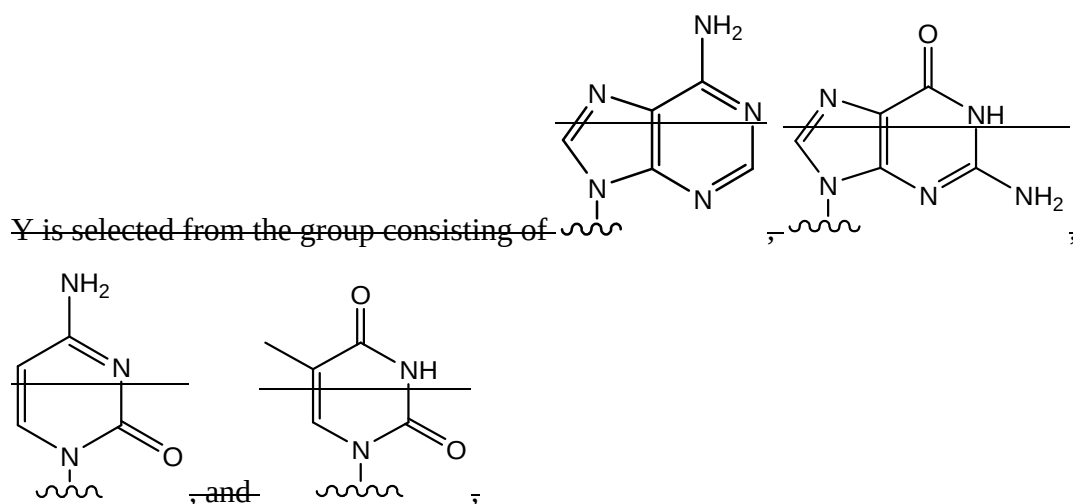
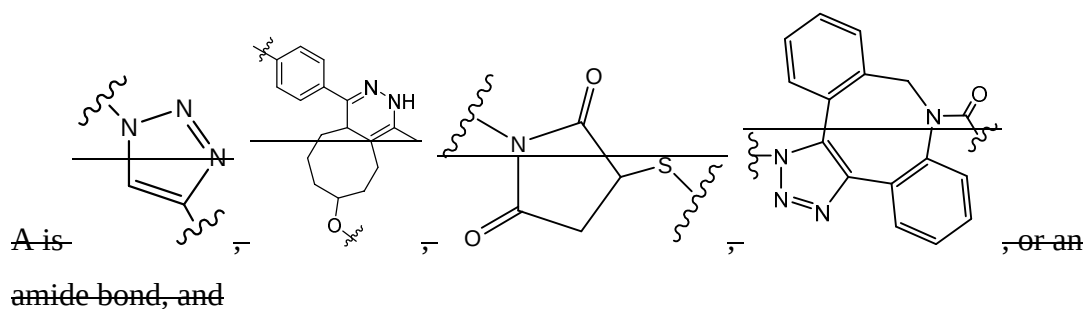
m is an integer from 1 to 10,

t is an integer from 0 to 50,

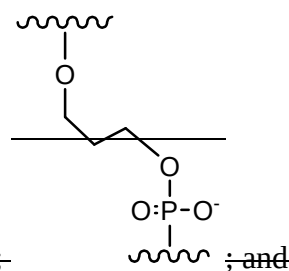
X₁ is a direct bond, a C₁-C₁₀ alkyl, a C₁-C₁₀ oxaalkyl, a C₁-C₁₀ thiaalkyl, or a C₁-C₁₀ azaalkyl,

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

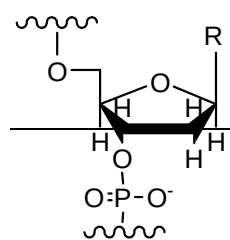
X₃ is a direct bond or an oligonucleotide,



q is an integer from 1 to 100, and



B is selected from the group consisting of a nucleotide;



wherein each R is independently selected from Y and hydrogen; and

the q number of B has a charge and a charge density.

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

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

60. The method of claim 57, wherein the charge is between about -200e and about +200e.

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

62. The method of any one of claims 57 through 61, wherein B comprises a q number of nucleotides and q is more than 1.

63. The method of claim 62, wherein the polynucleotide is selected from a branched polynucleotide and one or more hairpin loops.

64. The method of claim 62, wherein the polynucleotide comprises between two and five hairpin loops.

65. The method of any one of claims 57 through 64, wherein A is 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.

66. The method of any one of claims 57 through 65, wherein X_2 is $(-O-CH_2-CH_2-)_a$ wherein a is an integer from 1 to 24.

67. — The method of claim 66, wherein a is 24.

68. — The method of claim 66, wherein a is 16.

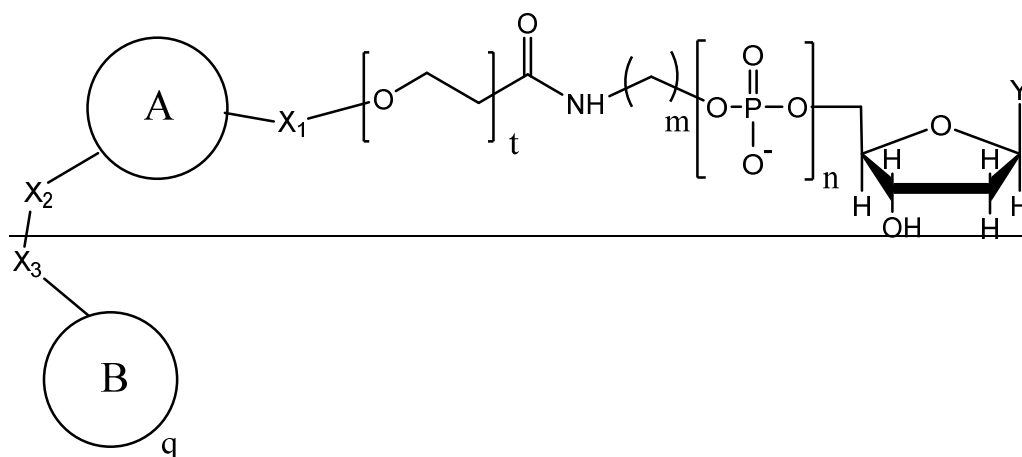
69. — The method of claim 66, wherein a is 12.

70. — The method of claim 66, wherein a is 8.

71. — The method of claim 66, wherein a is 4.

72. — The method of any one of claims 57 through 71, wherein the template is attached to a substrate and comprises linking nucleotides wherein the linking nucleotides are between the second template nucleotide and the substrate, and X_3 comprises an oligonucleotide, wherein the oligonucleotide hybridizes to a plurality of the linking nucleotides when the charge tag is in proximity to the substrate.

73. — The method of any one of claims 1 through 17, wherein the charge-tagged nucleotide is a compound of Formula I



wherein n is an integer from 3 to 10,

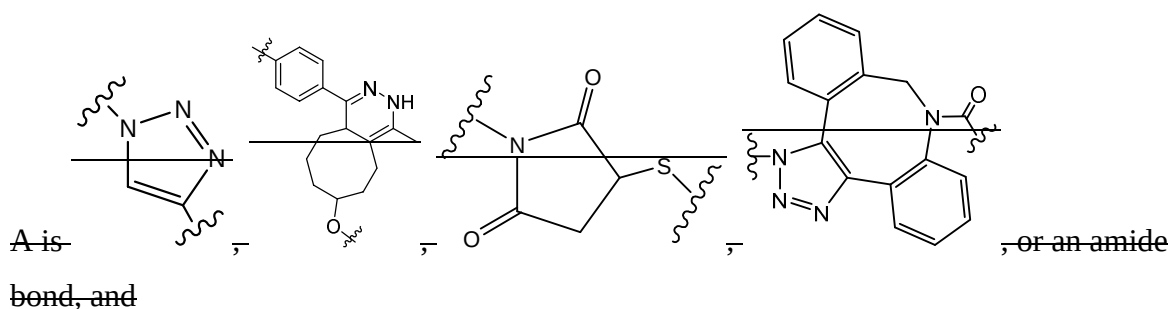
m is an integer from 1 to 10,

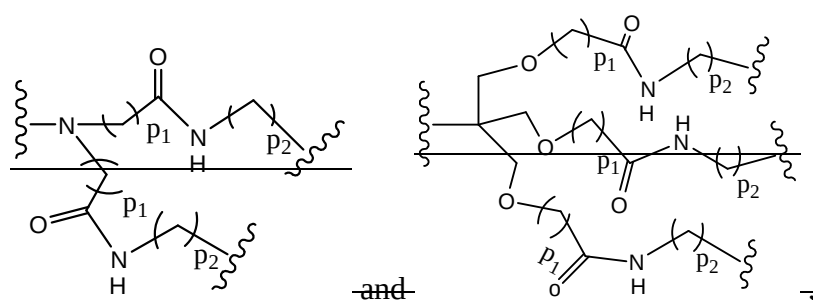
t is an integer from 0 to 50,

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,

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

X_3 is a direct bond or an oligonucleotide,





wherein

p_1 is an integer from 1 to 3, wherein any one or more of the p_1 -CH₂-groups is optionally replaced with from 1 to 3 -O-CH₂-CH₂-groups,

p_2 is an integer from 1 to 3, wherein any one or more of the p_2 -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.

79.—The method of any one of claims 73 through 78, 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.

80.—The method of any one of claims 73 through 78 wherein X₂ is (-O-CH₂-CH₂)_a wherein a is an integer from 1 to 24.

81.—The method of claim 80, wherein a is 24.

82.—The method of claim 80, wherein a is 16.

83.—The method of claim 80, wherein a is 12.

84.—The method of claim 80, wherein a is 8.

85.—The method of claim 80, wherein a is 4.

86.—The method of any one of claims 73 through 85, wherein the template is attached to a substrate and comprises linking nucleotides wherein the linking nucleotides are between the second template nucleotide and the substrate, and X₃ comprises an oligonucleotide, wherein the oligonucleotide hybridizes to a plurality of the linking nucleotides when the charge tag is in proximity to the substrate.

87.—The method of any one of claims 1 through 86, wherein the template further comprises a second template nucleotide 5-prime adjacent to the first template nucleotide, the method further comprising contacting the second template nucleotide with a fluorescently-

~~tagged nucleotide wherein the fluorescently tagged nucleotide is complementary to the second template nucleotide but not to the first template nucleotide, and~~

~~attaching the fluorescently tagged nucleotide to the polynucleotide with a polymerase.~~

~~88.—The method of claim 87, further comprising measuring attachment of the fluorescently tagged nucleotide to the polynucleotide by measuring fluorescence emitted from the polynucleotide.~~

~~89.—The method of claim 88, further comprising eluting the polynucleotide from the template before measuring.~~