

CLAIMS

WHAT IS CLAIMED IS:

1. A method of determining an identity of a nucleotide in a target nucleic acid sequence comprising:
 - a. providing a composition comprising:
 - i. two or more copies of said target nucleic acid sequence;
 - ii. two or more primer nucleic acid molecules that are complementary to one or more regions of said target nucleic acid sequence; and
 - iii. two or more polymerase molecules;
 - b. contacting said composition with a polymer nucleotide conjugate under conditions sufficient to allow a multivalent binding complex to be formed between said polymer-nucleotide conjugate and said two or more copies of said target nucleic acid sequence in said composition of (a), wherein the polymer-nucleotide conjugate comprises two or more copies of a nucleotide moiety and optionally one or more detectable labels; and
 - c. detecting said multivalent binding complex, thereby determining the identity of said nucleotide in the target nucleic acid sequence.
2. The method of claim 1, wherein the target nucleic acid sequence is DNA.
3. The method of claim 1 or claim 2, wherein the detection of the multivalent binding complex is performed in the absence of unbound or solution-borne polymer nucleotide conjugates.
4. The method of any one of claims 1 to 3, wherein the target nucleic acid sequence has been replicated or amplified or has been produced by replication or amplification.
5. The method of any one of claims 1 to 4, wherein the one or more detectable labels are fluorescent labels.
6. The method of any one of claims 1 to 5, wherein detecting the multivalent complex comprises a fluorescence measurement.
7. The method of any one of claims 1 to 6, wherein the contacting comprises use of one type of polymer-nucleotide conjugate.
8. The method of any one of claims 1 to 7, wherein the contacting comprises use of two or more types of polymer-nucleotide conjugates.

9. The method of claim 8, wherein each type of the two or more types of polymer-nucleotide conjugate comprises a different type of nucleotide moiety.
10. The method of claim 9, wherein the contacting comprises use of three types of polymer-nucleotide conjugate and wherein each type of the three types of polymer-nucleotide conjugate comprises a different type of nucleotide moiety.
11. The method of any one of claims 1 to 10, wherein the polymer-nucleotide conjugate comprises a blocked nucleotide moiety.
12. The method of claim 11, wherein the blocked nucleotide is a 3'-O-azidomethyl nucleotide, a 3'-O-methyl nucleotide, or a 3'-O-alkyl hydroxylamine nucleotide.
13. The method of any one of claims 1 to 12, wherein said contacting occurs in the presence of an ion that stabilizes said multivalent binding complex.
14. The method of any one of claims 1 to 13, wherein the contacting is done in the presence of strontium ions, magnesium ions, calcium ions, or any combination thereof.
15. The method of any one of claims 1 to 14, wherein the polymerase molecules are catalytically inactive.
16. The method of any one of claims 1 to 15, wherein the polymerase molecules have been rendered catalytically inactive by mutation or chemical modification.
17. The method of any one of claims 1 to 16, wherein the polymerase molecules have been rendered catalytically inactive by the absence of a necessary ion or cofactor.
18. The method of any one of claims 1 to 17, wherein the polymerase molecules are catalytically active.
19. The method of any one of claims 1 to 18, wherein the polymer-nucleotide conjugate does not comprise a blocked nucleotide moiety.
20. The method of any one of claims 1 to 19, wherein the multivalent binding complex has a persistence time of greater than 2 seconds.
21. The method of any one of claims 1 to 20, wherein the method can be carried out at a temperature within a range of 25°C to 62°C.
22. The method of any one of claims 1 to 21, wherein the polymer-nucleotide conjugate further comprises one or more fluorescent labels and the two or more copies of the target nucleic acid sequence are deposited on, attached to, or hybridized to a surface, wherein a fluorescence image of

the multivalent binding complex on the surface has a contrast to noise ratio in the detecting step of greater than 20.

23. The method of any one of claims 1 to 22, wherein the composition of (a) is deposited on a surface using a buffer that incorporates a polar aprotic solvent.

24. The method of any one of claims 1 to 23, wherein the contacting is performed under a condition that stabilizes said multivalent binding complex when said nucleotide moiety is complementary to a next base of said target nucleic acid sequence and destabilizes said multivalent binding complex when said nucleotide moiety is not complementary to said next base of said target nucleic acid sequence.

25. The method of any one of claims 1 to 24, wherein said polymer-nucleotide conjugate comprises a polymer having a plurality of branches and said two or more nucleotide moieties are attached to said branches.

26. The method of claim 25, wherein said polymer has a star, comb, cross-linked, bottle brush, or dendrimer configuration.

27. The method of any one of claims 1 to 26, wherein said polymer-nucleotide conjugate comprises one or more binding groups selected from the group consisting of an avidin, a biotin, an affinity tag, and combinations thereof.

28. The method of any one of claims 1 to 27, further comprising a dissociation step that destabilizes said multivalent binding complex formed between the composition of (a) and the polymer-nucleotide conjugate, said dissociation step enabling removal of said polymer-nucleotide conjugate.

29. The method of claim 28, further comprising an extension step to incorporate a nucleotide that is complementary to a next base of the target nucleic acid sequence into said two or more primer nucleic acid molecules.

30. The method of claim 29, wherein the extension step occurs concurrently with or after said dissociation step.

31. A method of determining an identity of a nucleotide in a target nucleic acid sequence comprising:

a. providing a composition comprising:

- i. two or more copies of said target nucleic acid sequence;
- ii. two or more primer nucleic acid molecules that are complementary to one or more regions of said target nucleic acid sequence; and

- iii. two or more polymerase molecules;
 - b. contacting said composition with a polymer nucleotide conjugate under conditions sufficient to allow a multivalent complex to be formed between said polymer-nucleotide conjugate and said two or more copies of said target nucleic acid sequence in said composition of (a), wherein the polymer-nucleotide conjugate comprises two or more copies of a reversibly terminated nucleotide moiety and optionally one or more cleavable detectable labels; and
 - c. detecting said multivalent complex, thereby determining the identity of said nucleotide in the target nucleic acid sequence.
32. The method of claim 31, wherein the target nucleic acid sequence is DNA.
33. The method of claim 31 or claim 32, further comprising contacting the composition of (a) with reversibly terminated nucleotides or polymer-nucleotide conjugates comprising two or more copies of a reversibly terminated nucleotide following the detection of said multivalent binding complex.
34. The method of any one of claims 31 to 33, wherein the target nucleic acid sequence has been replicated or amplified or has been produced by replication or amplification.
35. The method of any one of claims 31 to 34, wherein the one or more detectable labels are fluorescent labels.
36. The method of any one of claims 31 to 35, wherein detecting the multivalent complex comprises a fluorescence measurement.
37. The method of any one of claims 31 to 36, wherein the contacting comprises use of one type of polymer-nucleotide conjugate.
38. The method of any one of claims 31 to 37, wherein the contacting comprises use of two or more types of polymer-nucleotide conjugates.
39. The method of claim 38, wherein each type of the two or more types of polymer-nucleotide conjugate comprises a different type of nucleotide moiety.
40. The method of claim 39, wherein the contacting comprises use of three types of polymer-nucleotide conjugate and wherein each type of the three types of polymer-nucleotide conjugate comprises a different type of nucleotide moiety.
41. The method of any one of claims 31 to 40, wherein the polymer-nucleotide conjugate comprises a blocked nucleotide moiety.

42. The method of claim 41, wherein the blocked nucleotide is a 3'-O-azidomethyl, 3'-O-methyl, or 3'-O-alkyl hydroxylamine.
43. The method of any one of claims 31 to 42, wherein said contacting occurs in the presence of an ion that stabilizes said multivalent binding complex.
44. The method of any one of claims 31 to 43, wherein the polymerase molecules are catalytically inactive.
45. The method of any one of claims 31 to 44, wherein the polymerase molecules have been rendered catalytically inactive by mutation or chemical modification.
46. The method of any one of claims 31 to 45, wherein the polymerase molecules are catalytically active.
47. The method of any one of claims 31 to 46, wherein the polymer-nucleotide conjugate does not comprise a blocked nucleotide moiety.
48. The method of any one of claims 31 to 47, wherein the method can be carried out at a temperature within a range of 25°C to 80°C.
49. The method of any one of claims 31 to 48, wherein the polymer-nucleotide conjugate further comprises one or more fluorescent labels and the two or more copies of the target nucleic acid sequence are deposited on, attached to, or hybridized to a surface, wherein a fluorescence image of the multivalent binding complex on the surface has a contrast to noise ratio in the detecting step of greater than 20.
50. A system comprising:
- a) one or more computer processors individually or collectively programmed to implement a method comprising:
 - i) contacting a substrate comprising multiple copies of a target nucleic acid sequence tethered to a surface of the substrate with a reagent comprising a polymerase and one or more primer nucleic acid sequences that are complementary to one or more regions of said target nucleic acid sequence to form a primed target nucleic acid sequence;
 - ii) contacting the substrate surface with a reagent comprising a polymer nucleotide conjugate under conditions sufficient to allow a multivalent binding complex to be formed between said polymer-nucleotide conjugate and two or more copies of said

primed target nucleic acid sequence, wherein the polymer-nucleotide conjugate comprises two or more copies of a known nucleotide moiety and a detectable label;

iii) acquiring and processing an image of the substrate surface to detect said multivalent binding complex, thereby determining the identity of a nucleotide in the target nucleic acid sequence.

51. The system of claim 50, further comprising a fluidics module configured to deliver a series of reagents to the substrate surface in a specified sequence and for specified time intervals.

52. The system of claim 50 or claim 51, further comprising an imaging module configured to acquire images of the substrate surface.

53. The system of any one of claims 50 to 52, wherein (ii) and (iii) are repeated two or more times thereby determining the identity of a series of two or more nucleotides in the target nucleic acid sequence.

54. The system of any one of claims 50 to 53, wherein the series of steps further comprises a dissociation step that destabilizes said multivalent binding complex, said dissociation step enabling removal of said polymer-nucleotide conjugate.

55. The system of claim 54, wherein the series of steps further comprises an extension step to incorporate a nucleotide that is complementary to a next base of the target nucleic acid sequence into said two or more primer nucleic acid molecules.

56. The system of claim 55, wherein the extension step occurs concurrently with or after said dissociation step.

57. The system of any one of claims 50 to 56, wherein the detectable label comprises a fluorophore and the images comprise fluorescence images.

58. The system of claim 57, wherein the fluorescence images of the multivalent binding complex on the substrate surface has a contrast-to-noise ratio of greater than 20 when the fluorophore is cyanine dye 3 (Cy3) and the image is acquired using an inverted fluorescence microscope equipped with a 20X objective, NA = 0.75, dichroic mirror optimized for 532 nm light, a bandpass filter optimized for Cyanine dye-3 emission, and a camera, under non-signal saturating conditions while the surface is immersed in 25 mM ACES, pH 7.4 buffer.

59. The system of any one of claims 50 to 58, wherein the series of steps is completed in less than 60 minutes.

60. The system of any one of claims 50 to 59, wherein the series of steps is completed in less than 30 minutes.
61. The system of any one of claims 50 to 60, wherein the series of steps is completed in less than 10 minutes.
62. The system of any one of claims 50 to 61, wherein an accuracy of base-calling is characterized by a Q-score of greater than 25 for at least 80% of the nucleotide identities determined.
63. The system of any one of claims 50 to 62, wherein an accuracy of base-calling is characterized by a Q-score of greater than 30 for at least 80% of the nucleotide identities determined.
64. The system of any one of claims 50 to 63, wherein an accuracy of base-calling is characterized by a Q-score of greater than 40 for at least 80% of the nucleotide identities determined.
65. A composition comprising:
- a) a polymer core; and
 - b) two or more nucleotide, nucleotide analog, nucleoside, or nucleoside analog moieties attached to the polymer core;
- wherein the length of the linker is dependent on the nucleotide, nucleotide analog, nucleoside, or nucleoside analog moiety that is attached to the polymer core.
66. A composition comprising:
- a) a mixture of polymer-nucleotide conjugates, wherein each polymer-nucleotide conjugate comprises:
 - i) a polymer core; and
 - ii) two or more nucleotide, nucleotide analog, nucleoside, or nucleoside analog moieties attached to the polymer core, wherein the length of the linker is dependent on the nucleotide, nucleotide analog, nucleoside, or nucleoside analog moiety that is attached to the polymer core; and
- wherein the mixture comprises polymer-nucleotide conjugates having at least two different types of attached nucleotide, nucleotide analog, nucleoside, or nucleoside analog moiety.
67. The composition of claim 65 or claim 66, wherein the polymer core comprises a polymer having a plurality of branches and the two or more nucleotide, nucleotide analog, nucleoside, or nucleoside analog moieties are attached to said branches.

68. The composition of claim 67, wherein the polymer has a star, comb, cross-linked, bottle brush, or dendrimer configuration.
69. The composition of any one of claims 65 to 68, wherein the polymer-nucleotide conjugate comprises one or more binding groups selected from the group consisting of an avidin, a biotin, an affinity tag, and combinations thereof.
70. The composition of any one of claims 65 to 69, wherein the polymer core comprises a branched polyethylene glycol (PEG) molecule.
71. The composition of any one of claims 65 to 70, wherein the polymer-nucleotide conjugate comprises a blocked nucleotide moiety.
72. The composition of claim 71, wherein the blocked nucleotide is a 3'-O-azidomethyl nucleotide, a 3'-O-methyl nucleotide, or a 3'-O-alkyl hydroxylamine nucleotide.
73. The composition of any one of claims 65 to 72, wherein the polymer-nucleotide conjugate further comprises one or more fluorescent labels.