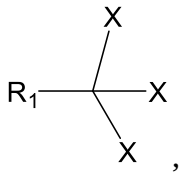
CLAIMS

1. A nanoparticle, comprising

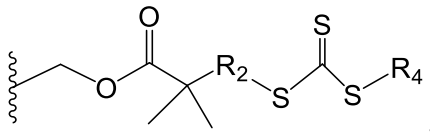
a scaffold, a single template site for bonding a template polynucleotide to the scaffold selected from a covalent template bonding site and a noncovalent template bonding site, and a plurality of accessory sites for bonding accessory oligonucleotides to the scaffold selected from covalent accessory oligonucleotide bonding sites and noncovalent accessory oligonucleotide bonding sites, wherein

the scaffold is a compound of Formula I:



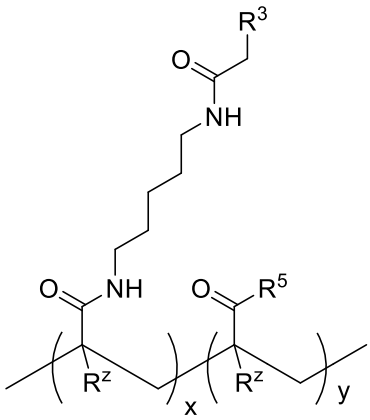
,

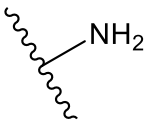
each X is a compound of formula II:



,

wherein R_2 is selected from Formula IIIa:



, wherein R^5 is selected from 

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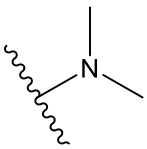
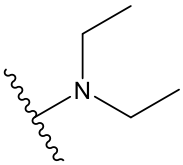
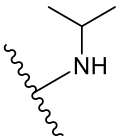
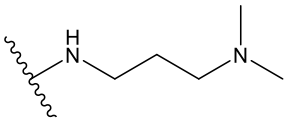
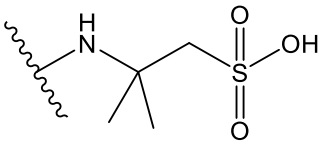


, 

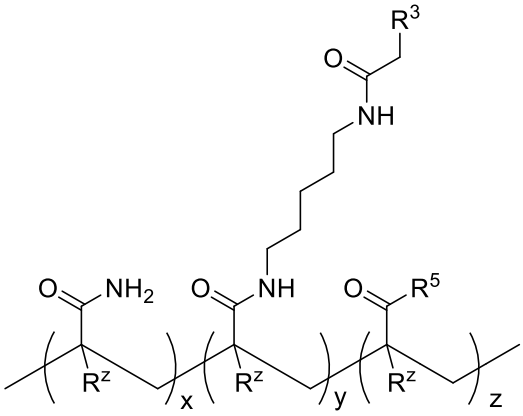
, 

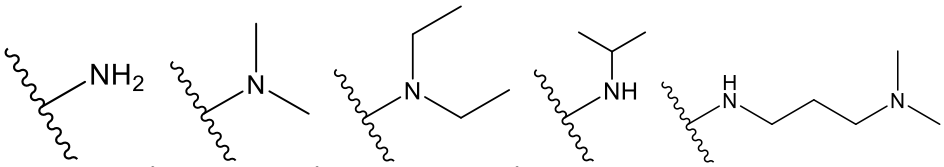


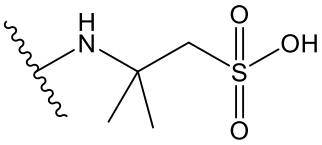
, and 

,

x is an integer in the range of from 1-20,000 and y is an integer in the range of from 1-100,000 and a ratio of x:y may be from approximately 10:90 to approximately 1:99, and wherein each R^z is independently H or C_{1-4} alkyl, and Formula IIIb:


, wherein R^5 is selected from


, and


, y is an integer in the range of from 1-2,000 and x and z are integers whose sum is in a range of from 1-10,000 and a ratio of (x:y):z may be from approximately (85):15 to approximately (95):5, and wherein each R^z is independently H or C_{1-4} alkyl,

R_1 includes the single template site for bonding a template polynucleotide to the scaffold, R^4 is selected from an optionally substituted C_1-C_{20} alkyl, an optionally substituted C_1-C_{20} alkenyl, an optionally substituted C_1-C_{20} alkynyl, an optionally substituted C_1-C_{20} oxaalkyl, an optionally substituted C_1-C_{20} thiaalkyl, and an optionally substituted C_1-C_{20} azaalkyl, wherein substituted comprises substitution with one or more of a C_1-C_{20} alkyl, a double-bonded oxygen, and a hydroxyl group, and R^3 includes the accessory site for bonding accessory oligonucleotides.

2. The nanoparticle of claim 1, wherein the single template site comprises a covalent template bonding site, and optionally wherein the covalent template bonding site is selected from amine-NHS ester bonding site, an amine-imidoester bonding site, an amine-pentofluorophenyl ester bonding site, an amine-hydroxymethyl phosphine bonding site, a carboxyl-carbodiimide bonding site, a thiol-maleimide bonding site, a thiol-haloacetyl bonding site, a thiol-pyridyl disulfide bonding site, a thiol-thiosulfonate bonding site, a thiol-vinyl sulfone bonding site, an aldehyde-hydrazide bonding site, an aldehyde-alkoxyamine bonding site, an aldehyde-NHS ester bonding site, a hydroxy-isocyanate bonding site, an azide-alkyne bonding site, an azide-phosphine bonding site, a transcyclooctene-tetrazine bonding site, a norbornene-tetrazine bonding site, an azide-cyclooctyne bonding site, an

azide-norbornene bonding site, an oxime bonding site, a SpyTag-SpyCatcher bonding site, a Snap-tag-O^6-Benzylguanine bonding site, a CLIP-tag-O^2-benzylcytosine bonding site, and a sortase-coupling bonding site.

3. The nanoparticle of any one of claim 1, wherein the single template site comprises a noncovalent template bonding site, and optionally wherein the noncovalent template bonding site comprises a polynucleotide hybridization site, or wherein the noncovalent template bonding site comprises a noncovalent peptide binding site and the noncovalent peptide binding site is selected from a coiled-coil bonding site and an avidin-biotin bonding site.

4. The nanoparticle of any preceding claim, wherein the plurality of accessory sites for bonding accessory oligonucleotides to the scaffold comprise covalent accessory oligonucleotide bonding sites, and optionally wherein the covalent accessory oligonucleotide bonding sites are selected from amine-NHS ester bonding sites, amine-imidoester bonding sites, amine-pentofluorophenyl ester bonding sites, amine-hydroxymethyl phosphine bonding sites, carboxyl-carbodiimide bonding sites, thiol-maleimide bonding sites, thiol-haloacetyl bonding sites, thiol-pyridyl disulfide bonding sites, thiol-thiosulfonate bonding sites, thiol-vinyl sulfone bonding sites, aldehyde-hydrazide bonding sites, aldehyde-alkoxyamine bonding sites, hydroxy-isocyanate bonding sites, azide-alkyne bonding sites, azide-phosphine bonding sites, transcyclooctene-tetrazine bonding sites, norbornene-tetrazine bonding sites, an azide-cyclooctyne bonding sites, azide-norbornene bonding sites, oxime bonding sites, SpyTag-SpyCatcher bonding sites, Snap-tag-O^6-Benzylguanine bonding sites, CLIP-tag-O^2-benzylcytosine bonding sites, sortase-coupling bonding sites, and any combination of two or more of the foregoing.

5. The nanoparticle of any one of claims 1 through 3, wherein the accessory oligonucleotide bonding sites comprise noncovalent accessory oligonucleotide bonding sites and optionally wherein the noncovalent accessory oligonucleotide bonding sites comprise polynucleotide hybridization sites, or wherein the noncovalent accessory oligonucleotide bonding sites comprise noncovalent peptide binding sites and the noncovalent peptide binding sites are selected from one or both of coiled-coil bonding sites and avidin-biotin bonding sites.

6. The nanoparticle of any one of claims 1 through 5, further comprising a single template polynucleotide bonded to the single template site, optionally further comprising a plurality of accessory oligonucleotides bonded to the plurality of accessory sites, optionally wherein the nanoparticle is at least about 10 nm in diameter.

7. A method, comprising bonding a single template polynucleotide to the single template site of the nanoparticle of any one of claims 1 through 6.

8. A method, comprising bonding a plurality of accessory oligonucleotides to the plurality of accessory sites of the nanoparticle of any one of claims 1 through 7.

9. The method of claim 7 or 8, further comprising synthesizing one or more scaffold-attached copies selected from copies of the template polynucleotide, copies of the polynucleotides complementary to the template polynucleotide, and copies of both, wherein the scaffold-attached copies extend from the accessory oligonucleotides, optionally further comprising attaching the scaffold to a substrate, wherein attaching comprises hybridizing accessory oligonucleotides with oligonucleotides attached to the substrate., and optionally wherein the substrate comprises a plurality of nanowells and the oligonucleotides attached to the substrate are attached within the plurality of nanowells, optionally, wherein no more than one scaffold binds within any one of the nanowells.

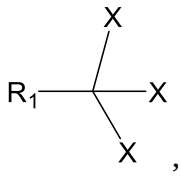
10. The method of claim 9 further comprising synthesizing one or more substrate-attached copies selected from copies of the template polynucleotide, copies of the polynucleotides complementary to the template polynucleotide, and copies of both, wherein the substrate-attached copies extend from oligonucleotides attached to a substrate.

11. The method of claim 9 or 10, further comprising sequencing at least one of scaffold-attached copies and substrate-attached copies, wherein sequencing comprises sequencing by synthesis.

12. A nanoparticle, comprising

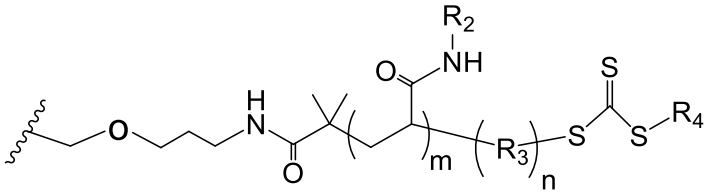
a scaffold, a single template site for bonding a template polynucleotide to the scaffold selected from a covalent template bonding site and a noncovalent template bonding site, and a plurality of accessory sites for bonding accessory oligonucleotides to the scaffold selected from covalent accessory oligonucleotide bonding sites and noncovalent accessory oligonucleotide bonding sites, wherein

the scaffold is a compound of Formula IV:



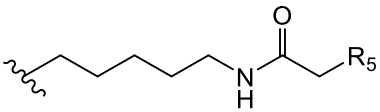
,

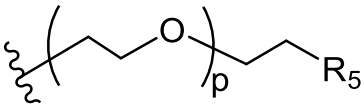
each X is a compound of formula V:

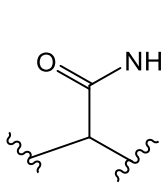


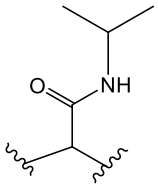
,

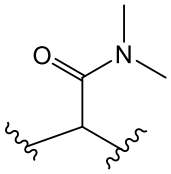
wherein R_2 is selected from Formula VIa:

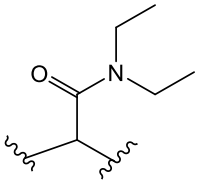


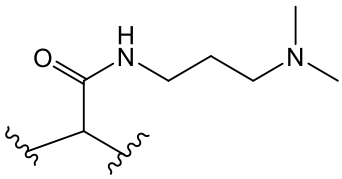
and Formula VIb: 

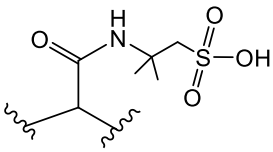
wherein p is an integer selected from 1 to 20, and R_5 comprises the accessory site for bonding accessory oligonucleotides, R_3 is selected from a direct bond, 

, 

, 

, 

, 

, and 

, m is an integer from 1 to 20,000 and n is an integer from 1 to 100,000,

R^1 comprises the single template site for bonding a template polynucleotide to the scaffold, R^4 is selected from an optionally substituted C_1-C_{20} alkyl, an optionally substituted C_1-C_{20} alkenyl, an optionally substituted C_1-C_{20} alkynyl, an optionally substituted C_1-C_{20} oxaalkyl, an optionally substituted C_1-C_{20} thiaalkyl, and an optionally substituted C_1-C_{20} azaalkyl, wherein substituted comprises substitution with one or more of a C_1-C_{20} alkyl, a double-bonded oxygen, and a hydroxyl group, and R^3 comprises the accessory site for bonding accessory oligonucleotides.

13. The nanoparticle of claim 12, wherein the single template site comprises a covalent template bonding site, and optionally wherein the covalent template bonding site is selected from an amine-NHS ester bonding site, an amine-imidoester bonding site, an amine-pentofluorophenyl ester bonding site, an amine-hydroxymethyl phosphine bonding site, a carboxyl-carbodiimide bonding site, a thiol-maleimide bonding site, a thiol-haloacetyl bonding site, a thiol-pyridyl disulfide bonding site, a thiol-thiosulfonate bonding site, a thiol-vinyl sulfone bonding site, an aldehyde-hydrazide bonding site, an aldehyde-alkoxyamine bonding site, a hydroxy-isocyanate bonding site, an azide-alkyne bonding site, an azide-phosphine bonding site, a transcyclooctene-tetrazine bonding site, a norbornene-

tetrazine bonding site, an azide-cyclooctyne bonding site, an azide-norbornene bonding site, an oxime bonding site, a SpyTag-SpyCatcher bonding site, a Snap-tag-O^6-Benzylguanine bonding site, a CLIP-tag-O^2-benzylcytosine bonding site, and a sortase-coupling bonding site.

14. The nanoparticle of claim 12, wherein the single template site comprises a noncovalent template bonding site, and optionally wherein the noncovalent template bonding site comprise a polynucleotide hybridization site, or wherein the noncovalent template bonding site comprises a noncovalent peptide binding site and the noncovalent peptide binding site is selected from one or both of a coiled-coil bonding site and an avidin-biotin bonding site.

15. The nanoparticle of any one of claims 12 through 14, wherein the plurality of accessory sites for bonding accessory oligonucleotides to the scaffold comprise covalent accessory oligonucleotide bonding sites, optionally wherein the covalent accessory oligonucleotide bonding sites are selected from amine-NHS ester bonding sites, amine-imidoester bonding sites, amine-pentofluorophenyl ester bonding sites, amine-hydroxymethyl phosphine bonding sites, carboxyl-carbodiimide bonding sites, thiol-maleimide bonding sites, thiol-haloacetyl bonding sites, thiol-pyridyl disulfide bonding sites, thiol-thiosulfonate bonding sites, thiol-vinyl sulfone bonding sites, aldehyde-hydrazide bonding sites, aldehyde-alkoxyamine bonding sites, hydroxy-isocyanate bonding sites, azide-alkyne bonding sites, azide-phosphine bonding sites, transcyclooctene-tetrazine bonding sites, norbornene-tetrazine bonding sites, an azide-cyclooctyne bonding sites, azide-norbornene bonding sites, oxime bonding sites, SpyTag-SpyCatcher bonding sites, Snap-tag-O^6-Benzylguanine bonding sites, CLIP-tag-O^2-benzylcytosine bonding sites, sortase-coupling bonding sites, and any combination of two or more of the foregoing.

16. The nanoparticle of any one of claims 12 through 14, wherein the accessory oligonucleotide bonding sites comprises noncovalent accessory oligonucleotide bonding sites, and optionally wherein the noncovalent accessory oligonucleotide bonding sites comprise polynucleotide hybridization sites, or wherein the noncovalent accessory oligonucleotide bonding sites comprise noncovalent peptide binding sites and the noncovalent peptide binding sites are selected from one or both of coiled-coil bonding sites and avidin-biotin bonding sites.

17. The nanoparticle of any one of claims 12 through 16, further comprising a single template polynucleotide bonded to the single template site, optionally

further comprising a plurality of accessory oligonucleotides bonded to the plurality of accessory sites, optionally wherein the nanoparticle is at least about 10 nm in diameter.

18. A method, comprising bonding a single template polynucleotide to the single template site of the nanoparticle of any one of claims 12 through 17.

19. A method, comprising bonding a plurality of accessory oligonucleotides to the plurality of accessory sites of the nanoparticle of any one of claims 12 through 18.

20. The method of claim 18 or 19, further comprising synthesizing one or more scaffold-attached copies selected from copies of the template polynucleotide, copies of the polynucleotides complementary to the template polynucleotide, and copies of both, wherein the scaffold-attached copies extend from the accessory oligonucleotides.

21. The method of any one of claims 18-20, further comprising attaching the scaffold to a substrate, wherein attaching comprises hybridizing accessory oligonucleotides with oligonucleotides attached to the substrate.

22. The method of claim 21, wherein the substrate comprises a plurality of nanowells and the oligonucleotides attached to the substrate are attached within the plurality of nanowells, and optionally wherein no more than one scaffold binds within any one of the nanowells.

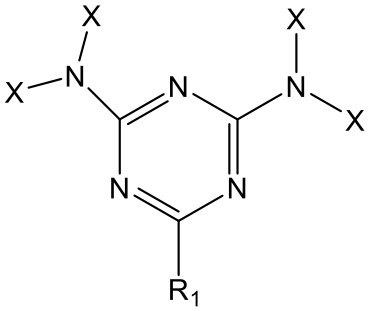
23. The method of claim 21 or claim 22 further comprising synthesizing one or more substrate-attached copies selected from copies of the template polynucleotide, copies of the polynucleotides complementary to the template polynucleotide, and copies of both, wherein the substrate-attached copies extend from oligonucleotides attached to a substrate.

24. The method of claim 20 or 23, further comprising sequencing at least one of scaffold-attached copies and substrate-attached copies, wherein sequencing comprises sequencing by synthesis.

25. A nanoparticle, comprising

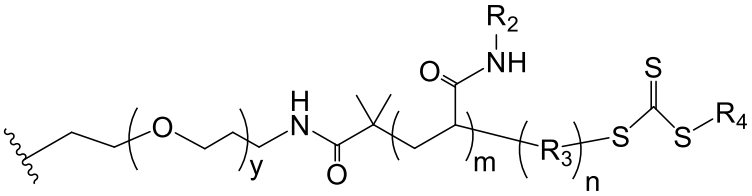
a scaffold, a single template site for bonding a template polynucleotide to the scaffold selected from a covalent template bonding site and a noncovalent template bonding site, and a plurality of accessory sites for bonding accessory oligonucleotides to the scaffold selected from covalent accessory oligonucleotide bonding sites and noncovalent accessory oligonucleotide bonding sites, wherein

the scaffold is a compound of Formula VII:



,

each X is a compound of formula VIII:



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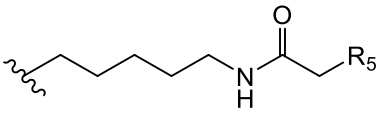
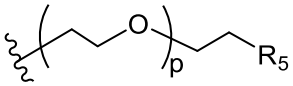
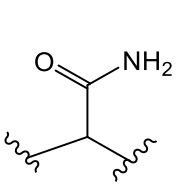
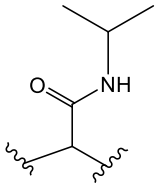
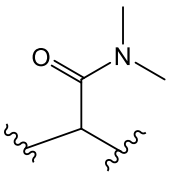
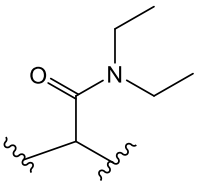
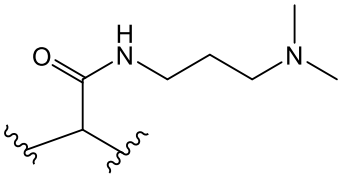
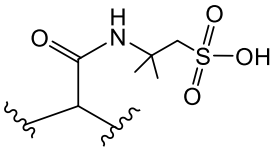
wherein y is an integer from 1 to 20, R_2 is selected from Formula IXa:



and Formula IXb:  wherein p is an integer selected from 1 to 20, and R_5 comprises the accessory site for bonding accessory oligonucleotides, R_3 is selected from a direct bond,  ,  ,  ,  ,  , and  , m is an integer from 1 to 2,000 and n is an integer from 1 to 10,000,

R^1 comprises the single template site for bonding a template polynucleotide to the scaffold, R^4 is selected from an optionally substituted C_1-C_{20} alkyl, an optionally substituted C_1-C_{20} alkenyl, an optionally substituted C_1-C_{20} alkynyl, an optionally substituted C_1-C_{20} oxaalkyl, an optionally substituted C_1-C_{20} thiaalkyl, and an optionally substituted C_1-C_{20} azaalkyl, wherein substituted comprises substitution with one or more of a C_1-C_{20} alkyl, a double-bonded oxygen, and a hydroxyl group, and R^3 comprises the accessory site for bonding accessory oligonucleotides.

26. The nanoparticle of claim 25, wherein the single template site comprises a covalent template bonding site, optionally wherein the covalent template bonding

site is selected from an amine-NHS ester bonding site, an amine-imidoester bonding site, an amine-pentofluorophenyl ester bonding site, an amine-hydroxymethyl phosphine bonding site, a carboxyl-carbodiimide bonding site, a thiol-maleimide bonding site, a thiol-haloacetyl bonding site, a thiol-pyridyl disulfide bonding site, a thiol-thiosulfonate bonding site, a thiol-vinyl sulfone bonding site, an aldehyde-hydrazide bonding site, an aldehyde-alkoxyamine bonding site, a hydroxy-isocyanate bonding site, an azide-alkyne bonding site, an azide-phosphine bonding site, a transcyclooctene-tetrazine bonding site, a norbornene-tetrazine bonding site, an azide-cyclooctyne bonding site, an azide-norbornene bonding site, an oxime bonding site, a SpyTag-SpyCatcher bonding site, a Snap-tag-O^6-Benzylguanine bonding site, a CLIP-tag-O^2-benzylcytosine bonding site, and a sortase-coupling bonding site.

27. The nanoparticle of any one of claim 25, wherein the single template site comprises a noncovalent template bonding site, and optionally wherein the noncovalent template bonding site comprises a polynucleotide hybridization site or wherein the noncovalent template bonding site comprises a noncovalent peptide binding site and the noncovalent peptide binding site is selected from a coiled-coil bonding site and an avidin-biotin bonding site.

28. The nanoparticle of any one of claims 25 through 27, wherein the plurality of accessory sites for bonding accessory oligonucleotides to the scaffold comprise covalent accessory oligonucleotide bonding sites optionally wherein the covalent accessory oligonucleotide bonding sites are selected from amine-NHS ester bonding sites, amine-imidoester bonding sites, amine-pentofluorophenyl ester bonding sites, amine-hydroxymethyl phosphine bonding sites, carboxyl-carbodiimide bonding sites, thiol-maleimide bonding sites, thiol-haloacetyl bonding sites, thiol-pyridyl disulfide bonding sites, thiol-thiosulfonate bonding sites, thiol-vinyl sulfone bonding sites, aldehyde-hydrazide bonding sites, aldehyde-alkoxyamine bonding sites, hydroxy-isocyanate bonding sites, azide-alkyne bonding sites, azide-phosphine bonding sites, transcyclooctene-tetrazine bonding sites, norbornene-tetrazine bonding sites, an azide-cyclooctyne bonding sites, azide-norbornene bonding sites, oxime bonding sites, SpyTag-SpyCatcher bonding sites, Snap-tag-O^6-Benzylguanine bonding sites, CLIP-tag-O^2-benzylcytosine bonding sites, sortase-coupling bonding sites, and any combination of two or more of the foregoing.

29. The nanoparticle of any one of claims 25 through 27, wherein the accessory oligonucleotide bonding sites comprises noncovalent accessory oligonucleotide bonding sites.

30. The nanoparticle of claim 29, wherein the noncovalent accessory oligonucleotide bonding sites comprise polynucleotide hybridization sites or wherein the noncovalent accessory oligonucleotide bonding sites comprise noncovalent peptide binding sites and the noncovalent peptide binding sites are selected from one or both of coiled-coil bonding sites and avidin-biotin bonding sites.

31. The nanoparticle of any one of claims 25 through 30, further comprising a single template polynucleotide bonded to the single template site optionally further comprising a plurality of accessory oligonucleotides bonded to the plurality of accessory sites and optionally wherein the nanoparticle is at least about 10 nm in diameter.

32. A method, comprising bonding a single template polynucleotide to the single template site of the nanoparticle of any one of claims 25 through 31.

33. A method, comprising bonding a plurality of accessory oligonucleotides to the plurality of accessory sites of the nanoparticle of any one of claims 25 through 32.

34. The method of claim 32 or 33, further comprising synthesizing one or more scaffold-attached copies selected from copies of the template polynucleotide, copies of the polynucleotides complementary to the template polynucleotide, and copies of both, wherein the scaffold-attached copies extend from the accessory oligonucleotides.

35. The method of any one of claims 32-34, further comprising attaching the scaffold to a substrate, wherein attaching comprises hybridizing accessory oligonucleotides with oligonucleotides attached to the substrate.

36. The method of claim 35, wherein the substrate comprises a plurality of nanowells and the oligonucleotides attached to the substrate are attached within the plurality of nanowells, optionally wherein no more than one scaffold binds within any one of the nanowells.

37. The method of claim 35 or 36 further comprising synthesizing one or more substrate-attached copies selected from copies of the template polynucleotide, copies of the polynucleotides complementary to the template polynucleotide, and copies of both, wherein the substrate-attached copies extend from oligonucleotides attached to a substrate.

38. The method of claim 34 or 37, further comprising sequencing at least one of scaffold-attached copies and substrate-attached copies, wherein sequencing comprises sequencing by synthesis.

39. A nanoparticle, comprising

a scaffold, a single template site for bonding a template polynucleotide to the scaffold selected from a covalent template bonding site and a noncovalent template bonding site, and a plurality of accessory sites for bonding accessory oligonucleotides to the scaffold selected from covalent accessory oligonucleotide bonding sites and noncovalent accessory oligonucleotide bonding sites, wherein

the scaffold comprises a dendrimer wherein the dendrimer comprises from 2 to 10 generations of constitutional repeating units, the constitutional repeating units comprise lysine wherein a lysine of an upstream generation forms a peptide bond with a first lysine of an immediately downstream generation and an isopeptide bond with a second lysine of the immediately downstream generation,

the single template site extends from the C-terminal end of the lysine of the first generation of the dendrimer and the plurality of accessory sites extend from NH_2 groups of lysines of the last generation of the dendrimer.

40. The nanoparticle of claim 39, wherein the single template site comprises a covalent template bonding site, optionally wherein the covalent template bonding site is selected from an amine-NHS ester bonding site, an amine-imidoester bonding site, an amine-pentofluorophenyl ester bonding site, an amine-hydroxymethyl phosphine bonding site, a carboxyl-carbodiimide bonding site, a thiol-maleimide bonding site, a thiol-haloacetyl bonding site, a thiol-pyridyl disulfide bonding site, a thiol-thiosulfonate bonding site, a thiol-vinyl sulfone bonding site, an aldehyde-hydrazide bonding site, an aldehyde-alkoxyamine bonding site, a hydroxy-isocyanate bonding site, an azide-alkyne bonding site, an azide-phosphine bonding site, a transcyclooctene-tetrazine bonding site, a norbornene-tetrazine bonding site, an azide-cyclooctyne bonding site, an azide-norbornene bonding site, an oxime bonding site, a SpyTag-SpyCatcher bonding site, a Snap-tag-O^6-Benzylguanine bonding site, a CLIP-tag-O^2-benzylcytosine bonding site, and a sortase-coupling bonding site.

41. A method, comprising bonding a single template polynucleotide to the single template site of the nanoparticle of any one of claims 39 through 40.

42. A method, comprising bonding a plurality of accessory oligonucleotides to the plurality of accessory sites of the nanoparticle of any one of claims 39 through 41.