

CLAIMS

1. A nanoparticle, comprising:
 - i. a scaffold comprising a DNA dendrimer, wherein the DNA dendrimer comprises from 2 to 100 generations of bifurcating constitutional repeating units,
 - ii. a single template site for bonding a template polynucleotide to the scaffold; and
 - iii. a plurality of accessory sites for bonding accessory oligonucleotides to the scaffold,wherein the plurality of accessory sites comprise a different chemistry or structure from the single template site, such that the plurality of accessory bonding sites are incapable of binding or attaching to the template polynucleotide,wherein the single template site for bonding a template polynucleotide to the scaffold is selected from a covalent template bonding site and a noncovalent template bonding site,wherein the plurality of accessory sites for bonding accessory oligonucleotides to the scaffold is selected from covalent accessory oligonucleotide bonding sites and noncovalent accessory oligonucleotide bonding sites,wherein the scaffold is configured to attach one template polynucleotide to the single template site and prevent attachment of a second template polynucleotide to the same nanoparticle.
2. The nanoparticle of claim 1, wherein the bifurcating constitutional repeating units each comprise three constitutional repeating unit oligodeoxyribonucleotides hybridized to each other to form an adapter comprising one upstream overhang and two downstream overhangs, wherein the upstream overhang of each adapter in generation 2 and higher is complementary to a downstream overhang of an immediately upstream constitutional repeating unit, and the downstream overhang of the adapter in generation 1 comprises the single template site.
3. The nanoparticle of claim 1, wherein the scaffold comprises a single-stranded DNA.
4. The nanoparticle of claim 1, wherein the scaffold comprises one or more scaffold polypeptides.

5. The nanoparticle of claim 4, wherein the scaffold polypeptide comprises a green fluorescent protein.
6. The nanoparticle of any one of claims 1 through 5, wherein the single template site comprises a covalent template bonding site.
7. The nanoparticle of claim 6, 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-O⁶-Benzylguanine bonding site, a CLIP-tag-O²-benzylcytosine bonding site, and a sortase-coupling bonding site.
8. The nanoparticle of any one of claims 1 through 5, wherein the single template site comprises a noncovalent template bonding site.
9. The nanoparticle of claim 8, wherein the noncovalent template bonding site comprises a polynucleotide hybridization site.
10. The nanoparticle of claim 8, wherein the noncovalent template bonding site is selected from 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.
11. The nanoparticle of any one of claims 1 through 10, wherein the plurality of accessory sites for bonding accessory oligonucleotides to the scaffold comprise covalent accessory oligonucleotide bonding sites.

12. The nanoparticle of claim 11, 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-O⁶-Benzylguanine bonding sites, CLIP-tag-O²-benzylcytosine bonding sites, sortase-coupling bonding sites, and any combination of two or more of the foregoing.
13. The nanoparticle of any one of claims 1 through 10, wherein the accessory oligonucleotide bonding sites comprise noncovalent accessory oligonucleotide bonding sites.
14. The nanoparticle of claim 13, wherein the noncovalent accessory oligonucleotide bonding sites comprise polynucleotide hybridization sites.
15. The nanoparticle of claim 13, 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.
16. The nanoparticle of any one of claims 1 through 15, further comprising a single template polynucleotide bonded to the single template site.
17. The nanoparticle of any one of claims 1 through 16, further comprising a plurality of accessory oligonucleotides bonded to the plurality of accessory sites.
18. The nanoparticle of any one of claims 1 through 17, wherein the nanoparticle is at least about 10 nm in diameter.
19. A method, comprising:

- a. bonding a single template polynucleotide to the single template site of the nanoparticle of any one of claims 1 through 18;
- b. bonding a plurality of accessory oligonucleotides to the plurality of accessory sites of the nanoparticle; and
- c. 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.

20. The method of claim 19, further comprising attaching the scaffold to a substrate, wherein attaching comprises hybridizing accessory oligonucleotides with oligonucleotides attached to the substrate.

21. The method of claim 20, wherein the substrate comprises a plurality of nanowells and the oligonucleotides attached to the substrate are attached within the plurality of nanowells.

22. The method of claim 21, wherein no more than one scaffold binds within any one of the nanowells.

23. The method of any one of claims 20 through 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 19 or 23, further comprising sequencing at least one of scaffold-attached copies and substrate-attached copies, wherein sequencing comprises sequencing by synthesis.