

1. WO2012125987 - DELIVERY SYSTEM

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[EN]

We claim:

1. A delivery system comprising:

a nanoparticle comprised of a plurality of DNA oligonucleotides, each of which has a structure comprising:

a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form the nanoparticle, which is a three-dimensional tetrahedral structure comprising a plurality of faces defined by substantially double stranded arms,

wherein at least some oligonucleotides within the plurality have a structure that further comprises:

a second portion that does not hybridize with another oligonucleotide in the plurality, so that such second portions comprises a ligand hybridization element, available for hybridization to a ligand associated with a payload moiety.

2. The delivery system of claim 1, wherein the ligand hybridization element comprises a single stranded overhang.

3. The delivery system of claim 1, wherein the ligand hybridization element comprises a single stranded 5' overhang.

4. The delivery system of claim 3, wherein the nanoparticle comprises at least six ligand hybridization elements.

5. The delivery system of claim 1, wherein the delivery system further comprises at least six siRNA payload moieties.

6. The delivery system of claim 5, wherein the siRNA payload moieties each have a 3' overhang.



7. The delivery system of claim 6, wherein at least one siRNA payload moiety is hybridized to the nanoparticle by way of its 3' overhang.

8. The delivery system of claim 7, wherein the at least one siRNA payload moiety is hybridized to the nanoparticle by way of hybridization between its 3' overhang and one of the nanoparticle's ligand hybridization elements, so that the 3' overhang is the ligand hybridization element's complementary ligand.

9. The delivery system of claim 8, wherein the ligand and ligand hybridization element each have a sequence selected from the group consisting of poly A and polyT.

10. The delivery system of claim 9, further comprising at least three targeting payloads, wherein the oligonucleotides within the plurality being designed and constructed such that, when the at least three targeting payload moieties hybridize to the ligand hybridization elements, the at least three targeting payload moieties are displayed on the same face of the nanoparticle.

11. A delivery system comprising:

a nanoparticle comprised of a plurality of oligonucleotides, each of which has a structure comprising:

a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form the nanoparticle, which has a three-dimensional structure comprising a plurality of faces defined by substantially double stranded arms,

wherein at least some oligonucleotides within the plurality have a structure that further comprises:

a second portion that does not hybridize with another oligonucleotide in the plurality, so that such second portions are ligand hybridization elements, available for hybridization to complementary ligands,

oligonucleotides within the plurality being designed and constructed such that the nanoparticle includes at least three ligand hybridization elements.

12. The delivery system of claim 1, wherein the oligonucleotides within the plurality are designed and constructed such that the nanoparticle includes at least three ligand

hybridization elements arranged relative to one another in the nanoparticle such that, when ligands associated with payload moieties hybridize to the ligand hybridization elements, a

13. A delivery system comprising:

a nanoparticle comprised of a plurality of oligonucleotides, each of which has a structure comprising:

a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form the nanoparticle, which has a three-dimensional structure comprising a plurality of faces defined by substantially double stranded arms,

wherein at least some oligonucleotides within the plurality have a structure that further comprises:

a second portion that does not hybridize with another oligonucleotide in the plurality, so that such second portions are single stranded ligand hybridization elements, available for hybridization to complementary ligands, which ligand hybridization elements have a sequence comprising polyT or polyA.



14. A delivery system comprising:

a nanoparticle comprised of a plurality of oligonucleotides, each of which has a structure comprising:

a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form the nanoparticle, which has a three-dimensional structure comprising a plurality of faces defined by substantially double stranded arms,

wherein at least some oligonucleotides within the plurality have a structure that further comprises:

a second portion that does not hybridize with another oligonucleotide in the plurality, so that such second portions are single stranded ligand hybridization elements, available for hybridization to complementary ligands,

oligonucleotides within the plurality being designed and constructed such that at least one ligand hybridization element is or comprises a single stranded sequence with a 5' end.

15. A delivery system comprising:

a nanoparticle comprised of a plurality of oligonucleotides, each of which has a structure comprising:

a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form the nanoparticle, which has a three-dimensional structure comprising a plurality of faces defined by substantially double stranded arms,

wherein at least some oligonucleotides within the plurality have a structure that further comprises:

a second portion that does not hybridize with another oligonucleotide in the plurality, so that such second portions are single stranded ligand hybridization elements, available for hybridization to complementary ligands,

oligonucleotides within the plurality being designed and constructed such that the nanoparticle includes at least three ligand hybridization elements arranged relative to one another in the nanoparticle such that, when ligands associated with payload moieties hybridize to the ligand hybridization elements, a plurality of ligands is displayed on the same face of the nanoparticle, each of which ligand hybridization elements is or comprises a single stranded sequence with a 5' end.

16. The delivery system of any one of claims 11-15, wherein the nanoparticle contains a plurality of different ligand hybridization sites.

17. The delivery system of any one of claims 11-15, wherein the different ligand

hybridization sites differ from one another in length or sequence.

18. The delivery system of any one of claims 11-15, wherein oligonucleotides in the plurality are DNA.

19. The delivery system of any one of claims 11-15, wherein oligonucleotides in the plurality are RNA.

20. The delivery system of any one of claims 11-15, wherein some oligonucleotides in the plurality are DNA and some oligonucleotides in the plurality are RNA.



21. The delivery system of any one of claims 11-15, wherein at least one oligonucleotide in the plurality comprises at least one non-natural residue.
22. The delivery system of any one of claims 11-15, further comprising at least one hybridized entity comprising:
a ligand oligonucleotide whose sequence includes a ligand sequence substantially complementary to at least one ligand hybridization element in the nanoparticle; associated with a payload moiety.
23. The delivery system of claim 22, wherein the payload moiety is covalently attached to the ligand oligonucleotide.
24. The delivery system of claim 23, wherein the payload moiety is releasably attached to the ligand oligonucleotide.
25. The delivery system of claim 24, wherein the hybridized entity comprises a cleavage site.
26. The delivery system of claim 22, wherein the moiety is attached to the ligand oligonucleotide by hybridization.
27. The delivery system of claim 22, comprising at least 1, 2, 3, 4, 5, 6 payload moieties, or more.
28. The delivery system of claim 22, wherein the payload moiety is or comprises an oligonucleotide.
29. The delivery system of claim 28, wherein the payload moiety is selected from the group consisting of an siRNA, shRNA, miRNA, ribozyme and combinations thereof.
30. The delivery system of claim 22, wherein the payload moiety is selected from the group consisting of therapeutic payloads, detectable payloads, targeting payloads and delivery payload.
31. The delivery system of any one of claims 11-15, wherein at least one ligand hybridization element has a length greater than at least 10 base pairs.
32. The delivery system of any one of claims 11-15, wherein at least one ligand hybridization element has a length greater than at least 15 base pairs.
33. The delivery system of any one of claims 11-15, wherein at least one ligand hybridization element has a length greater than at least 20 base pairs.
34. The delivery system of any one of claims 11-15, wherein at least one ligand hybridization element has a length greater than at least 25 base pairs.
35. The delivery system of any one of claims 11-15, wherein at least one ligand



hybridization element includes at least one non-natural nucleotide residue.

36. The delivery system of any one of claims 11-12 or 14-15, wherein at least one ligand hybridization element has a nucleotide sequence comprising a plurality of purine residues.

37. The delivery system of claim 36, wherein at least one ligand hybridization element has a nucleotide sequence consisting of a plurality of purine residues.

38. The delivery system of claim 36, wherein at least one ligand hybridization element has a nucleotide sequence selected from the group consisting of polyA and polyT.

39. The delivery system of any one of claims 11-15, wherein oligonucleotides in the plurality are selected to form a nanoparticle in the shape of a regular polyhedron.

40. The delivery system of any one of claims 11-15, wherein oligonucleotides in the plurality are selected to form a nanoparticle in the shape of an irregular polyhedron.

41. A method for generating a delivery system comprising the step of:

(a) providing a plurality of oligonucleotides, each of which has a structure comprising a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form a three-dimensional structure whose arms are substantially double stranded, wherein at least some oligonucleotides within the plurality have a structure that further comprises a second portion (b) combining said plurality of oligonucleotides in a reaction buffer to form a reaction mixture;

(c) maintaining the combination for a time and under conditions sufficient to permit self-assembly of the nanoparticles.

42. The method of claim 41, wherein the step of maintaining further comprises:

subjecting the reaction mixture of step (b) to a heat treatment comprising: i. a heating step to denature the plurality of oligonucleotides and; and ii. a cooling step to anneal the plurality of oligonucleotides to promote self-assembly of said three-dimensional structure.

43. The method of any one of claims 41 or 42, wherein the plurality of oligonucleotides contains a plurality of different ligand hybridization sites.

44. The method of any one of claims 41 or 42, wherein the different ligand hybridization sites differ from one another in length or sequence.

45. The method of any one of claims 41 or 42, wherein oligonucleotides in the plurality are DNA.

46. The method of any one of claims 41 or 42, wherein oligonucleotides in the plurality are RNA.

47. The method of any one of claims 41 or 42, wherein some oligonucleotides in the plurality are DNA and some oligonucleotides in the plurality are RNA.

48. A kit for forming a self-assembling nanoparticle delivery system comprising:

a plurality of oligonucleotides, each of which has a structure comprising: a first portion that is complementary to a first portion of another oligonucleotide in the plurality such that the plurality of oligonucleotides self-assembles to form the nanoparticle, which has a three-dimensional structure comprising a



plurality of faces defined by substantially double stranded arms,

wherein at least some oligonucleotides within the plurality have a structure that further a second portion that does not hybridize with another oligonucleotide in the plurality, so that such second portions are single stranded ligand hybridization elements, available for hybridization to complementary ligands,

oligonucleotides within the plurality being designed and constructed such that the nanoparticle includes at least three ligand hybridization elements arranged relative to one another in the nanoparticle such that, when ligands associated with payload moieties hybridize to the ligand hybridization elements, a plurality of ligands is displayed on the same face of the nanoparticle, each of which ligand hybridization elements is or comprises a single stranded sequence with a 5' end; and

a buffer.

49. The kit of claim 48, wherein the plurality of oligonucleotides contains a plurality of different ligand hybridization sites.

50. The kit of claim 48, wherein the different ligand hybridization sites differ from one another in length or sequence.

51. The kit of claim 48, wherein oligonucleotides in the plurality are DNA.

52. The kit of claim 48, wherein oligonucleotides in the plurality are RNA.

53. The kit of claim 48, wherein some oligonucleotides in the plurality are DNA and some oligonucleotides in the plurality are RNA.

54. The kit of claim 48, wherein at least one oligonucleotide in the plurality comprises at least one non-natural residue.

