

WHAT IS CLAIMED IS:

1. A conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising a modified CH3 constant domain that specifically binds to a transferrin receptor (TfR) with an affinity of about 3nM to about 600nM;

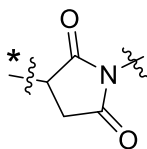
each y is independently at least 1; and

n is at least 1.

2. The conjugate of claim 1, wherein the engineered binding site binds TfR with an affinity of about 500nM to about 3nM, about 400nM to about 20nM, about 300nM to about 30nM, about 200nM to about 40nM, about 150nM to about 50nM, or about 130nM to about 80nM.
3. The conjugate of claim 1 or 2, wherein P comprises one or more modified sites that facilitate the attachment of P to each L.
4. The conjugate of any one of claims 1-3, wherein P comprises an Fc polypeptide dimer, comprising a first Fc polypeptide comprising the modified constant domain; and a second Fc polypeptide dimerized to the first Fc polypeptide.
5. The conjugate of claim 3 or 4, wherein the modified site is an amino acid substitution.
6. The conjugate of claim 5, wherein the modified site is a cysteine substitution.
7. The conjugate of claim 6, wherein the cysteine substitution is selected from the group consisting of: S239C, S442C, A330C, K149C, and T289C, according to EU numbering and A114C according to Kabat numbering.

8. The conjugate of any one of claims 1-7, wherein the oligonucleotide is an antisense oligonucleotide (ASO) or a siRNA.

9. The conjugate of any one of claims 1-8, wherein the linking group L comprises a moiety having the structure:



wherein, * denotes the attachment point to a sulfur atom of a modified site within P.

10. The conjugate of any one of claims 1-9, wherein y is an integer from 1 to 4, optionally wherein y is 1.

11. The conjugate of any one of claims 1-10, wherein n is an integer from 1 to 6, optionally wherein n is 1.

12. An Fc polypeptide dimer, or a Fab-Fc dimer fusion thereof, comprising:

(a) a first Fc polypeptide comprising a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM; and

(b) a second Fc polypeptide dimerized to the first Fc polypeptide of (a);

wherein the Fab-Fc dimer fusion further comprises a first Fab and a second Fab; and,

wherein the Fc polypeptide dimer, or a Fab-Fc dimer fusion comprises one or more cysteine substitutions.

13. The Fc polypeptide dimer of claim 12, wherein the Fab-Fc dimer fusion is a non-targeting Fab-Fc dimer fusion.

14. The Fc polypeptide dimer of claim 12 or 13, wherein the cysteine substitution(s) is selected from the group consisting of: S239C, S442C, A330C and T289C according to EU numbering, and A114C according to Kabat numbering.

15. The Fc polypeptide dimer of any one of claims 12-14, wherein the first and second Fc polypeptides each comprise a cysteine substitution; or wherein the first and second Fab-Fc fusions each comprise a cysteine substitution.
16. The Fc polypeptide dimer of claim 15, wherein
- (a) the first or second Fc polypeptides comprises a cysteine substitution at S239C;
 - (b) the first and second Fab-Fc fusions each comprise a cysteine substitution at S239C;
 - (c) the first and second Fc polypeptides each comprise two cysteine substitutions;
 - (d) the first and second Fab-Fc fusions each comprise two cysteine substitutions;
 - (e) the first and second Fc polypeptides each comprise a cysteine substitution at S239C and A330C; or
 - (f) the first and second Fab-Fc fusions each comprise a cysteine substitution at S239C and A330C.
17. The Fc polypeptide dimer of any one of claims 12-14, wherein the second Fc polypeptide comprises a cysteine substitution; or wherein the second Fab-Fc fusion comprises a cysteine substitution, optionally wherein the cysteine substitution is S239C.
18. An Fc polypeptide dimer comprising:
- (a) a first Fc polypeptide comprising a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM; and
 - (b) a second Fc polypeptide dimerized to the first Fc polypeptide of (a);
- wherein the first and/or the second Fc polypeptide comprises one or more substitutions selected from the group consisting of N297A and N297G.
19. The Fc polypeptide dimer of claim 18, wherein the first and the second Fc polypeptides are each joined to a non-targeting Fab fragment or a portion thereof, to produce a Fab-Fc dimer fusion.

20. The Fc polypeptide dimer of any one of claims 1-19, wherein the modified CH3 constant domain is a modified CH3 domain comprising two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen or sixteen positions selected from the following: position 153 is Trp, Tyr, Leu, Gln, or Glu; position 157 is Leu, Tyr, Met, Val, Phe or Trp; position 159 is Leu, Thr, His, Pro or Phe; position 160 is Val, Pro, or an acidic amino acid; position 161 is Trp ; position 162 is Val, Ser, Ala or Gly; position 163 is Asn, Gly, His, Gln, Leu, Lys, Val, Phe, Ser, Ala, Asp, Thr or Glu; position 164 is Ser, Thr, Gln, Phe, Tyr or Val; position 165 is Gln, Phe, or His; position 186 is Glu, Ala, Ser, Leu, Thr, Pro or Asp; position 187 is Lys, Arg, Gly, or Pro; position 188 is Glu or Ser; position 189 is Thr, Asn or an acidic amino acid; position 194 is Trp, Tyr, His, or Phe; position 197 is Ser, Thr, Glu, Lys or Trp; and position 199 is Ser, Trp, Gly, Cys, Pro or Met, as numbered with reference to SEQ ID NO:1.

21. The Fc polypeptide dimer of any one of claims 12-20,
wherein the first Fc polypeptide or Fab-Fc dimer fusion comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to the sequence of any one of SEQ ID NOS:279, 281, 361-366, 491-494, 702-718, 632, 645-649, 738-746, 804, and
wherein the second Fc polypeptide or Fab-Fc dimer fusion comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to the sequence of any one of SEQ ID NOS: 557-561, 627-631, 635-644, 719-723, 724-731, 733-737, and 810.

22. The Fc polypeptide dimer of claim 21, wherein the first Fc polypeptide or Fab-Fc dimer fusion comprises a Glu at position 153, Tyr at position 157, Thr at position 159, Glu at position 160, Trp at position 161, Ser, or Ala at position 162, Asn at position 163, Thr or Ser at position 186, Glu at position 188, Glu at position 189, and Phe at position 194, as numbered with reference to SEQ ID NO:1.

23. The Fc polypeptide dimer of claim 22, wherein:

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:362 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:559;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:702 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:559;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:708 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:722;

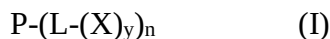
the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:718 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:559;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:362 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:560;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:362 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:561; or

the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:804 and the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:810.

24. A conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising 1) a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM; and 2) one

or more modified sites, wherein the one or more modified sites are amino acid substitutions that facilitate the attachment of P to each L;

each y is independently at least 1; and

n is at least 1.

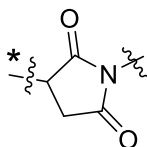
25. The conjugate of claim 24, wherein P comprises an Fc polypeptide comprising the modified constant domain.

26. The conjugate of claim 25, wherein the protein comprises an Fc polypeptide dimer, comprising a first Fc polypeptide comprising the modified constant domain; and a second Fc polypeptide dimerized to the first Fc polypeptide.

27. The conjugate of claim 25 or 26, wherein the Fc polypeptide is joined to a non-targeting Fab fragment or a portion thereof, to produce a Fab-Fc dimer fusion; or the first and second Fc polypeptides of the Fc polypeptide dimer are each joined to a non-targeting Fab fragment or a portion thereof, to produce a Fab-Fc dimer fusion.

28. The conjugate of any one of claims 24-27, wherein the modified site is a cysteine substitution, optionally wherein the cysteine substitution is selected from the group consisting of: S239C, S442C, A330C, K149C, and T289C, according to EU numbering and A114C according to Kabat numbering.

29. The conjugate of any one of claims 24-28, wherein the linking group L comprises a moiety having the structure:



wherein, * denotes the attachment point to a sulfur atom of a modified site within P.

30. The conjugate of claim 29, wherein the modified site is an alanine or glycine substitution, optionally wherein the alanine or glycine substitution is N297A or N297G, according to EU numbering.

31. The conjugate of any one of claims 24-27 and 30, wherein L is conjugated to P at Q295, optionally wherein L is conjugate to P by enzymatic conjugation, optionally wherein the enzymatic conjugation uses a bacterial transglutaminase (BTG).

32. The conjugate of any one of claims 24-31, wherein the modified constant domain is a modified CH3 domain comprising two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen or sixteen positions selected from the following: position 153 is Trp, Tyr, Leu, Gln, or Glu; position 157 is Leu, Tyr, Met, Val, Phe or Trp; position 159 is Leu, Thr, His, Pro or Phe; position 160 is Val, Pro, or an acidic amino acid; position 161 is Trp ; position 162 is Val, Ser, Ala or Gly; position 163 is Asn, Gly, His, Gln, Leu, Lys, Val, Phe, Ser, Ala, Asp, Thr or Glu; position 164 is Ser, Thr, Gln, Phe, Tyr or Val; position 165 is Gln, Phe, or His; position 186 is Glu, Ala, Ser, Leu, Thr, Pro or Asp; position 187 is Lys, Arg, Gly, or Pro; position 188 is Glu or Ser; position 189 is Thr, Asn or an acidic amino acid; position 194 is Trp, Tyr, His, or Phe; position 197 is Ser, Thr, Glu, Lys or Trp; and position 199 is Ser, Trp, Gly, Cys, Pro or Met, as numbered with reference to SEQ ID NO:1.

33. The conjugate of any one of claims 24-32,
wherein the first Fc polypeptide or Fab-Fc dimer fusion comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to the sequence of any one of SEQ ID NOS:279, 281, 361-366, 491-494, 702-718, 632, 645-649, 738-746, 804; and
wherein the second Fc polypeptide or Fab-Fc dimer fusion comprises a sequence having at least 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to the sequence of any one of SEQ ID NOS: 557-561, 627-631, 635-644, 719-723, 724-731, 733-737, and 810.

34. The conjugate of claim 33, wherein the first Fc polypeptide or Fab-Fc dimer fusion comprises a Glu at position 153, Tyr at position 157, Thr at position 159, Glu at position 160,

Trp at position 161, Ser, or Ala at position 162, Asn at position 163, Thr or Ser at position 186, Glu at position 188, Glu at position 189, and Phe at position 194, as numbered with reference to SEQ ID NO:1.

35. The conjugate of claim 33, wherein:

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:362 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:559;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:702 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:559;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:708 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:722;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:718 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:559;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:362 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:560;

the first Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:362 and the second Fc polypeptide or Fab-Fc dimer fusion comprises the amino acid sequence of SEQ ID NO:561; or

the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:804 and the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:810.

36. The conjugate of any one of claims 24-35, wherein the oligonucleotide is an antisense oligonucleotide (ASO) or a siRNA, wherein the oligonucleotide is an ASO, optionally wherein the ASO is a gapmer having a modification pattern of formula 5'-X_a-Y_a-Z_a-3', wherein X_a and Z_a

each comprise 3 LNA modified nucleotides, and wherein the gap region Y_a comprises PS linkages.

37. The conjugate of claim 36, wherein the oligonucleotide is an ASO, and

- (a) each L is linked to the 5' end of the ASO; or
- (b) each L is linked to the 3' end of the ASO.

38. The conjugate of any one of claims 24-37, wherein L comprises a C_6 amine group having the formula $-(CH_2)_6-NH-$.

39. The conjugate of any one of claims 24-38, wherein P binds TfR with an affinity of about 500nM to about 10nM; about 400nM to about 20nM, about 300nM to about 30nM, about 200nM to about 40nM, about 150nM to about 50nM, or about 100nM.

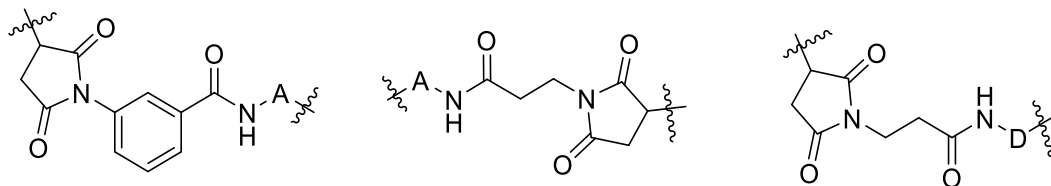
40. The conjugate of any one of claims 24-39, wherein y is an integer from 1 to 4, optionally wherein y is 1.

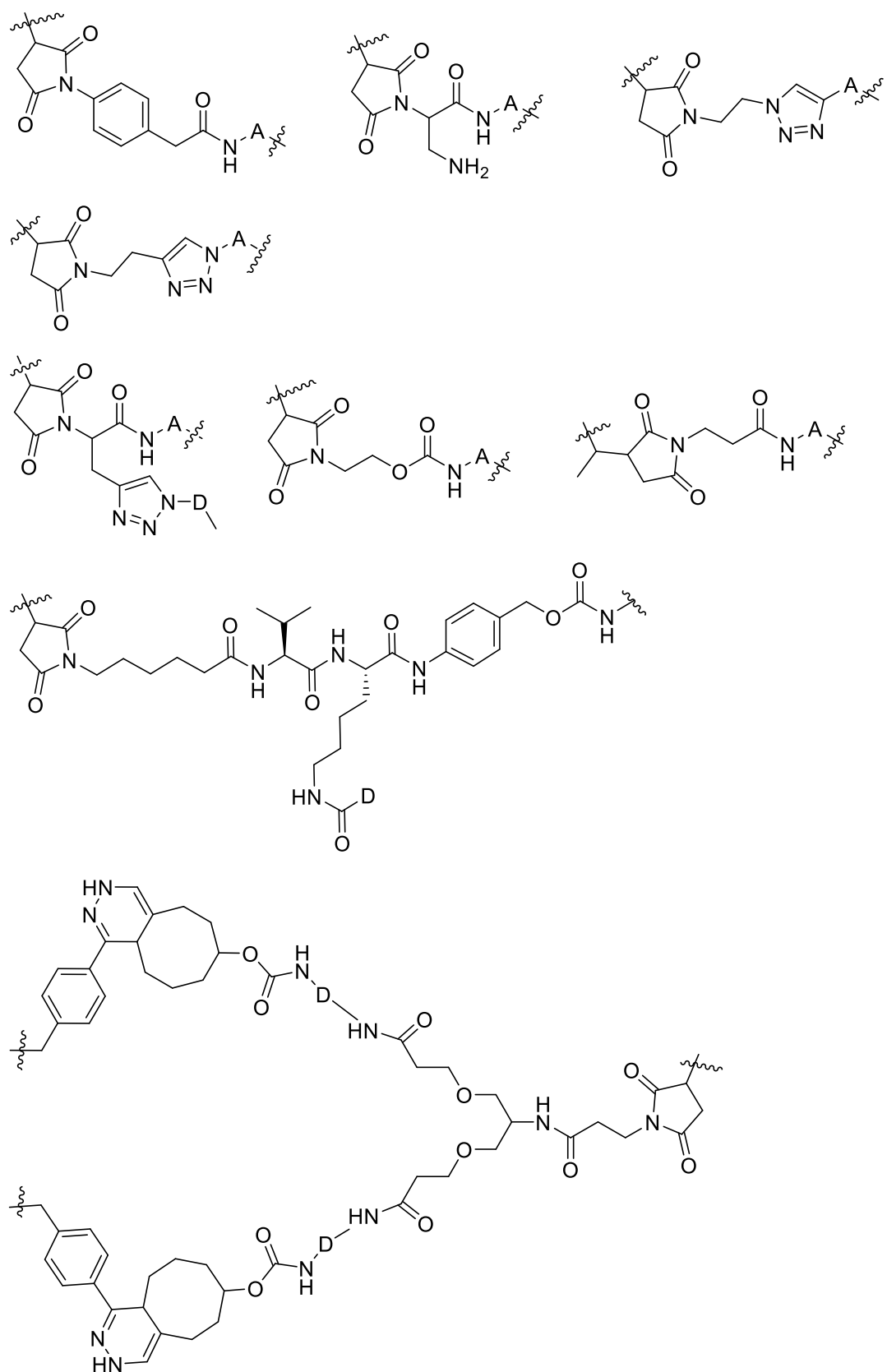
41. The conjugate of any one of claims 24-40, wherein n is an integer from 1 to 6, optionally wherein n is 1.

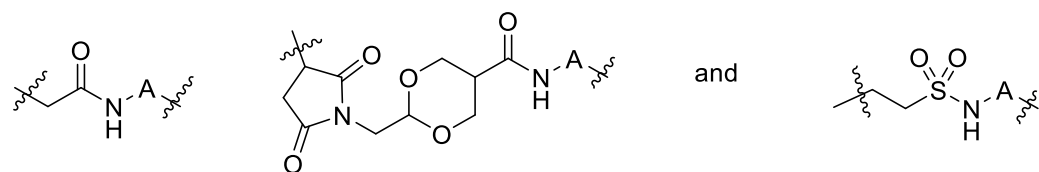
42. The conjugate of any one of claims 24-26 and 28-41, wherein P does not comprise a Fab fragment or an antigen-binding portion or variable domain portion a portion thereof.

43. The conjugate of any one of claims 1-11 and 24-42, wherein

each L is a linking group independently selected from the group consisting of:







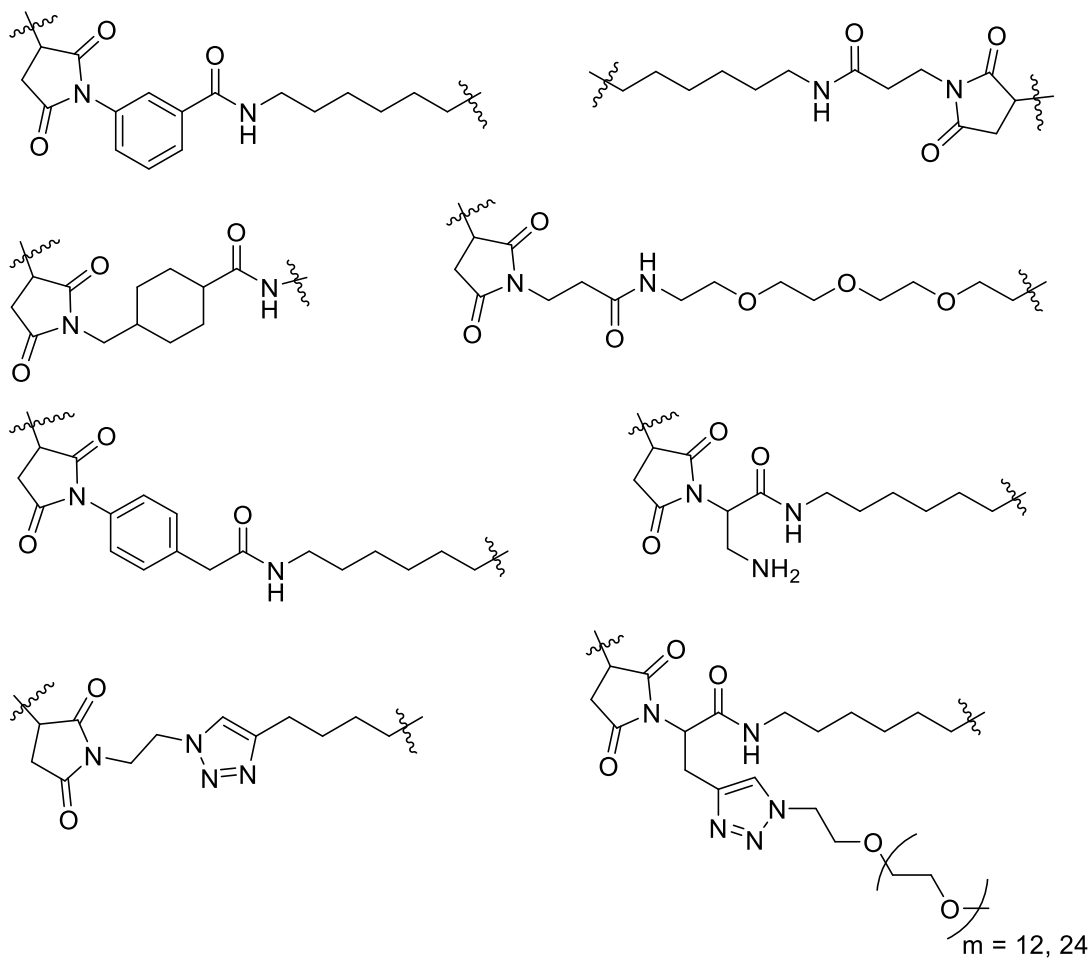
wherein,

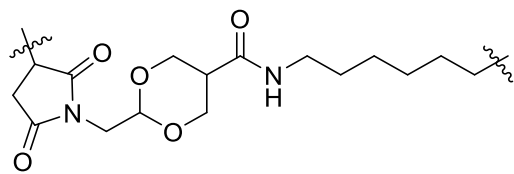
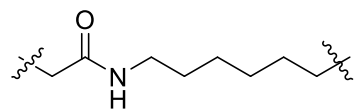
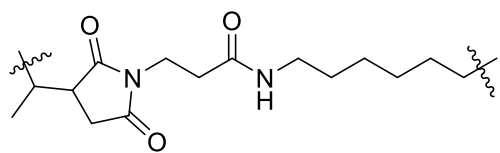
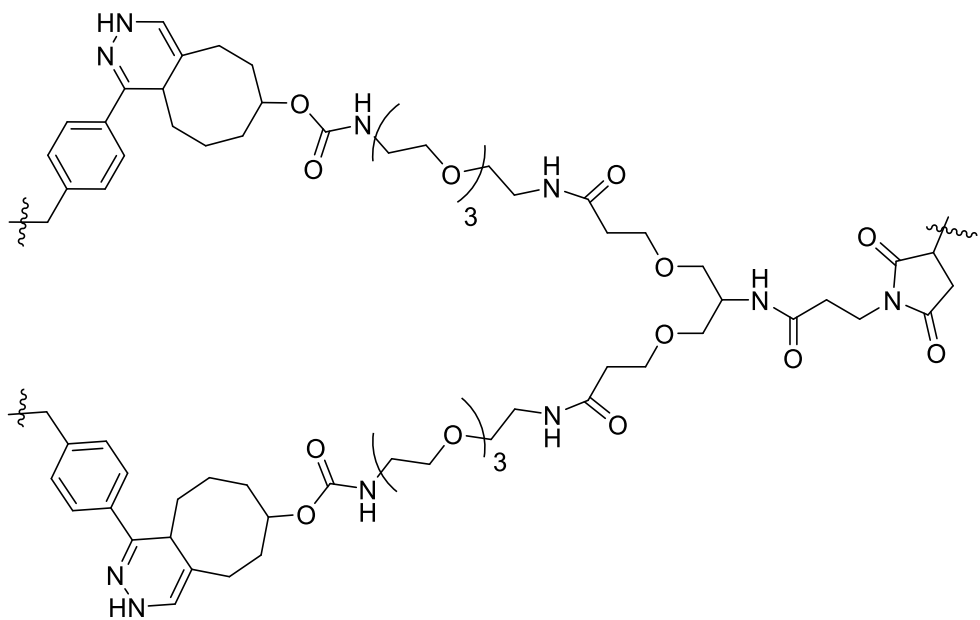
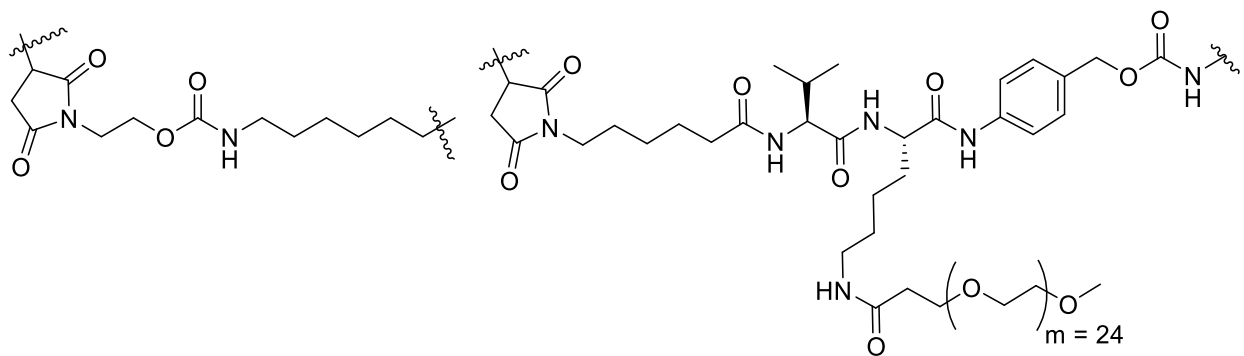
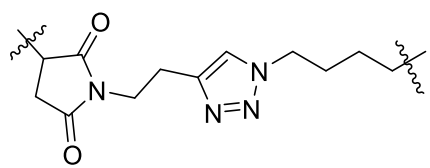
each A is independently (C₁-C₁₅)alkylene;

each D is -(CH₂-CH₂-O)_m-; and

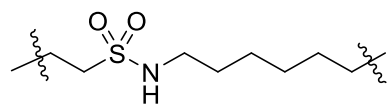
each m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24.

44. The conjugate of claim 43, wherein each L is a linking group independently selected from the group consisting of:





and



45. A conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising a modified CH3 constant domain that specifically binds to a transferrin receptor, wherein P does not comprise a Fab fragment or a portion thereof;

each y is independently 1 or more; and

n is 1 or more.

46. The conjugate of claim 45, wherein the oligonucleotide is an antisense oligonucleotide (ASO) or a siRNA.

47. The conjugate of claim 45, wherein the conjugate is greater than 50kDa in size.

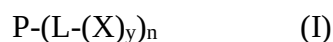
48. The conjugate of any one of claims 45-47, wherein P comprises an Fc polypeptide comprising the modified constant domain.

49. The conjugate of any one of claims 45-47, wherein P comprises an Fc polypeptide dimer, comprising a first Fc polypeptide comprising the modified constant domain; and a second Fc polypeptide dimerized to the first Fc polypeptide.

50. The conjugate of any one of claims 45-49, wherein P further comprises one or more modified sites, wherein the one or more modified site are amino acid substitutions that facilitate the attachment of P to each L.

51. The conjugate of claim 50, wherein the modified site is a cysteine substitution, optionally wherein the cysteine substitution is selected from the group consisting of: S239C, S442C, A330C, and T289C.

52. A conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising 1) a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM; and 2) one or more non-targeting Fab fragments or a portion thereof;

each y is independently 1 or more; and

n is 1 or more.

53. The conjugate of claim 52, wherein the oligonucleotide is an antisense oligonucleotide (ASO) or a siRNA.

54. The conjugate of claim 52 or 53, wherein P comprises

(a) an Fc polypeptide comprising the modified CH3 constant domain; or

(b) an Fc polypeptide dimer, comprising a first Fc polypeptide comprising the modified constant domain; and a second Fc polypeptide dimerized to the first Fc polypeptide.

55. The conjugate of claim 54, wherein the Fc polypeptide is joined to the non-targeting Fab fragment or a portion thereof to produce a Fab-Fc dimer fusion; or the first and second Fc polypeptides of the Fc polypeptide dimer are each joined to a non-targeting Fab fragment or a portion thereof to produce a Fab-Fc dimer fusion.

56. The conjugate of any one of claims 52-55, wherein P further comprises one or more modified sites, wherein the one or more modified sites are amino acid substitutions that facilitate the attachment of P to each L.

57. The conjugate of claim 56, wherein the modified site is a cysteine substitution, optionally wherein the cysteine substitution is selected from the group consisting of: S239C, S442C, A330C, K149C, A114C, and T289C.

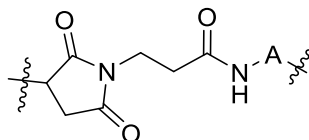
58. A conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently



wherein A is (C₁-C₁₅)alkylene;

P is a protein comprising a Fab-Fc dimer fusion, comprising 1) a modified constant domain that specifically binds to a transferrin receptor with an affinity of about 100nm; and 2) one or more modified sites, which facilitate the attachment of P to each L, and wherein the one or more modified sites are selected from the group consisting of S239C, S442C, A330C, K149C, and T289C, according to EU numbering and A114C according to Kabat numbering;

each y is independently at least 1; and

n is at least 1.

59. The conjugate of claim 58, wherein y is an integer from 1 to 4, optionally wherein y is 1.

60. The conjugate of claim 58 or 59, wherein n is an integer from 1 to 6, optionally wherein n is 1.

61. A pharmaceutical composition comprising a conjugate of formula (I) of any one of claims 1-22 and 24-60 and a pharmaceutically acceptable excipient.

62. The pharmaceutical composition of claim 61, comprising a plurality of conjugates of formula (I), optionally wherein the ratio of oligonucleotide to protein in the composition is about 1:1 to about 4:1, about 1:1 to about 2:1, or about 1.23.

63. The pharmaceutical composition of claim 62, wherein the ratio of oligonucleotide to protein in the composition is about 2:1 to about 3:1, or about 2.5.

64. The conjugate according to any one of claims 1-11 and 24-60 or the pharmaceutical composition of claim 61 for use in targeting delivery of an oligonucleotide to muscle tissue and/or brain tissue in a patient

65. The conjugate for use in modulating gene expression in a brain cell or a plurality of brain cells, wherein the conjugate comprises

(i) a protein that specifically binds TfR and;

(ii) an oligonucleotide,

wherein the protein binds TfR with a low affinity and the conjugate is transported across the BBB, and wherein in the cell, the oligonucleotide modulates the expression of a target gene.

66. The conjugate according to claim 65, wherein the low affinity is about 500nM to about 3nM, or about 400nM to about 20nM, or about 300nM to about 30nM, or about 200nM to about 40nM, or about 150nM to about 50nM.

67. The conjugate according to claim 65 or 66, wherein the modulation of target gene expression is gene knockdown or gene knockout,

optionally wherein the gene knockdown or gene knockout is at least 20%, at least 25%, at least 30%, at least 40%, at least 50% as compared to the expression without administering the oligonucleotide.

68. The conjugate according to any one of claims 65-67, wherein administration of the conjugate decreases mean cell volume by less than about 2 femtoliters.
69. The conjugate according to any one of claims 65-68, wherein administration of the conjugate decreases TfR protein levels on the brain vasculature by less than about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 50%, or about 60%.
70. The conjugate according to any one of claims 65-69, wherein the brain cell is a neuron, an endothelial cell, an oligodendrocyte, an astrocyte, or a microglia.
71. The conjugate according to any one of claims 65-69, wherein the plurality of cells comprises at least two, three, four, or five cell types selected from the group consisting of: a neuron, an endothelial cell, an oligodendrocyte, an astrocyte, and a microglia, optionally wherein the neuron is an excitatory neuron or inhibitory neuron.
72. The conjugate according to any one of claims 65-71, wherein the conjugate is formulated for intravenous administration.
73. A conjugate for use in delivering an oligonucleotide to deep brain regions, wherein the conjugate comprises:
- (i) a protein that specifically binds TfR and;
 - (ii) an oligonucleotide,
- wherein the protein binds TfR with a low affinity and the conjugate is transported across the BBB, and wherein in a brain cell in the deep brain regions, the oligonucleotide modulates the expression of a target gene.
74. The conjugate according to claim 73, wherein the low affinity is about 500nM to about 3nM, or about 400nM to about 20nM, or about 300nM to about 30nM, or about 200nM to about 40nM, or about 150nM to about 50nM.

75. The conjugate according to claim 73 or 74, wherein the deep brain regions comprise at least one of cortex, brainstem, hippocampus, striatum, cerebellum, or thalamus.

76. The conjugate according to any one of claims 73-75, wherein the conjugate is for further use in delivering the oligonucleotide to spinal cord, optionally wherein the spinal cord is a cervical spinal cord and/or a lumbar spinal cord.

77. A conjugate for use in delivering an antisense oligonucleotide across brain regions wherein the conjugate comprises:

(i) a protein that specifically binds TfR and;

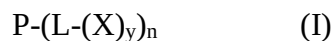
(ii) an oligonucleotide,

wherein the protein binds TfR with a low affinity and the conjugate is transported across the BBB, and wherein in a cell in a brain region, the oligonucleotide modulates the expression of a target gene.

78. A conjugate for use in transporting an oligonucleotide across the BBB and delivering the oligonucleotides to a neuron cell, wherein the conjugate comprises the oligonucleotide conjugated to a protein that binds TfR with a low affinity, wherein said oligonucleotide decreases the expression level of a target gene in the neuron cell, thereby generating a neuron cell with decreased target gene expression.

79. A conjugate for use in transporting an oligonucleotide across the BBB and delivering the oligonucleotide to a neuron cell, wherein the conjugate comprises the oligonucleotide conjugated to a protein that binds TfR with a low affinity, and wherein said oligonucleotide decreases the expression level of a target gene thereby modifying the neuron cell to decrease target gene expression.

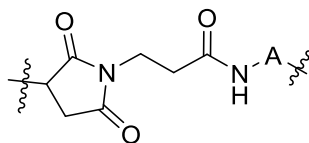
80. A conjugate for use in transporting an oligonucleotide across the blood brain barrier (BBB) of a patient, wherein the conjugate comprises a conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently



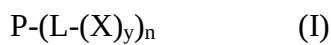
wherein A is (C₁-C₁₅)alkylene;

P is a protein comprising a Fab-Fc dimer fusion, comprising 1) a modified constant domain that specifically binds to a transferrin receptor with an affinity of about 100nm; and 2) one or more modified sites, which facilitate the attachment of P to each L, and wherein the one or more modified sites are selected from the group consisting of S239C, S442C, A330C, K149C, and T289C, according to EU numbering and A114C according to Kabat numbering;

each y is independently at least 1; and

n is at least 1.

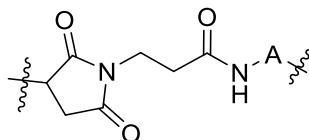
81. A conjugate for use in modulating the expression of a target gene in a cell within the brain of a patient, wherein the conjugate comprises a conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently



wherein A is (C₁-C₁₅)alkylene;

P is a protein comprising a Fab-Fc dimer fusion, comprising 1) a modified constant domain that specifically binds to a transferrin receptor with an affinity of about 100nm; and 2) one or more modified sites, which facilitate the attachment of P to each L,

selected from the group consisting of S239C, S442C, A330C, K149C, and T289C, according to EU numbering and A114C according to Kabat numbering;

each y is independently at least 1; and

n is at least 1.

82. The conjugate according to claim 81, wherein the modulation of target gene expression is gene knockdown or gene knockout.

83. A conjugate for use in modulating the expression of a target gene in cells within the brain of a patient, wherein the conjugate comprises a conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising 1) a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM; and 2) one or more modified sites, which facilitate the attachment of P to each L;

each y is independently at least 1; and

n is at least 1.

84. The conjugate according to claim 83, wherein the cells comprise at least two, three, four, or five cell types selected from the group consisting of: a neuron, an endothelial cell, an oligodendrocyte, an astrocyte, and a microglia, optionally wherein the neuron is an excitatory neuron or inhibitory neuron.

85. The conjugate according to claim 83 or 84, wherein the modulation of target gene expression is gene knockdown or gene knockout.

86. A conjugate for use in transporting oligonucleotides across the blood brain barrier (BBB) of a patient delivering the oligonucleotides to cells distributed throughout brain regions or the central nervous system, wherein the conjugate comprises a conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising 1) a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM; and 2) one or more modified sites, which facilitate the attachment of P to each L;

each y is independently at least 1; and

n is at least 1.

87. The conjugate according to claim 86, wherein brain regions comprise at least one deep brain region.

88. The conjugate according to claim 86 or 87, wherein the oligonucleotide modulates the expression of a target gene.

89. The conjugate according to claim 88, wherein the modulation of target gene expression is gene knockdown or gene knockout.

90. A conjugate for use in delivering oligonucleotides to the CNS cells of a patient, wherein the conjugate comprises a conjugate of formula I:



wherein,

each X is independently an oligonucleotide;

each L is independently a linking group;

P is a protein comprising a modified CH3 constant domain that specifically binds to a transferrin receptor with an affinity of about 3nM to about 600nM;

each y is independently at least 1; and

n is at least 1;

and,

wherein delivery of the oligonucleotides to the CNS modulates gene expression throughout brain regions or the central nervous system.

91. The conjugate according to claim 90, wherein the brain regions comprise at least one deep brain region, optionally wherein the at least one deep brain region is selected from: striatum, thalamus, caudate putamen, substantia nigra, and brainstem.

92. The conjugate according to claim 90 or 91, wherein the cells within the brain regions comprise endothelial cells, neurons, astrocytes, oligodendrocytes, and microglia.

93. The conjugate according to claim 92, wherein the oligonucleotide modulates gene expression in the endothelial cells, neurons, astrocytes, oligodendrocytes, and/or microglia, optionally wherein the oligonucleotide reduces gene expression in the cells by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or 100%, as compared to the expression without administering the oligonucleotide.

94. The conjugate according to any one of claims 80-93, wherein the conjugate is formulated for intravenous administration.

95. The Fab-Fc dimer of any one of claims 12-17 and 19-23 or the conjugate of any one of claims 27-42, and 56-63, wherein each of the Fab of the Fab-Fc dimer and the non-targeting Fab (NTF) of the conjugate comprises a light chain variable region and a heavy chain variable region, does not specifically bind to a naturally occurring epitope in a subject, and comprises three heavy chain CDRs and three light chain CDRs, wherein

CDR-H1 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 825 or 826,

CDR-H2 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 827, 828, or 869,

CDR-H3 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 829,

CDR-L1 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 819 or 820,

CDR-L2 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 821 or 822, and

CDR-L3 comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 823 or 824.

96. The Fab-Fc dimer or the conjugate of claim 95, wherein

(a) the heavy chain variable region comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of SEQ ID NO: 837 or 853; and

(b) the light chain variable region comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of SEQ ID NO: 832 or 851.

97. The Fab-Fc dimer or the conjugate of claim 96, wherein

(a) the heavy chain variable region comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of SEQ ID NO: 837 and the light chain variable region comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of SEQ ID NO: 832; or

(a) the heavy chain variable region comprises an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of SEQ ID NO: 853 and the light chain variable region comprises an amino

acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of SEQ ID NO: 851.

98. The Fab-Fc dimer or the conjugate of claim 96, wherein

(a) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 837 or 853; and

(b) the light chain variable region comprises the amino acid sequence of SEQ ID NO: 832 or 851.

99. The Fab-Fc dimer or the conjugate of claim 98, wherein

(a) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 837 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 832; or

(b) the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 853 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 851.

100. The Fab-Fc dimer or the conjugate of claim 95, wherein the NTF comprises

(a) a heavy chain comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of any one of SEQ ID NO: 838, 839, 840, 841, 844, 845, 846, 847, 854, 855, 856, 857, 859, 860, 861, and 862; and

(b) a light chain comprising an amino acid sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% identity to the amino acid sequence of any one of SEQ ID NO: 833, 835, 850, and 852.

101. The Fab-Fc dimer or the conjugate of claim 100, wherein

(a) the heavy chain comprises the amino acid sequence of any one of SEQ ID NO: 838, 839, 840, 841, 844, 845, 846, 847, 854, 855, 856, 857, 859, 860, 861, and 862; and

(b) the light chain comprises the amino acid sequence of any one of SEQ ID NO: 833, 835, 850, and 852.

102. The Fab-Fc dimer or the conjugate of claim 101, wherein

(a) the heavy chain comprises the amino acid sequence of SEQ ID NO: 838, 839, 840, or 841, and the light chain comprises the amino acid sequence of SEQ ID NO: 833;

(b) the heavy chain comprises the amino acid sequence of SEQ ID NO: 844, 845, 846, or 847, and the light chain comprises the amino acid sequence of SEQ ID NO: 833;

(c) the heavy chain comprises the amino acid sequence of SEQ ID NO: 838, 839, 840, or 841, and the light chain comprises the amino acid sequence of SEQ ID NO: 835;

(d) the heavy chain comprises the amino acid sequence of SEQ ID NO: 844, 845, 846, or 847, and the light chain comprises the amino acid sequence of SEQ ID NO: 835;

(e) the heavy chain comprises the amino acid sequence of SEQ ID NO: 854, 855, 856, or 857, and the light chain comprises the amino acid sequence of SEQ ID NO: 850;

(f) the heavy chain comprises the amino acid sequence of SEQ ID NO: 859, 860, 861, or 862, and the light chain comprises the amino acid sequence of SEQ ID NO: 850;

(g) the heavy chain comprises the amino acid sequence of SEQ ID NO: 854, 855, 856, or 857, and the light chain comprises the amino acid sequence of SEQ ID NO: 852;
and,

(h) the heavy chain comprises the amino acid sequence of SEQ ID NO: 859, 860, 861, or 862, and the light chain comprises the amino acid sequence of SEQ ID NO: 852.

103. The Fab-Fc dimer of any one of claims 12-17 and 19-23 or the conjugate of any one of claims 27-42, and 56-63, wherein each of the Fab of the Fab-Fc dimer and the non-targeting Fab (NTF) of the conjugate comprises a light chain variable region and a heavy chain variable region, does not specifically bind to a naturally occurring epitope in a subject, and comprises three light chain CDRs and three heavy chain CDRs, wherein

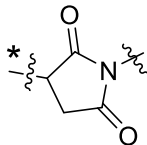
CDR-H1 comprises SEQ ID NO: 825 or 826,

CDR-H2 comprises SEQ ID NO: 827, 828, or 869,

CDR-H3 comprises SEQ ID NO: 829,

CDR-L1 comprises SEQ ID NO: 819 or 820,
CDR-L2 comprises SEQ ID NO: 821 or 822, and
CDR-L3 comprises SEQ ID NO: 823 or 824.

104. The conjugate for use according to any one of claims 65-79, wherein the oligonucleotide is conjugated to the protein via a linker having the structure:



wherein, * denotes the attachment point to a sulfur atom of a modified site within P.

105. The conjugate for according to claim 104, wherein the protein comprises an Fc peptide having a modified CH3 constant domain that specifically binds TfR.