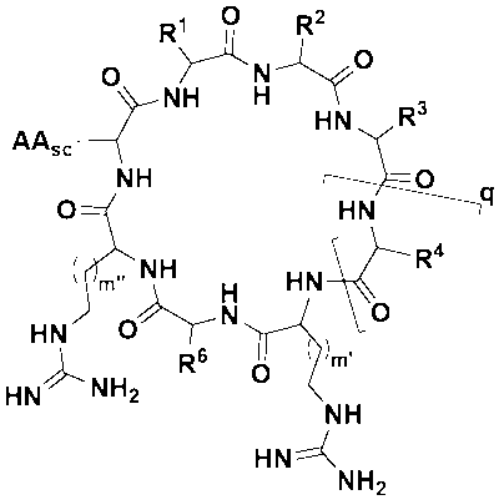
CLAIMS

1. An EEV-cargo conjugate comprising:

(a) a cyclic cell penetrating peptide (cCPP), wherein the cCPP has a structure of Formula (2):

Formula (2):



(2), or a protonated form thereof,

wherein:

R^1, R^2, and R^3 are each, independently, H or a side chain of an amino acid comprising an aryl or heteroaryl group;

at least two of R^1, R^2, and R^3 are, independently, a side chain of an amino acid comprising an aryl or heteroaryl group;

R^4 and R^6 are, independently, H or an amino acid side chain;

AA_{SC} is an amino acid side chain;

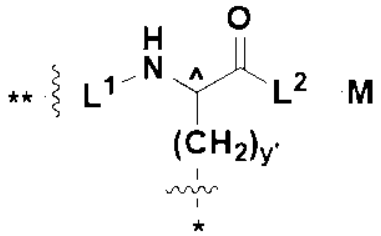
q is 1, 2, 3 or 4; and

m′ and m″ are each, independently, an integer from 0 to 3;

(b) a linear exocyclic peptide (EP) comprising from 2 to 10 amino acid residues;

(c) a linker of Formula (A′):

Formula (A'):



(A′)

wherein:

** is a point of attachment to the linear exocyclic peptide (EP);

* is a point of attachment to the cyclic cell penetrating peptide (cCPP);

L^1 and L^2, are, independently, a linker arm;

^ indicates L- or D-stereochemistry;

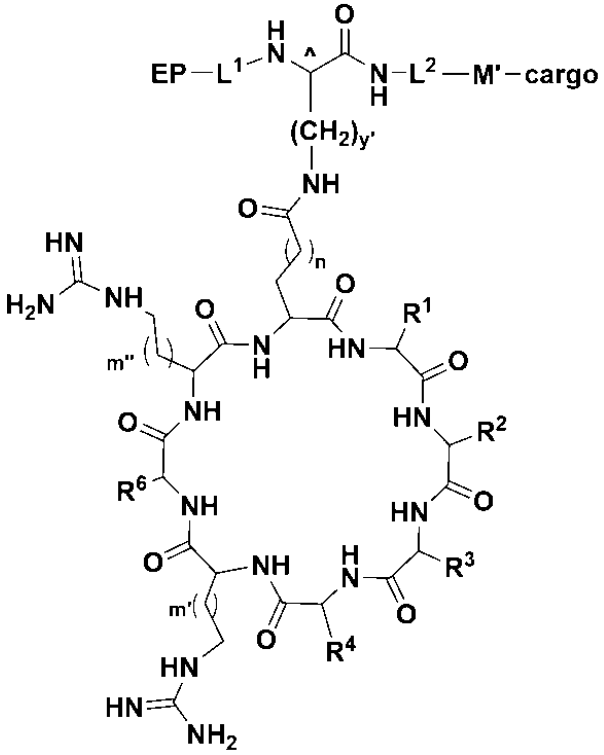
y′ is an integer from 1 to 5; and

M comprises a reactive handle comprising a functional group that reacts with a corresponding functional group on a cargo; and

(d) a cargo that comprises an oligonucleotide that binds to exon 50 in a pre-mRNA transcript of human dystrophin gene (*DMD*) to cause exon 50 skipping.

2. The compound of claim 1, comprising Formula (A-3c):

Formula (A-3c):



(A-3c)

wherein:

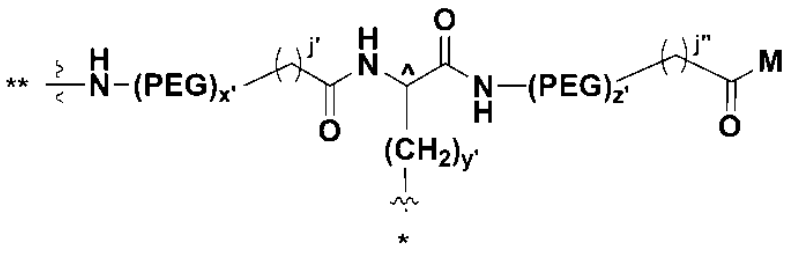
R^1, R^2, and R^3 are, independently, H or a side chain of an amino acid residue comprising an aryl or heteroaryl group;

at least two of R^1, R^2, and R^3 are a side chain of an amino acid residue comprising an aryl or heteroaryl group;

R^4 andR^6 are, independently, H or an amino acid side chain;

wherein the linker is of Formula (B′):

Formula (B'):



(B′), or a protonated form thereof;

wherein:

x′ is an integer from 0 to 12;

j′ is 0, 1, or 2, wherein j′ is 0 when x′ is 0;

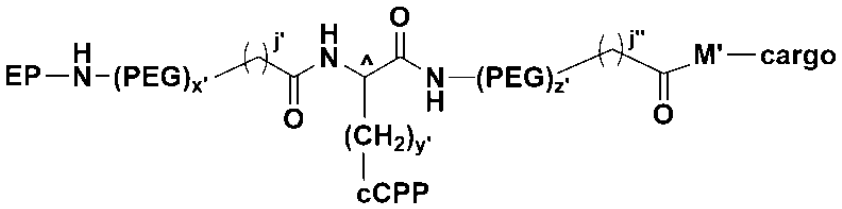
z′ is an integer from 0 to 12;

j″ is 0, 1, or 2, wherein j″ is 0 when z′ is 0; and

M′ is a bonding group comprising the reaction product between reactive handle M comprising a functional group of the linker of Formula (B′) and a corresponding functional group on the cargo.

3. The EEV-cargo conjugate of claim 1, comprising Formula (B-1):

Formula (B-1):



(B-1).

4. The EEV-cargo conjugate of any of claims 1-3, wherein:

R^4 and R^6 are, independently, H or an amino acid side chain;

q is 1, 2, 3, or 4; and

R^1, R^2, and R^3 are each, independently, a side chain comprising an aryl or heteroaryl group wherein:

(a) at least two of R^1, R^2, and R^3 are a side chain of phenylalanine and at least one of R^1, R^2, and R^3 is a side chain of naphthylalanine, or

(b) R^1, R^2, and R^3 are a side chain of phenylalanine.

5. The EEV-cargo conjugate of any of claims 1-3, wherein

one of R^1, R^2, and R^3 is H;

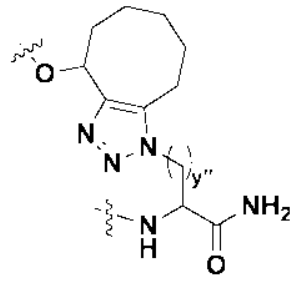
two of R^1, R^2, and R^3 are a side chain of phenylalanine; and

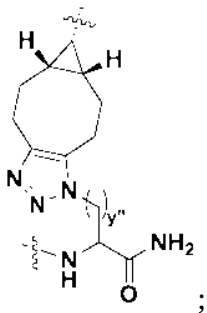
$R^4$and R^6 are, independently, H or a side chain of arginine.

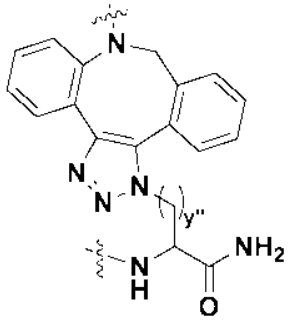
6. The EEV-cargo conjugate of any of claims 1-3, wherein the cCPP has a sequence selected from: fNalRrRrQ (SEQ ID NO: 77), Ff-Nal-Cit-r-Cit-r-Q (SEQ ID NO: 78), Ff-Nal-

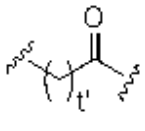
GrGrQ (SEQ ID NO: 43), FfFGRGRQ (SEQ ID NO: 35), FGFGRGRQ (SEQ ID NO: 33), and GfFGrGrQ (SEQ ID NO: 34).

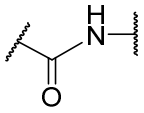
7. The EEV-cargo conjugate of any of the preceding claims, wherein M′ comprises:

-NH-, -C(O)-, -O-, 

; 

; 

; 

, or 

, wherein y″ is an integer from 1 to 4 and t′ is an integer from 0 to 10.

8. The EEV-cargo conjugate of any of claims 2-14, wherein z′ is 0, 2, 4, 8, or 12 and x’ is x’ is 0, 2, 4, 8, or 12.

9. The EEV-cargo conjugate of any of the preceding claims, wherein the linker is covalently bound to the 5' end, the 3’ end, or the backbone, of the oligonucleotide.

10. The EEV-cargo conjugate of any of the preceding claims, wherein:

(a) at least 1 amino acid residue of the EP comprises a side chain comprising a guanidine group, a terminal amine, an imidazole, or a protonated form thereof;

(b) the EP comprises 1, 2, 3, or 4 arginine residues;

(c) the EP comprises 1, 2, 3, or 4 lysine residues;

(d) the EP comprises 1 or 2 uncharged hydrophobic amino acid residues selected from valine, proline, b-alanine, glycine or a combination thereof; or

(e) any combination thereof.

11. The EEV-cargo conjugate of any one of claims 1-9, wherein the EP comprises one of the following sequences: PKKKRKV (SEQ ID NO: 184); KR; RR; KKK; KGK; KBK; KBR; KRK; KRR; RKK; RRR; KKKK (SEQ ID NO: 190); KKRK (SEQ ID NO: 201); KRKK (SEQ ID NO: 202); KRRK (SEQ ID NO: 203); RKKR (SEQ ID NO: 205); RRRR (SEQ ID NO: 191); KGKK (SEQ ID NO: 208); KKGK (SEQ ID NO: 209); KKKKK (SEQ ID NO: 212); KKKRK (SEQ ID NO: 213); KBKBK; KKKRKV (SEQ ID NO: 232); PGKKRKV (SEQ ID NO: 241); PKGKRKV (SEQ ID NO: 242); PKKGRKV (SEQ ID NO: 243); PKKKGKV (SEQ ID NO: 244); PKKKRGV (SEQ ID NO: 245; or PKKKRKG (SEQ ID NO: 246).

12. The EEV-cargo conjugate of any one of claims 1-9, wherein the EP has the sequence: Ac-PKKKRKV.

13. The EEV-cargo conjugate of any one of claims 2, 3, or 12, wherein:

(a) the EEV-cargo conjugate is selected from:

Ac-PKKKRKV-K(cyclo[Ff-Nal-RrRrQ])-PEG_{12}-M′-cargo ((SEQ ID NOS: 330 and 331, respectively); and

Ac-PKKKRKV-K(cyclo[Ff-Nal-GrGrQ])-PEG_{12}-M′-cargo (SEQ ID NOS: 332 and 333, respectively);

(b) the EEV-cargo conjugate is selected from:

Ac-PKKKRKV-PEG_2-K(cyclo[Ff-Nal-Cit-r-Cit-rQ])-PEG_{12}-M′-cargo (SEQ ID NOS 334 and 335, respectively);

Ac-PKKKRKV-PEG_2-K(cyclo[Ff-Nal-GrGrQ])-PEG_{12}-M′-cargo (SEQ ID NOS 336 and 337, respectively);

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-M′-cargo (SEQ ID NOS 338 and 339, respectively); and

Ac-PKKKRKV-PEG_2-K(cyclo[GfFGrGrQ])-PEG_{12}-M′-cargo (SEQ ID NOS 340 and 341, respectively); or

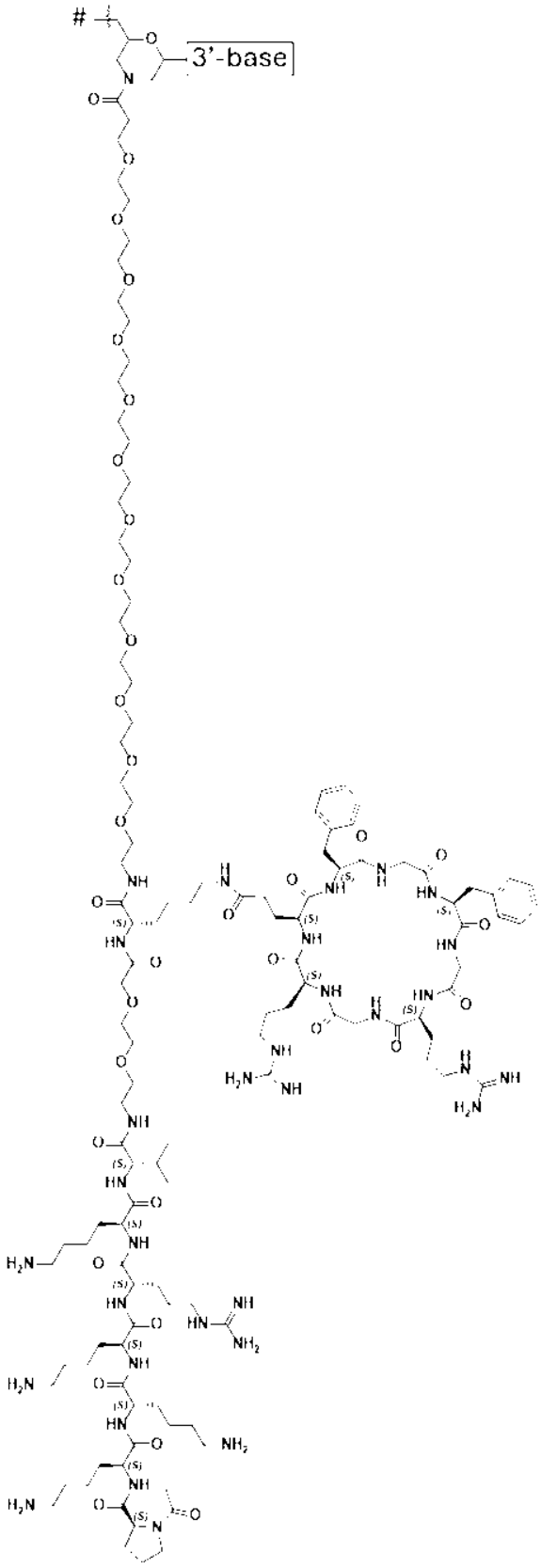
(c) the EEV-cargo conjugate is selected from:

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-M′-cargo (SEQ ID NOS: 342 and 343, respectively);

Ac-PKKKRKV-PEG_2-K(cyclo[GfFGrGrQ])-PEG_2-M′-cargo (SEQ ID NOS: 344 and 345, respectively); and

Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_2-M′-cargo (SEQ ID NOS: 346 and 347, respectively).

14. The EEV-cargo conjugate of claim 1, wherein the oligonucleotide comprises a PMO and wherein the EEV-cargo conjugate has the structure



(SEQ ID NOS: 338 and 339, respectively),

wherein # is a phosphorodiamidate morpholino oligonucleotide (PMO) and 3’ base is a nucleobase at the 3’ end of the PMO.

15. The EEV-cargo conjugate of any of claims 1-13, wherein the oligonucleotide comprises a from 15 to 30 nucleotides and wherein phosphorodiamidate morpholino oligonucleotide (PMO).

16. The EEV-cargo conjugate of any of claims 1-15, wherein the oligonucleotide comprises (a) a sequence comprising: 5’-XXACCGCCXXCCACXCAGAGCXCAGA-3’ (SEQ ID NO: 368), wherein X = U or T, the reverse complement thereof, or a sequence with at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% o4 100% sequence identity thereto, or
(b) a sequence comprising at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 contiguous nucleic acids of a base sequence comprising: 5’-XXACCGCCXXCCACXCAGAGCXCAGA-3’ (SEQ ID NO: 368), wherein X = U or T.

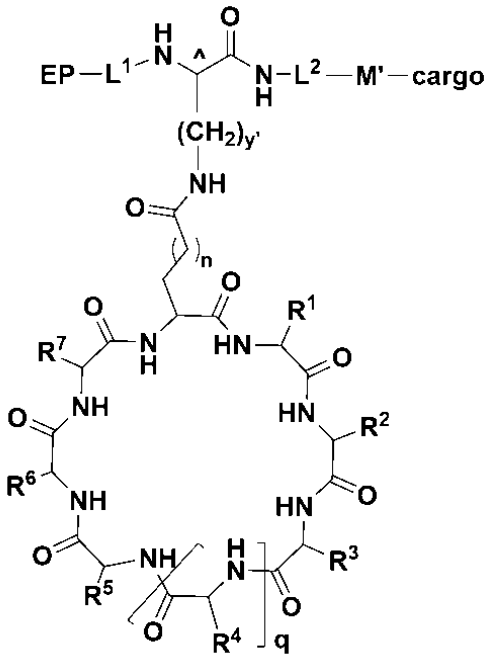
17. The EEV-cargo conjugate of any of claims 1-15, wherein the oligonucleotide comprises a sequence selected from:

5’-AGTGGTCAGTCCAGGAGCTAGGTC-3’ (SEQ ID NO: 358),
5’-GTGGTCAGTCCAGGAGCTAGG-3’ (SEQ ID NO: 359),
5’-GGTCAGTCCAGGAGCTAGGTCA-3’ (SEQ ID NO: 360),
5’-TAGTGGTCAGTCCAGGAGCTAGGT-3’(SEQ ID NO: 361),
5’-GCTCCAATAGTGGTCAGTCCAG-3’(SEQ ID NO: 362),
5’-ACCGCCTTCCACTCAGAGCTCAGA-3’ (SEQ ID NO: 363),
5’-TTACCGCCTTCCACTCAGAGCTCA-3’(SEQ ID NO: 364),
5’-GGAGCTAGGTCAGGCTGCTTTG-3’(SEQ ID NO: 365),
5’-GTGGTCAGTCCAGGAGCTAGGTC-3’ (SEQ ID NO: 366), and
5’-AGGAGCTAGGTCAGGCTGCTTT-3’ (SEQ ID NO: 367).

18. The EEV-cargo conjugate of any of claims 1-15, wherein the oligonucleotide comprises:
5’-ACCGCCTTCCACTCAGAGCTCAGA-3’ (SEQ ID NO: 363) or 5’-TTACCGCCTTCCACTCAGAGCTCA-3’ (SEQ ID NO: 364).

19. An EEV-cargo conjugate comprising a compound of Formula (A-3):

Formula (A-3):



(A-3)

wherein:

R^1, R^2, and R^3 are, independently, H or a side chain of an amino acid comprising an aryl or heteroaryl group;

at least two of R^1, R^2, and R^3 are a side chain of an amino acid comprising an aryl or heteroaryl group;

R^4, R^5, R^6, and R^7 are, independently, H or a side chain of an amino acid;

L^1 and L^2, are, independently, a linker arm;

M′ is a bonding group;

y′ is an integer from 1 to 5;

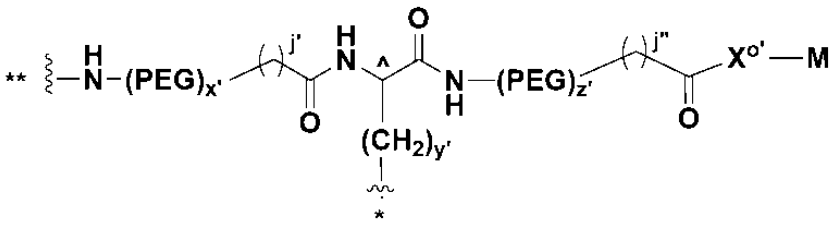
^ indicates L- or D-stereochemistry;

q is 1, 2, 3 or 4;

n is an integer from 1-4; and

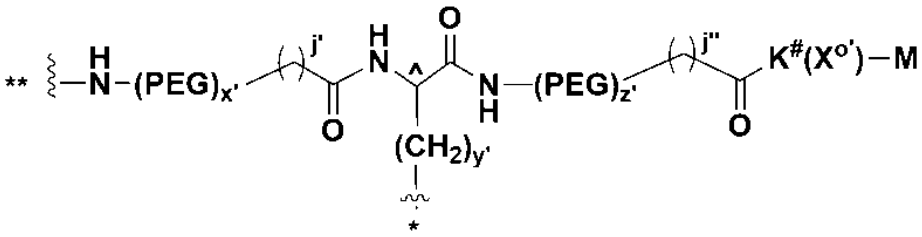
wherein the linker comprises Formula (C′) of (D’):

Formula (C'):



(C′)

Formula (D'):



(D′); and

wherein:

x′ is an integer from 0 to 12;

j′ is 0, 1, or 2, wherein j′ is 0 when x′ is 0;

z′ is an integer from 0 to 12;

j″ is 0, 1, or 2, wherein j″ is 0 when z′ is 0; and

$X^{o\prime}$ is a hydrophobic component;

$K^{\#}$ is D-lysine or L-lysine residue; and

M is a reactive handle on the linker of Formula (C′) comprising a functional group that reacts with a corresponding functional group on a cargo to form a reaction product comprising M'.

20. The EEV-cargo conjugate of claim 19 wherein:

(a) the linker comprises Formula (C′) and the EEV-cargo conjugate is selected from:

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Nal-M′-cargo (SEQ ID NOS: 354 and 355, respectively); and

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Bip-M′-cargo (SEQ ID NOS: 356 and 357, respectively); or

(b) the linker comprises Formula (D') and the EEV-cargo conjugate is selected from

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-K(Bip)-M′-cargo (SEQ ID NOS: 350 and 351, respectively); and

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-K(Nal)-M′-cargo (SEQ ID NOS: 352 and 353, respectively).

21. A pharmaceutical composition comprising the EEV-cargo conjugate of any one of claims 1-20 and a pharmaceutically acceptable carrier.

22. The EEV-cargo conjugate of any one of claims 1 to 20 or the pharmaceutical composition of claim 21 for use in the treatment of DMD.

23. The use of claim 22, wherein the EEV-cargo conjugate or the pharmaceutical composition is to be administered by parenteral administration.

24. The use of claim 23, wherein parenteral administration is selected from: subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, intrasternal, and intrathecal administration.

25. Use of the EEV-cargo conjugate of any one of claims 1 to 20 or the pharmaceutical composition of claim 21 for the manufacture of a medicament for the treatment of DMD.