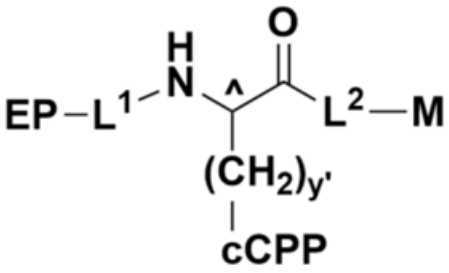
INTRACELLULAR THERAPEUTICS, the disclosures of which are hereby incorporated by reference in their entireties.

[0005] While not wishing to be bound by theory, it is believed that the linkers can influence efficacy and tolerability of the resultant construct, for example to increase efficacy.

[0006] Therapeutics can be conjugated to CPPs or EEVs using a variety of chemistries, including, for example, click chemistry or amide chemistry. Although both methods are effective at conjugating the therapeutic to the EEV, the different chemistries result in different linkages that may impart different properties to the resulting molecule. EEV generated using click chemistry for cargo conjugation include a hydrophobic component at the point of conjugation that is absent when amide chemistry is used. While not wishing to be bound by theory, molecules that include the hydrophobic component at the point of conjugation appear to have improved efficacy as compared to similar molecules in which the therapeutic is conjugated to the EEV using amide chemistry. However, there are problems associated with click chemistry that are not experienced with amide chemistry, for example, formation of regioisomers. While amide chemistry eases the synthetic process, it results in the loss of the hydrophobic component at the point of conjugation. EEVs incorporating a hydrophobic component are provided herein that demonstrate enhanced efficacy as compared to a similar EEVs in which the hydrophobic component is absent. In examples, the EEVs incorporating the hydrophobic component do not show any decrease in tolerability as compared to the similar EEV in which the hydrophobic component is absent.

[0007] While not wishing to be bound by theory, it is also believed that the linker length can influence efficacy and tolerability. In some instances, as linker length decreases, efficacy increases and tolerability decreases; and as linker length increases, efficacy decreases and tolerability increases. EEVs incorporating different linker lengths are provided herein. Additionally, it is believed that including one or more amino acid residues in the linker can influence efficacy and tolerability. EEVs incorporating one or more amino acid residues are provided herein. Furthermore, it is believed that the chirality of the amino acids in a cell-penetrating peptide (CPP) or endosomal escape vehicle (EEV) can impact cytosolic delivery efficiency, as well as toxicity.

[0007a] In one aspect, the disclosure provides an endosomal escape vehicle (EEV) comprising the structure:



(A)

wherein

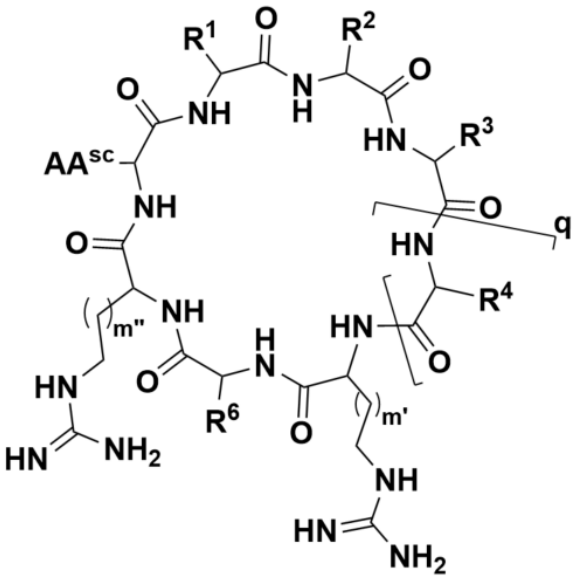
EP is a linear exocyclic peptide comprising from 2 to 8 amino acid residues, comprising

1, 2, 3, or 4 arginine residues,

1, 2, 3, or 4 lysine residues, and

1 or 2 uncharged hydrophobic amino acid residues selected from valine, proline, b-alanine, glycine or a combination thereof;

cCPP is a cyclic cell penetrating peptide of formula (2)



R^1, R^2, and R^3 can each independently be H or an amino acid residue having a side chain comprising an aryl or heteroaryl group;

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

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

AA^{SC} is an amino acid side chain, wherein the cCPP is linked to the -$(CH_2)_{y'}$- group through

the AA^{SC} of the cCPP;

m′ is an integer from 0 to 3;

m″ is an integer from 0 to 3; and

q is 1, 2, 3, or 4;

L^1 is a linker arm comprising a polyethylene glycol component (PEG)x′, wherein x′ is an integer from 1-12;

L^2 comprises one or more polyethylene glycol components (PEG)z′ and (PEG)z″, and

(i) one or more amino acid components (AA)b′ and (AA)b″,

(ii) one or more hydrophobic components ($X^{o\prime}$) and ($X^{o\prime\prime}$), or

(iii) combinations thereof

wherein each of $X^{o\prime}$ and $X^{o\prime\prime}$ is independently an amino acid residue with an aromatic side chain,

wherein

each of b′ and b″ is, independently, an integer from 0-12 and

each of z′ and z″ is, independently, an integer from 0-12;

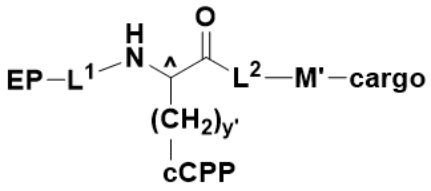
(AA)b′ and (AA)b″ independently comprise one or more amino acid residues selected from lysine (K), arginine (R), histidine (H), glycine (G), β-alanine (B), phenylalanine (F), proline (P), valine (V), or combinations thereof;

^ indicates L- or D-stereochemistry;

y′ is an integer from 1 to 5; and

M comprises a reactive handle.

[0007b] In one aspect, the disclosure provides an EEV-cargo conjugate comprising the EEV as disclosed herein conjugated to a cargo, wherein the EEV-cargo conjugate has the structure of Formula (A-1):



(A-1)

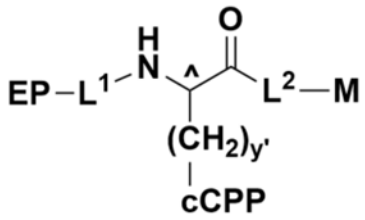
wherein M' is a bonding group.

[0007c] In one aspect, the disclosure provides a pharmaceutical composition comprising the EEV-cargo conjugate as disclosed herein and a pharmaceutically acceptable carrier.

[0007d] In one aspect, the disclosure provides a use of the pharmaceutical composition as disclosed herein or the EEV-cargo conjugate as disclosed herein in the manufacture of a medicament for treating a disease in a subject in need thereof.

[0008] An EEV can comprise a cyclic cell penetrating peptide (cCPP), an exocyclic peptide (EP) and trivalent linker comprising the structure of Formula (A):

Formula (A):



(A);

wherein:

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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

^ indicates L- or D-stereochemistry;

y′ is an integer from 1 to 5; and

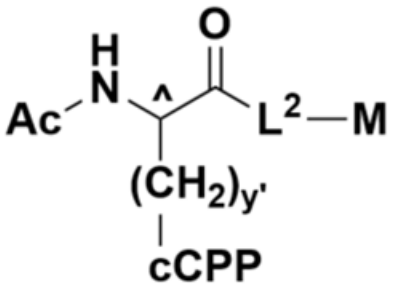
M comprises a reactive handle.

[0009] In examples, L^1 and L^2, are each, independently, absent, or comprise hydrocarbon component (e.g., NRH-$(CH_2)_n$-COOH), a polyethylene glycol (PEG) component, a hydrophobic component (X), an amino acid component comprising one or more amino acid residues (AA), or any combination thereof.

[0010] In examples, L^1 for Formula (A) comprises (AA)a′ or (PEG)x′, any combination thereof, and L^2 comprises one or more amino acid components (AA)b′, (AA)b″, one or more PEG components (PEG)z′, (PEG)z″, one or more hydrophobic components (e.g., $X^{o\prime}$, $X^{o\prime\prime}$, etc.) or any combination thereof, wherein (AA)a′, (AA)b′, and (AA)b″ are independently an amino acid component comprising at least one amino acid residue.

[0011] In examples, the EEV can comprise a cyclic cell penetrating peptide (cCPP) and a linker. In examples, the EEV does not include a linear exocyclic peptide (EP). In examples, the EEV has the structure shown in Formula (AA):

Formula (AA):



(AA);

wherein:

Ac is acetyl;

cCPP is a cyclic cell penetrating peptide;

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

^ indicates L- or D-stereochemistry;

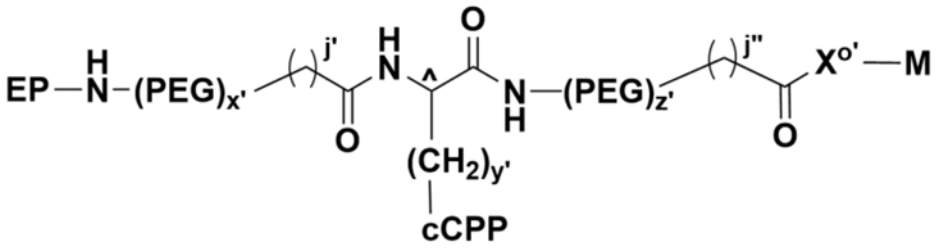
y′ is an integer from 1 to 5; and

M comprises a reactive handle.

[0012] In examples, the linker arm L^2 of Formula (AA), comprises one or more amino acid components (e.g., (AA)b′, (AA)b′, etc.), one or more PEG components (e.g., (PEG)z′, (PEG)z″, etc.), one or more hydrophobic components (e.g., $X^{o\prime}$, $X^{o\prime\prime}$, etc.), or any combination thereof, wherein (AA)b′ and (AA)b″ are independently an amino acid component comprising at least one amino acid residue, b′ and b″ are independently an integer from 0-12, and z′ and z″ are independently an integer from 0-12.

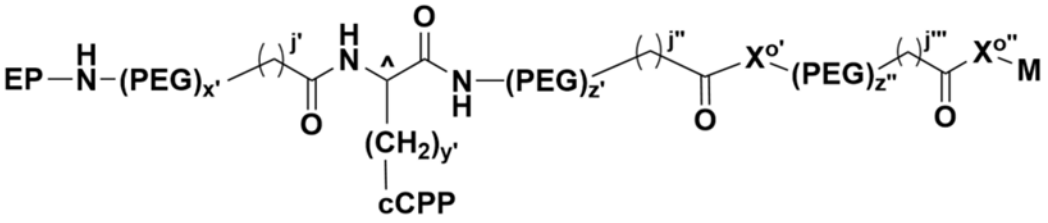
[0013] In examples, the EEV of Formula (A) comprises a structure selected from:

Formula (B):



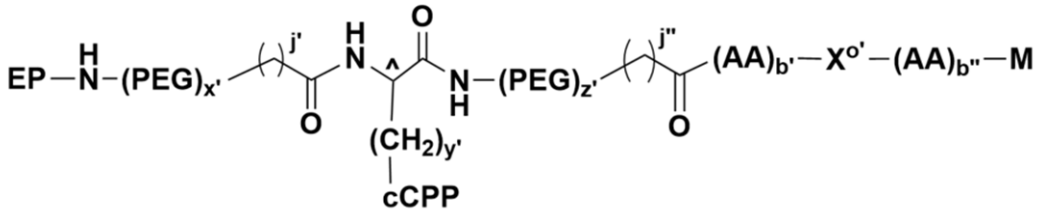
(B);

Formula (BB):



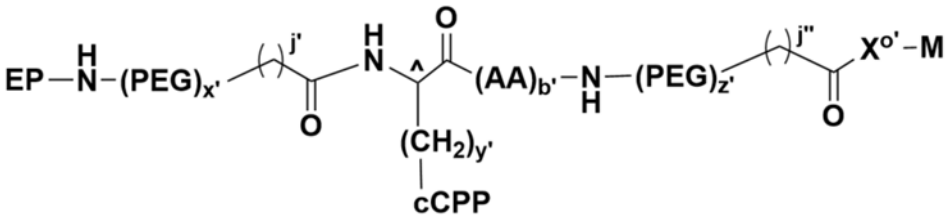
(BB);

Formula (C):



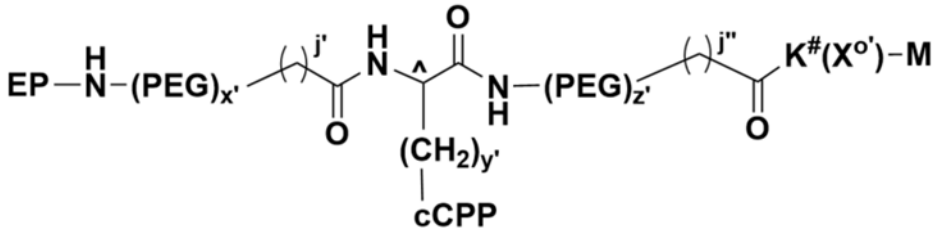
(C);

Formula (D):



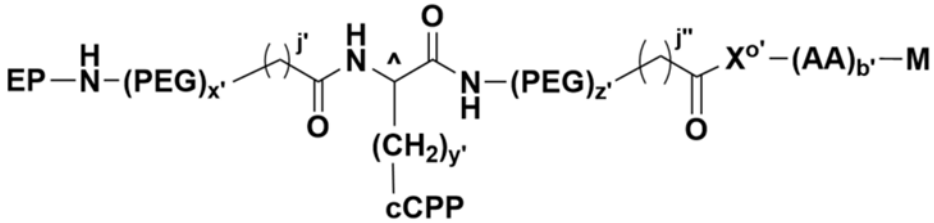
(D);

Formula (E):



(E); or

Formula (F):



(F);

wherein:

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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;

z″ is an integer from 0 to 12;

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

$X^{o\prime}$ and $X^{o\prime\prime}$ are each, independently, a hydrophobic component;

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

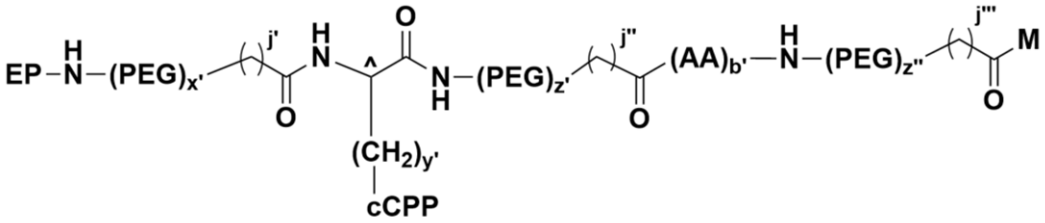
(AA)b′ and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

b′ is an integer from 0 to 12; and

b″ is an integer from 0 to 12.

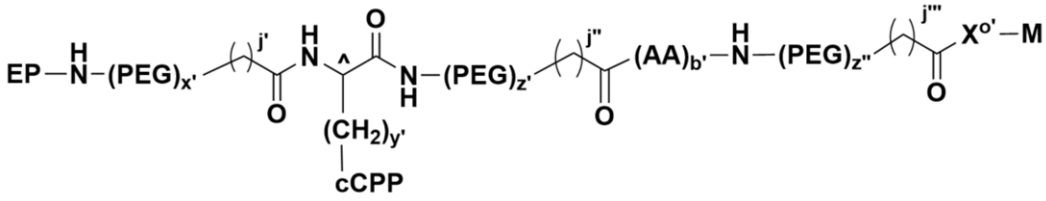
[0014] In examples, the EEV of Formula (A) comprises a structure selected from:

Formula (G):



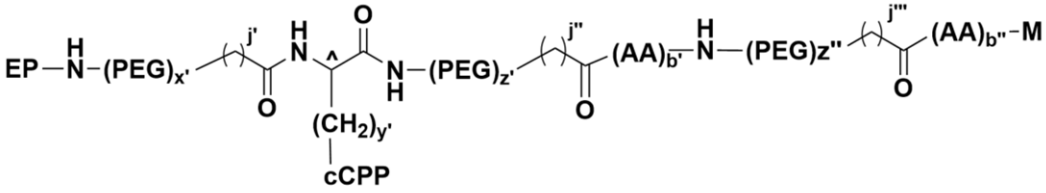
(G);

Formula (H):



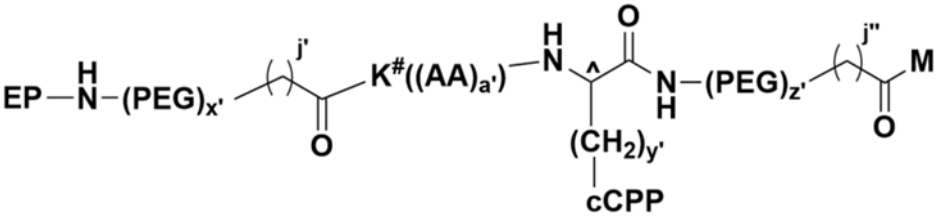
(H);

Formula (I):



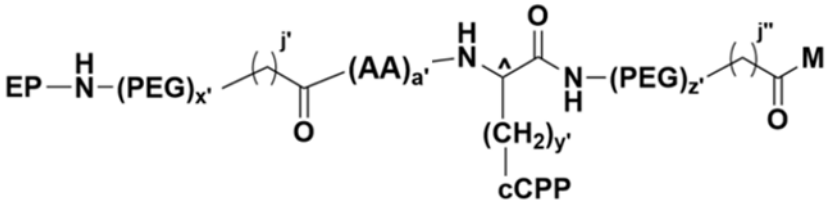
(I);

Formula (J):



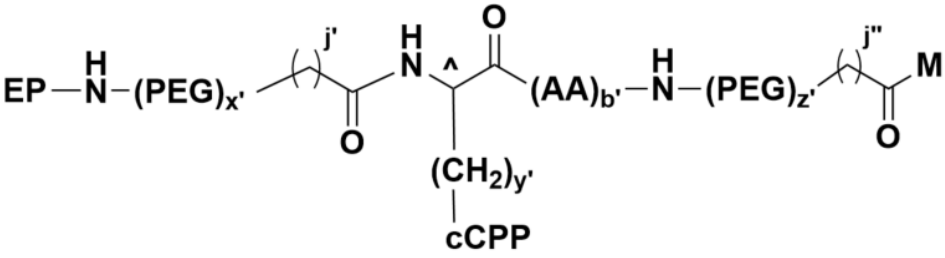
(J);

Formula (JJ):



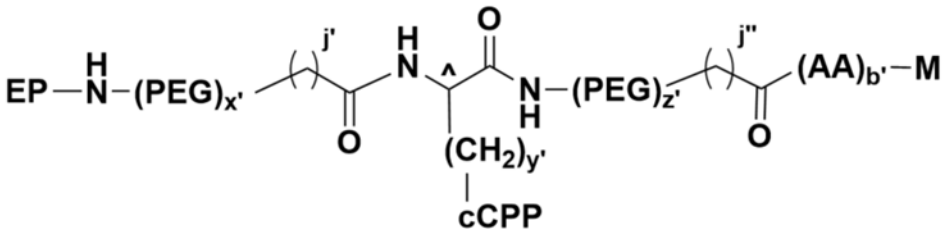
(JJ);

Formula (K):



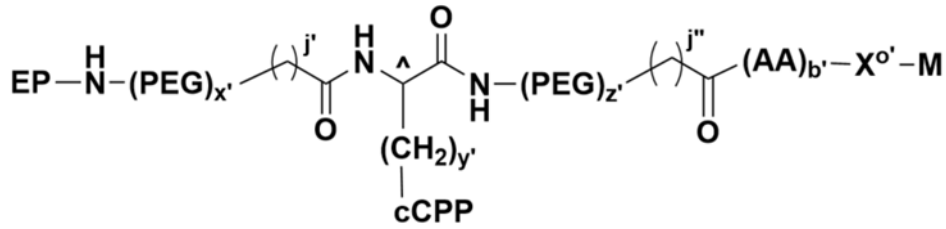
(K);

Formula (L):



(L); or

Formula (M):



(M);

wherein:

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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;

z″ is an integer from 0 to 12;

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

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

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

(AA)a′, (AA)b′, and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

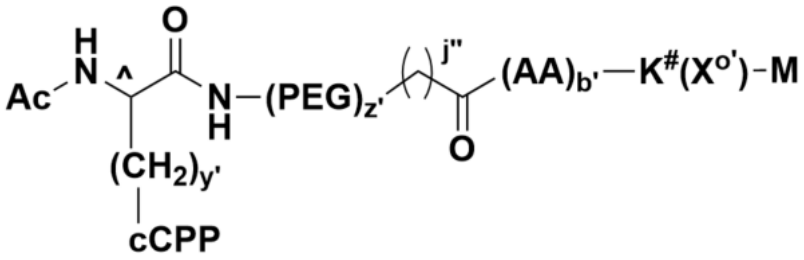
a′ is an integer from 0 to 12;

b′ is an integer from 0 to 12; and

b″ is an integer from 0 to 12.

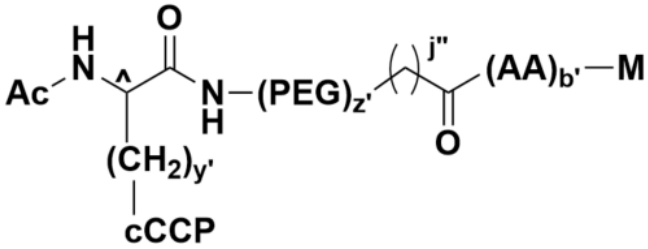
[0015] In examples, the EEV of Formula (AA) comprises a structure selected from:

Formula (N):



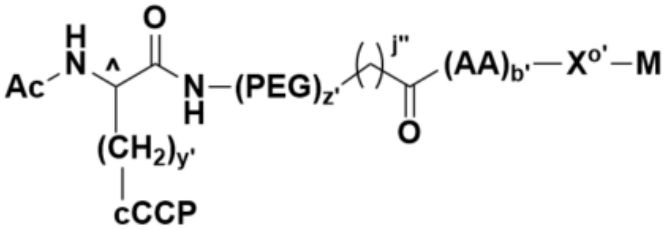
(N);

Formula (O):



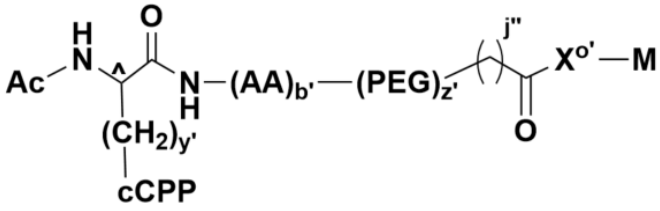
(O);

Formula (P):



(P); or

Formula (Q):



(Q);

wherein:

Ac is acetyl;

cCPP is a cyclic cell penetrating peptide;

z′ is an integer from 0 to 12;

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

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

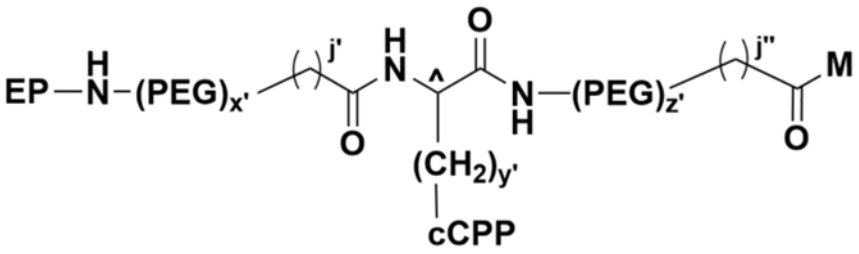
$K^{\#}$ is D-lysine or L-lysine residue;

(AA)b′ is an amino acid (AA) component comprising at least one amino acid residue; and

b′ is an integer from 0 to 12.

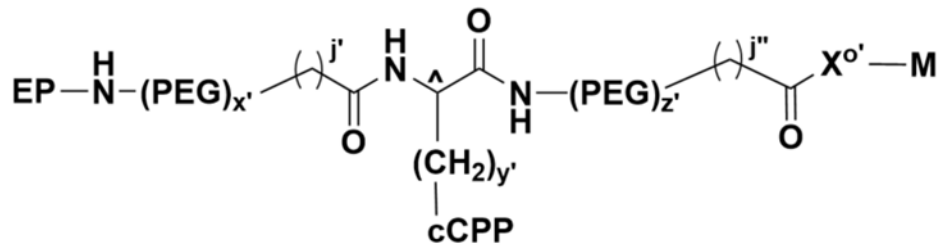
[0016] In examples, the EEV comprises a structure selected from Formula (R) and Formula (B):

Formula (R):



(R); or

Formula (B):



(B);

wherein

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

x′ is an integer from 0 to 12;

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

^ indicates L- or D-stereochemistry;

y′ is an integer from 1 to 5;

z′ is an integer from 0 to 12;

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

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

M comprises a reactive handle.

[0017] An EEV can comprise a cyclic cell penetrating peptide (cCPP), an exocyclic peptide (EP) and linker, wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to

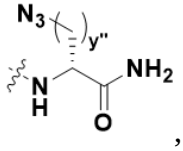
the linker. In examples, cCPP comprises at least 2 D-amino acids with a hydrophobic side chain and at least 2 D-arginine amino acids. In examples, cCPP comprises at least 2 D-phenylalanine. In examples, L^2 comprises a hydrophobic component ($X^{o\prime}$). In examples, $X^{o\prime}$ is Nal, dNal, or BiP. In examples, AAsc is a side chain of glutamine. In examples, AAsc is a side chain of L-glutamine. In examples, AAsc is a side chain of D-glutamine.

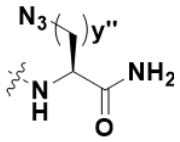
[0018] In examples, the EEV comprises a structure selected from Formula (B), (BB), (C), (D), (E), or (F), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

[0019] In examples, the EEV comprises a structure selected from Formula (G), (H), (I), (J), (JJ), (K), (L), or (M), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

[0020] In examples, the EEV comprises a structure selected from Formula (N), (O), (P), or (Q), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

[0021] In examples, the EEV comprises a structure selected from Formula (R) or (B), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

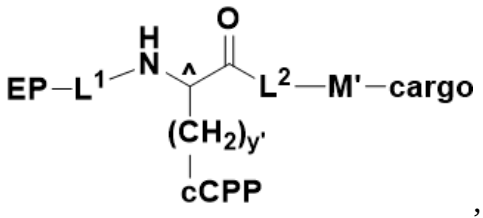
[0022] In examples, M comprises -OH, 

, or 

, wherein y'' is an integer from 1-4.

[0023] In examples, the EEV can be conjugated to a cargo to form an EEV-cargo conjugate comprising the structure of Formula (A-1):

Formula (A-1):



, wherein

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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

^ indicates L- or D-stereochemistry;

y′ is an integer from 1 to 5;

M′ comprises a bonding group; and

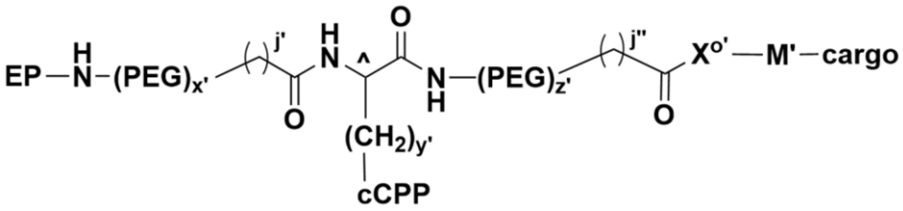
cargo is a peptide, an oligonucleotide, a small molecule, or any combinations thereof.

[0024] In examples, L^1 and L^2, are each, independently, absent, or comprise one or more hydrocarbon components (e.g., NRH-$(CH_2)_n$-COOH), one or more polyethylene glycol components, one or more hydrophobic components, one or more amino acid components, or any combination thereof.

[0025] In examples, L^1 comprises (AA)a′ or (PEG)x′, or any combination thereof, and L^2 comprises (AA)b′, (AA)b″, (PEG)z′, (PEG)z″, one or more hydrophobic components (e.g., X^{o}′, X^{o}″, etc.), or any combination thereof.

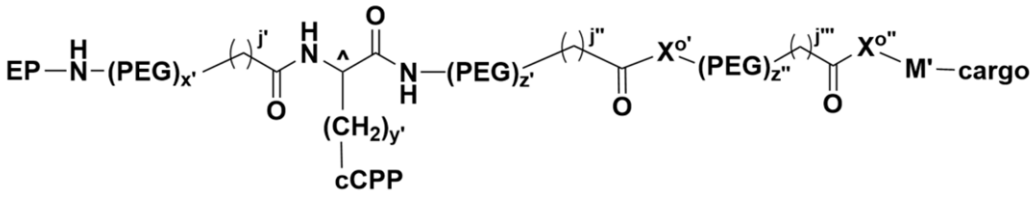
[0026] In examples, the compound of Formula (A-1) comprises a structure selected from:

Formula (B-1):



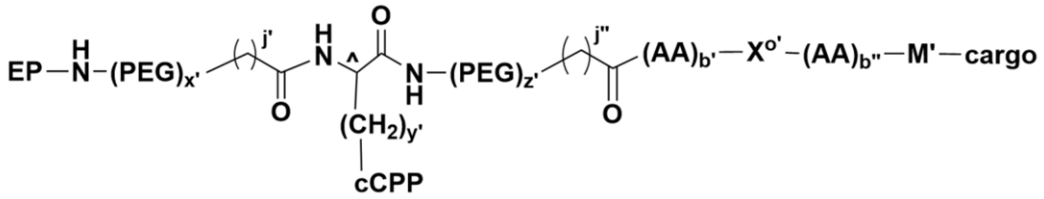
(B-1);

Formula (BB-1):



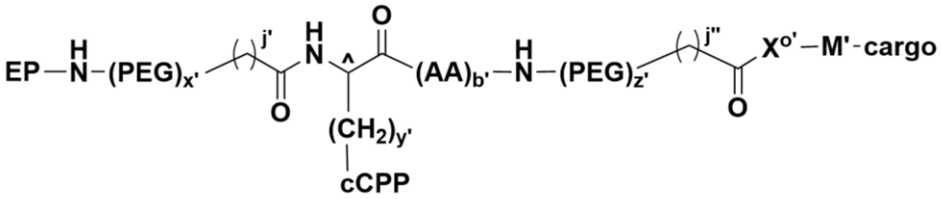
(BB-1);

Formula (C-1):



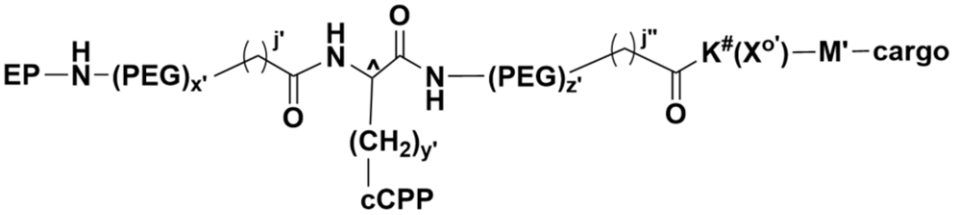
(C-1);

Formula (D-1):



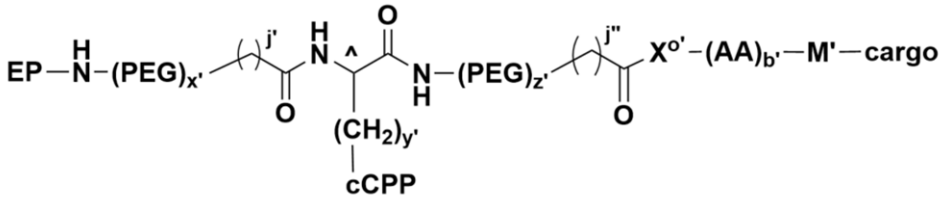
(D-1);

Formula (E-1):



(E-1); or

Formula (F-1):



(F-1);wherein:

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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;

z″ is an integer from 0 to 12;

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

$X^{o\prime}$ and $X^{o\prime\prime}$ are each, independently, a hydrophobic component;

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

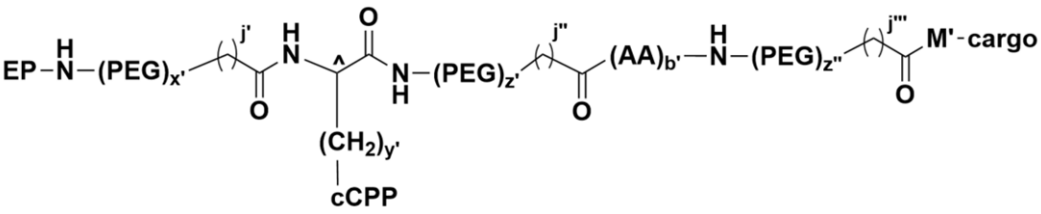
(AA)b′ and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

b′ is an integer from 0 to 12; and

b″ is an integer from 0 to 12.

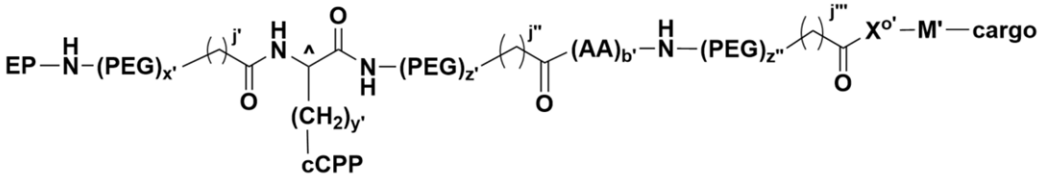
[0027] In examples, the compound of Formula (A-1) comprises a structure selected from:

Formula (G-1):



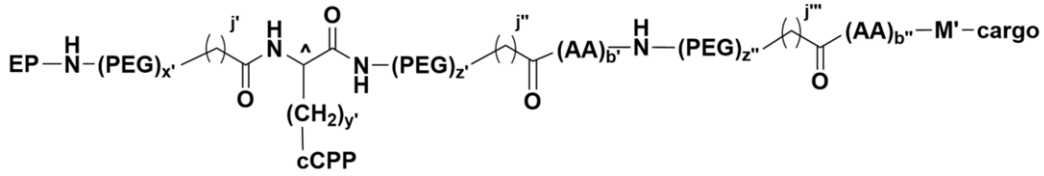
(G-1);

Formula (H-1):



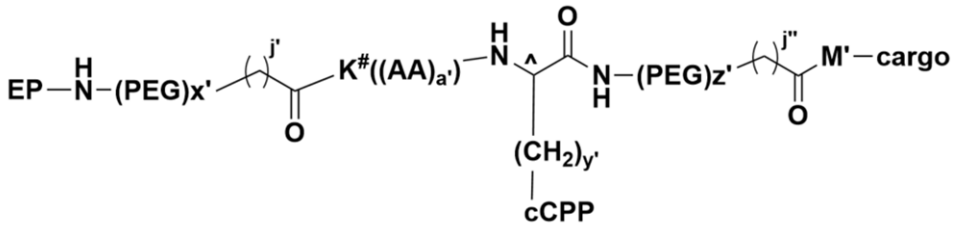
(H-1);

Formula (I-1):



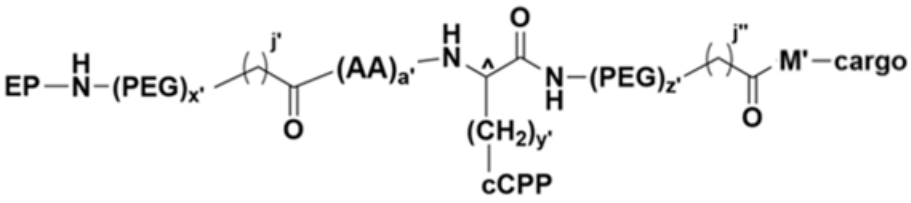
(I-1);

Formula (J-1):



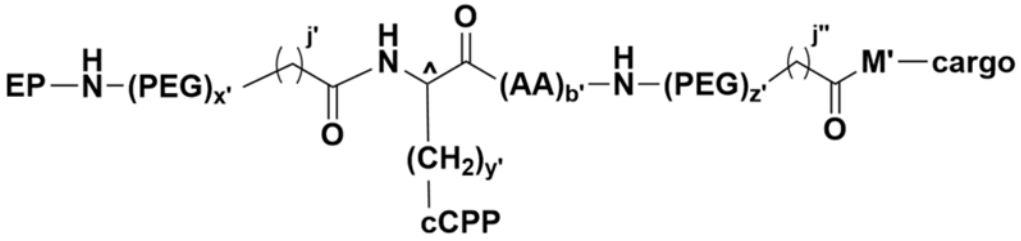
(J-1);

Formula (JJ-1):



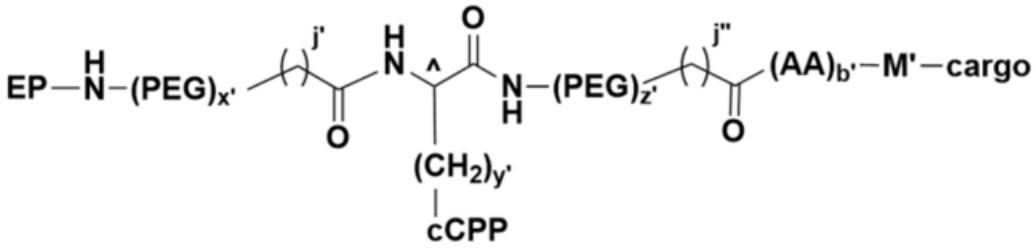
(JJ-1);

Formula (K-1):



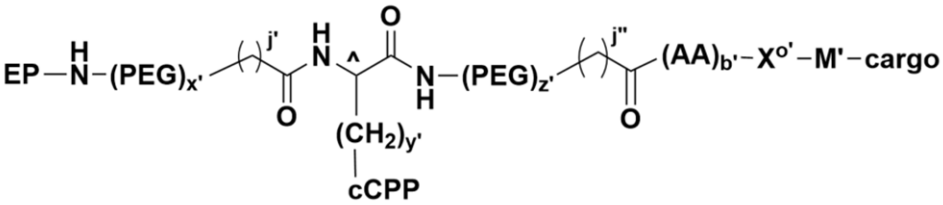
(K-1);

Formula (L-1):



(L-1); or

Formula (M-1):



(M-1);

wherein:

EP is a linear exocyclic peptide;
cCPP is a cyclic cell penetrating peptide;
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;
z″ is an integer from 0 to 12;

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

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

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

(AA)a′, (AA)b′, and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

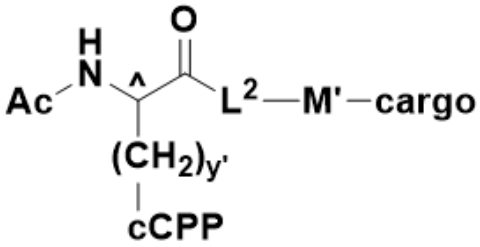
a′ is an integer from 0 to 12;

b′ is an integer from 0 to 12; and

b″ is an integer from 0 to 12.

[0028] In examples, the EEV can be conjugated to a cargo to form an EEV-cargo conjugate comprising the structure of Formula (AA-1):

Formula (AA-1):



(AA-1);

wherein:

cCPP is a cyclic cell penetrating peptide;

Ac is acetyl;

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

^ indicates L- or D-stereochemistry;

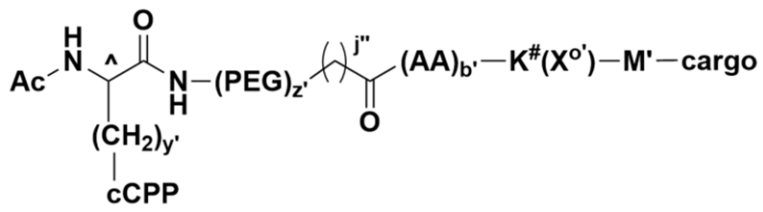
y′ is an integer from 1 to 5;

M′ comprises a bonding group; and

cargo is a peptide, an oligonucleotide, a small molecule, or any combinations thereof.

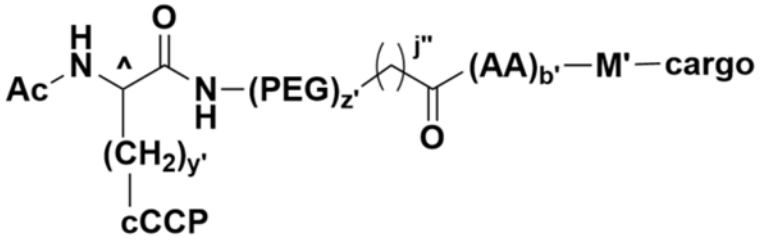
[0029] In examples, the compound of Formula (AA-1) comprises a structure selected from:

Formula (N-1):



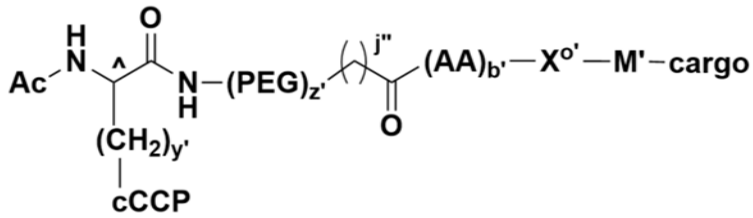
(N-1);

Formula (O-1):



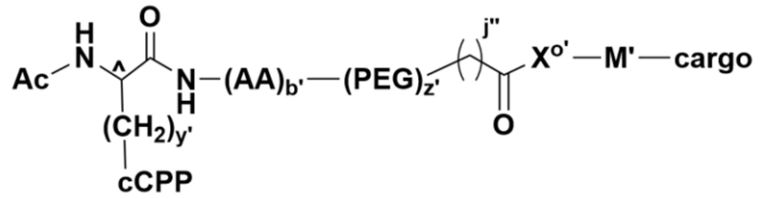
(O-1);

Formula (P-1):



(P-1); or

Formula (Q-1):



(Q-1);

wherein;

Ac is acetyl;

cCPP is a cyclic cell penetrating peptide;

z′ is an integer from 0 to 12;

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

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

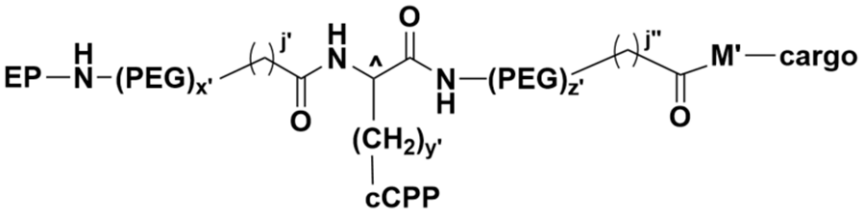
$K^{\#}$ is D-lysine or L-lysine residue;

(AA)b′ is an amino acid (AA) component comprising at least one amino acid residue; and

b′ is an integer from 0 to 12.

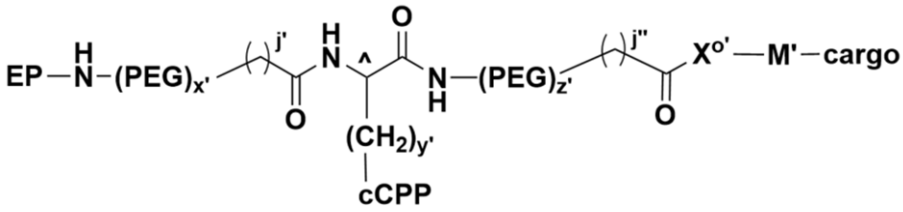
[0030] In examples, the EEV of Formula (A-1) comprises a structure selected from Formula (R-1) and Formula (B-1):

Formula (R-1):



(R-1); or

Formula (B-1):



(B-1);

wherein

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

x′ is an integer from 0 to 12;

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

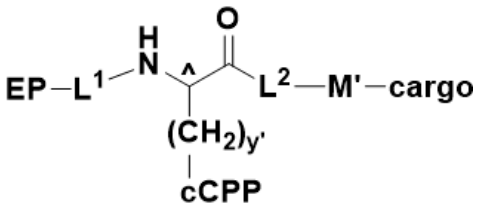
z′ is an integer from 0 to 12;

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

$X^{o\prime}$ comprises a hydrophobic component.

[0031] In examples, the EEV can be conjugated to a cargo to form an EEV-cargo conjugate comprising the structure of Formula (A-1):

Formula (A-1):



(A-1);

wherein

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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

^ indicates D-stereochemistry;

y′ is an integer from 1 to 5;

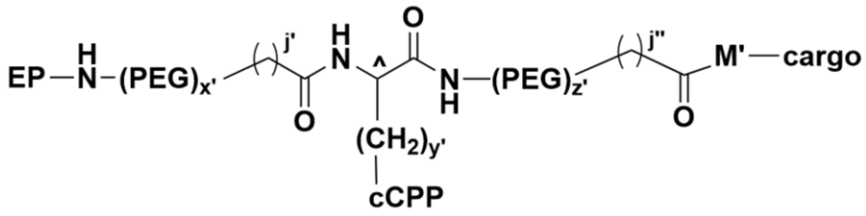
M′ comprises a bonding group; and

cargo is a peptide, an oligonucleotide, a small molecule, or any combinations thereof;

wherein EP comprises all D-amino acids; cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

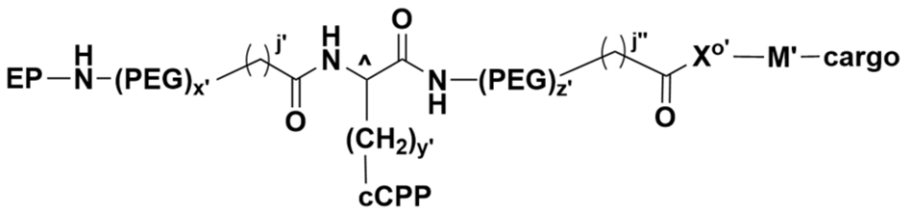
[0032] In examples, the EEV-cargo conjugate of Formula (A-1) comprises a structure selected from Formula (R-1) and Formula (B-1):

Formula (R-1):



(R-1); or

Formula (B-1):



(B-1);

wherein

EP is a linear exocyclic peptide;

cCPP is a cyclic cell penetrating peptide;

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;

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

wherein EP comprises all D-amino acids; cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AA_{sc} conjugates cCPP to the linker.

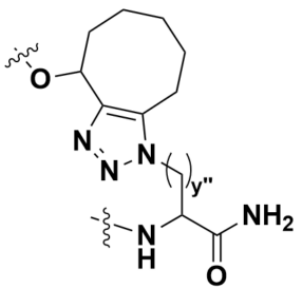
[0033] In examples, the EEV-cargo conjugate comprises a structure selected from Formula (B-1), (BB-1), (C-1), (D-1), (E-1), or (F-1), wherein EP comprises all D-amino acids; and cCPP

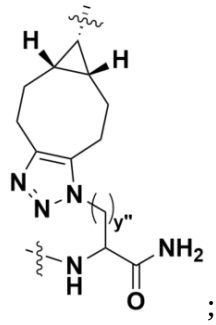
comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

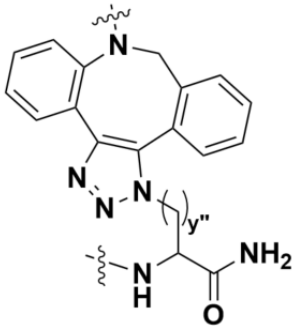
[0034] In examples, the EEV-cargo conjugate comprises a structure selected from Formula (G-1), (H-1), (I-1), (J-1), (JJ-1), (K-1), (L-1), or (M-1), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

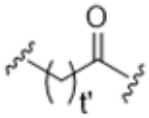
[0035] In examples, the EEV-cargo conjugate comprises a structure selected from Formula (N-1), (O-1), (P-1), or (Q-1), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

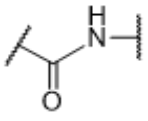
[0036] In examples, the EEV-cargo conjugate comprises a structure selected from Formula (R-1) or (B-1), wherein EP comprises all D-amino acids; and cCPP comprises D-amino acids, achiral amino acids and AAsc, wherein AAsc is an amino acid side chain and AAsc conjugates cCPP to the linker.

[0037] In examples, 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.

[0038] In examples, the cargo is a peptide, oligonucleotide, a small molecule, or a combination thereof. In examples, the cargo is an oligonucleotide. In examples, the oligonucleotide is an antisense oligonucleotide. In examples, the oligonucleotide is a phosphorodiamidate morpholino oligonucleotide (PMO).

[0039] In examples, the EP comprises from 2 to 10 amino acid residues. In examples, the EP comprises from 2 to 8 amino acid residues. In examples, the EP comprises from 2 to 6 amino acid residues. In examples, the EP comprises 6 amino acid residues. In examples, the EP comprises from 7 amino acid residues. In examples, the EP comprises from 8 amino acid residues. In examples, at least 1 amino acid residue comprises a side chain comprising a guanidine group, a terminal amine, an imidazole, or a protonated form thereof. In examples, the EP comprises 1, 2, 3, or 4 lysine residues. In examples, the EP comprises 1, 2, 3 or 4 arginine residues. In examples, the EP comprises 1 or 2 uncharged hydrophobic amino acid residues. In examples, the EP comprises at least 1 uncharged hydrophobic amino acid residues. In examples, the EP comprises at least 2 uncharged hydrophobic amino acid residues. In examples, the EP comprises 1-5 uncharged hydrophobic amino acid residues. In examples, the EP comprises 1-3 uncharged hydrophobic amino acid residues. In examples, the EP comprises 1 uncharged hydrophobic amino acid residue. In examples, the EP comprises 2 uncharged hydrophobic amino acid residues. In examples, the EP comprises 3 uncharged hydrophobic amino acid residues. In examples, the EP comprises 4 uncharged hydrophobic amino acid residues. In examples, the EP comprises 5 uncharged hydrophobic amino acid residues. In examples, the uncharged hydrophobic amino acid residue is selected from valine, proline, β-alanine, glycine, or a combination thereof.

[0040] Also provided herein is a pharmaceutical composition comprising an EEV-cargo conjugate as described herein and a pharmaceutically acceptable carrier and a method of treating a disease in a subject in need thereof comprising administering to the subject an effective amount of the pharmaceutical composition. In examples, administering comprises parenteral administration. In examples, parenteral administration is selected from: subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, intrasternal, and intrathecal administration, and intracerebroventricular injection.

BRIEF DESCRIPTION OF THE DRAWINGS

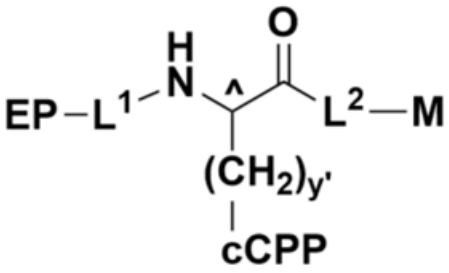
[0041] FIG. 1 shows the effects of linker and conjugation chemistry on efficacy and tolerability.

[0042] FIG. 2 shows the effects of including a hydrophobic component in a linker.

[0043] FIG. 3 shows examples of structural modifications to linkers that mimic the hydrophobic component generated using click chemistry.

CLAIMS

1. An endosomal escape vehicle (EEV) comprising the structure:



(A)

wherein

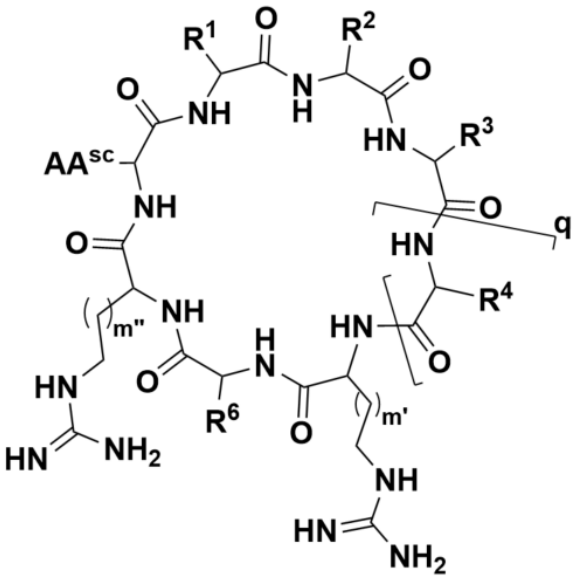
EP is a linear exocyclic peptide comprising from 2 to 8 amino acid residues, comprising

1, 2, 3, or 4 arginine residues,

1, 2, 3, or 4 lysine residues, and

1 or 2 uncharged hydrophobic amino acid residues selected from valine, proline, b-alanine, glycine or a combination thereof;

cCPP is a cyclic cell penetrating peptide of formula (2)



R^1, R^2, and R^3 can each independently be H or an amino acid residue having a side chain comprising an aryl or heteroaryl group;

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

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

AA^{SC} is an amino acid side chain, wherein the cCPP is linked to the -$(CH_2)_{y'}$- group through the AA^{SC} of the cCPP;

m′ is an integer from 0 to 3;

m″ is an integer from 0 to 3; and

q is 1, 2, 3, or 4;

L^1 is a linker arm comprising a polyethylene glycol component (PEG)x′, wherein x′ is an integer from 1-12;

L^2 comprises one or more polyethylene glycol components (PEG)z′ and (PEG)z″, and

(i) one or more amino acid components (AA)b′ and (AA)b″,

(ii) one or more hydrophobic components ($X^{o\prime}$) and ($X^{o\prime\prime}$), or

(iii) combinations thereof

wherein each of $X^{o\prime}$ and $X^{o\prime\prime}$ is independently an amino acid residue with an aromatic side chain,

wherein

each of b′ and b″ is, independently, an integer from 0-12 and

each of z′ and z″ is, independently, an integer from 0-12;

(AA)b′ and (AA)b″ independently comprise one or more amino acid residues selected from lysine (K), arginine (R), histidine (H), glycine (G), β-alanine (B), phenylalanine (F), proline (P), valine (V), or combinations thereof;

^ indicates L- or D-stereochemistry;

y′ is an integer from 1 to 5; and

M comprises a reactive handle.

2. The EEV of claim 1, wherein x′ is 2.

3. The EEV of any of claims 1-2, wherein

b′ is an integer from 1-8; and

b″ is an is an integer from 1-8.

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

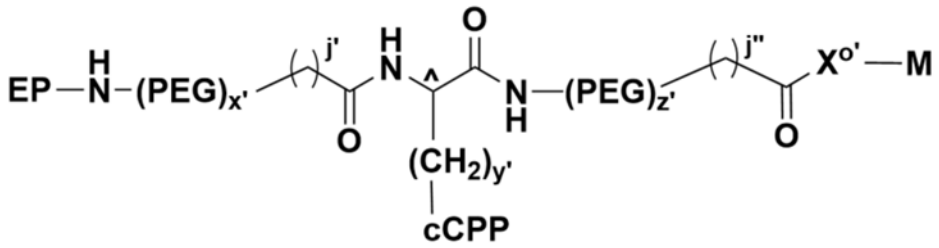
b′ is 2; or

b″ 2.

5. The EEV of any of claims 1-4, wherein ($X^{o\prime}$) and ($X^{o\prime}$) are independently selected from Nal, dNal, or Bip.

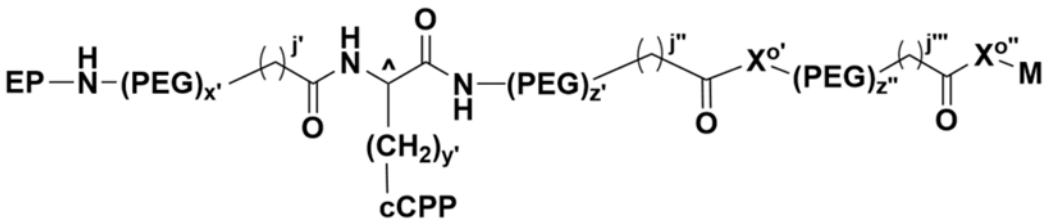
6. The EEV of claim 1, comprising a structure selected from:

Formula (B):



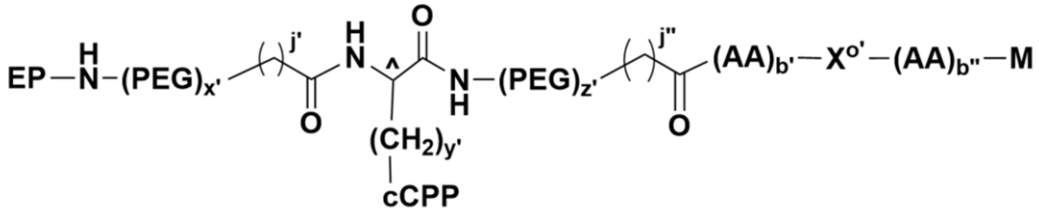
(B);

Formula (BB):



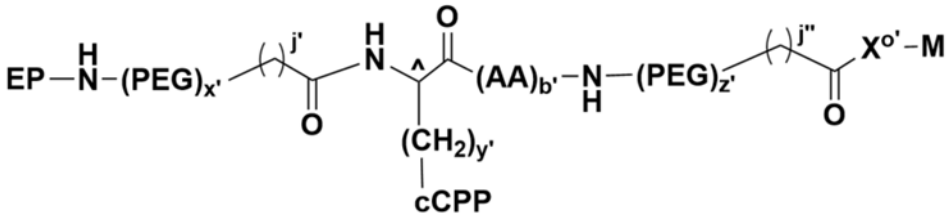
(BB);

Formula (C):



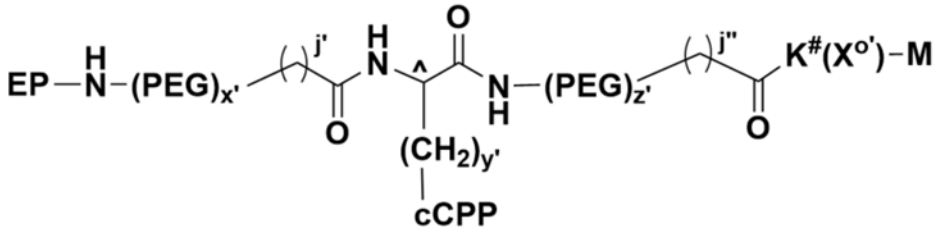
(C);

Formula (D):



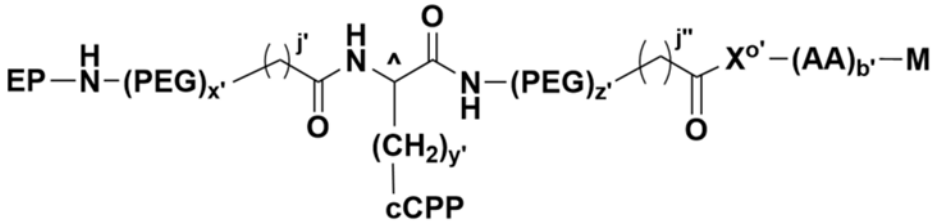
(D);

Formula (E):



(E); and

Formula (F):



(F);

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;

z″ is an integer from 0 to 12;

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

$X^{o\prime}$ and $X^{o\prime\prime}$ each, individually, comprises a hydrophobic component;

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

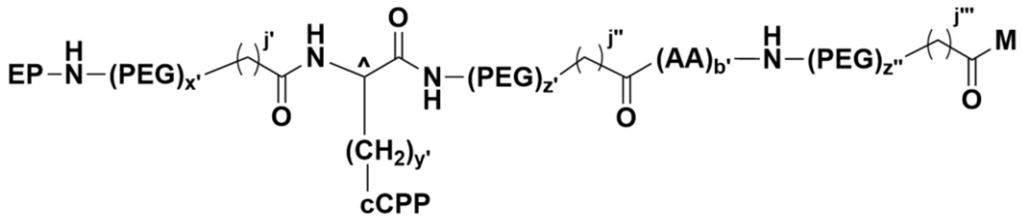
(AA)b′ and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

b′ is an integer from 0 to 12; and

b″ is an integer from 0 to 12.

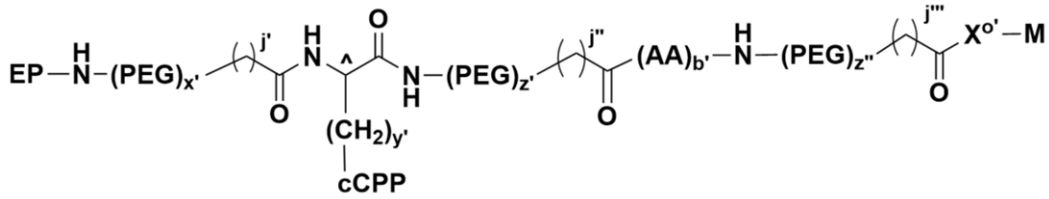
7. The EEV of claim 1, comprising a structure selected from:

Formula (G):



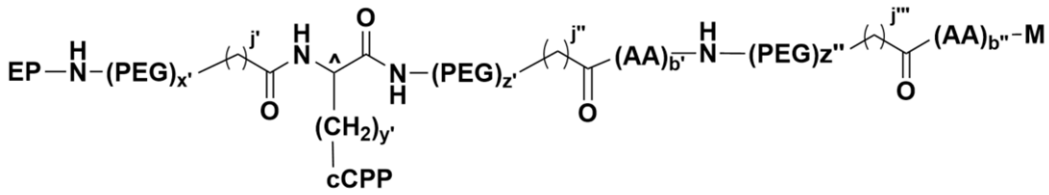
(G);

Formula (H):



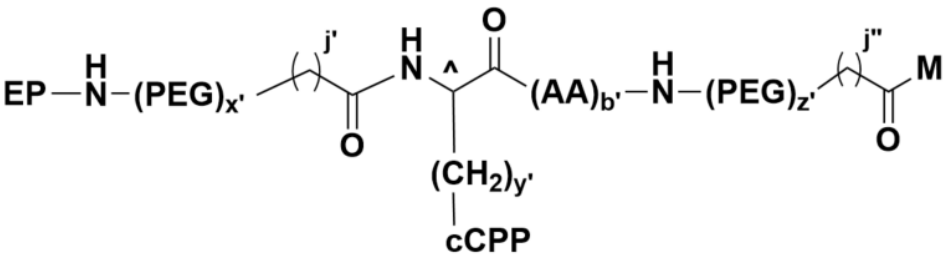
(H);

Formula (I):



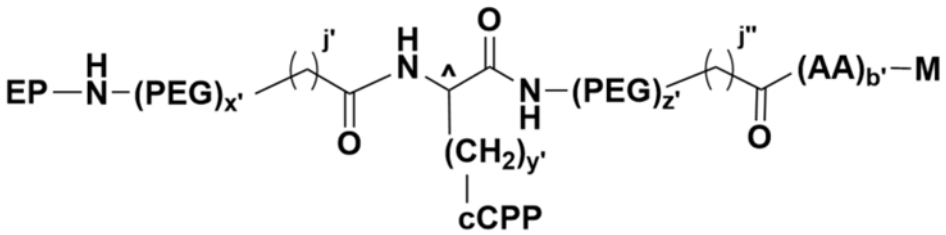
(I);

Formula (K):



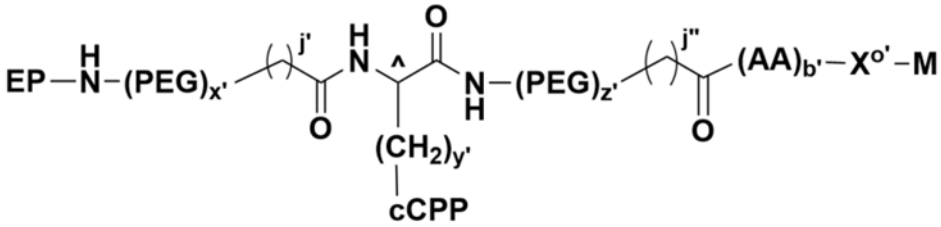
(K);

Formula (L):



(L); and

Formula (M):



(M);

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;

z″ is an integer from 0 to 12;

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

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

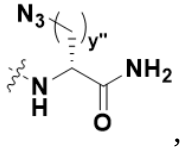
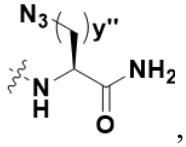
$K^{\#}$ is D-lysine or L-lysine residue;

(AA)a′, (AA)b′, and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

a′ is an integer from 0 to 12;

b′ is an integer from 0 to 12; and

b″ is an integer from 0 to 12.

8. The EEV of any of claims 1 to 8, wherein M comprises -OH, , or , wherein y″ is an integer from 1 to 4.

9. The EEV of any of claims 1-5, wherein L^2 comprises (PEG)z′ and (PEG)z″ and each of z′ and z″ is, independently, 2, 4, 8, or 12.

10. The EEV of any of claims 1-3, wherein b′ and b″ are, independently, an integer from 2-5.

11. The EEV of any of claims 1-10, wherein the EP comprises a sequence selected from:

(a) HBH, HBHBH, or HBRBH;

(b) HHRBFBR;

(c) KBK, KBKBK or KBR;

(d) KGK or KGKK, KKGK;

(e) KKKK, KKRK, KKKKR, KKKRK, or KKKRKG;

(f) KR, KRK, or KRKKK;

(g) PGKKRKV;

(h) PKGKRKV, PKK, PKKGRKV, PKKK, PKKKGKV, PKKKRGV, PKKKRKG, PKKRGV, PKKRKG, or PKKRKV;

(i) RBHBH, RBHBR, RBR, RBRBR, or RBRRBR;

(j) RFR;

(k) RHR;

(l) RK or RKKK;

(m) RR, RF, RRR, RFRRF, or RRRRFFF,

(n) RIRRI;

(o) KRKIL; and

(p) KFK, KRKF, or KKFRK;

wherein the amino acid residues in the EP can have D- or L-stereochemistry.

12. The EEV of any of claims 1-11, wherein the EP has the structure: Ac-PKKKRKV-.

13. The EEV of any of claims 1-12, wherein:

R_1, R_2, and R_3 are a side chain of phenylalanine.

14. The EEV of any of claims 1-12, wherein:

R_1, R_2, and R_3 are each independently a side chain comprising an aryl or heteroaryl group; and

at least two of R_1, R_2, and R_3 are a side chain of phenylalanine.

15. The EEV of any of claims 1-14, wherein R_4 and R_6 are H or an amino acid side chain of arginine, serine, histidine or citrulline.

16. The EEV of any of claims 1-15, wherein R_4 and R_6 are H or an amino acid side chain of arginine.

17. The EEV of any of claims 1-15, wherein R_4 and R_6 are H.

18. The EEV of any of claims 1-12, wherein the cyclic cell penetrating peptide is selected from:

Ff-Nal-GrGrQ;
FfFGRGRQ;
FGFGRGRQ;
GfFGrGrQ;
FGFGRRRQ;
FGFRRRRQ;
FAFARARQ;
FfFRRRRQ;
FfFRrRrQ;
fffrrrrQ;
FfFRrRrQ;
FG-Nal-GRGRQ;
fGfGrGrQ;
fGfGrGrQ;
FGFGRQ;
fGfrrrrQ;
FGWRGRQ;
RGRGRGRQ;
FFFGRGRQ;

FGFRGRGQ;

FRGRGRGQ;

FRFGRGRQ;

FRFRFRFQ;

FGFLRLRQ; and

FGFVRVRQ.

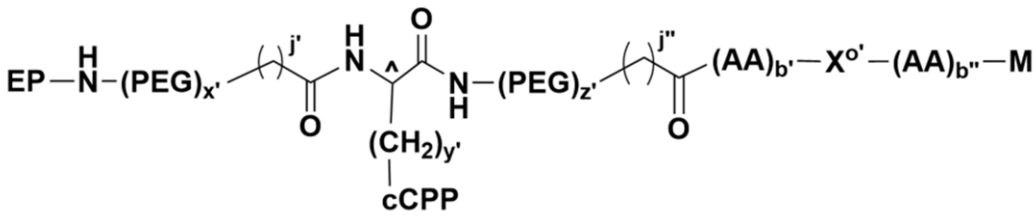
19. The EEV of claim 6, comprising Formula (B), wherein the EEV is selected from:

Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFRRRRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFRRRGQ])-PEG_{12}-Nal-OH
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FGFGRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FGFGRGRQ])-PEG_8-Nal-OH
Ac-PKKKRKV-K(cyclo[FGFGRGRQ])-PEG_4-Nal-OH
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_8-Nal-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_4-Nal-OH
Ac-PKKKRKV-K(cyclo[fFRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FAFARARQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FGFGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FGWRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FfFGRGRQ])-PEG_{12}-Nal-OH
Ac-PKKKRKV-K(cyclo[FG-Nal-GRGRQ])-PEG_{12}-Nal-OH; and
Ac-pkkkrkv-PEG_2-k(cyclo[fGfrrrrQ])-PEG_{12}-Nal-OH.
Ac-RBRRBR-PEG_2-K(cyclo[FfFGRGRQ)]-PEG_{12}-Nal-OH
Ac-RBRRBR-PEG_2-K(cyclo[FfFRRRRQ])-PEG_{12}-Nal-OH
Ac-RBRRBR-PEG_2-K(cyclo[FfFRrRrQ])-PEG_{12}-Nal-OH
Ac-rbrrbr-PEG_2-K(cyclo[fGfGrGrQ)]-PEG_{12}-Nal-OH

Ac-pkkkrkv-PEG_2-k(cyclo[fGfrrrrQ])-PEG_{12}-Nal-OH
Ac-pkkkrkv-PEG_2-k(cyclo[fGfrrrrQ])-PEG_{12}-dNal-OH
Ac-pkkkrkv-PEG_2-k(cyclo[fffrrrrQ])-PEG_{12}-dNal-OH
Ac-pkkkrkv-PEG_2-k(cyclo[FGFRRRRQ])-PEG_{12}-dNal-OH
Ac-pkkkrkv-PEG_2-k(cyclo[fGfGrGrQ])-PEG_{12}-dNal-OH
Ac-PKKKRKV-PEG_2-K(cyclo[RGRGRGRQ])-PEG_{12}-dNal-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-Bip-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-C_6-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-F-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-W-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-Y-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-V-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-I-OH
Ac-PKKKRKV-PEG_2-K(*cyclo*[FGFGRGRQ])-PEG_{12}-L-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_{12}-H-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-G-OH

20. The EEV of claim 6, comprising Formula (C):

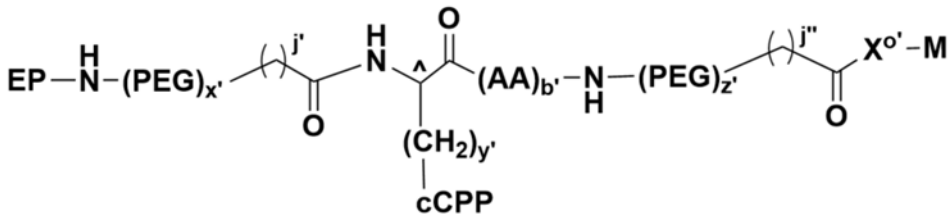
Formula (C):



(C).

21. The EEV of claim 6, comprising Formula (D):

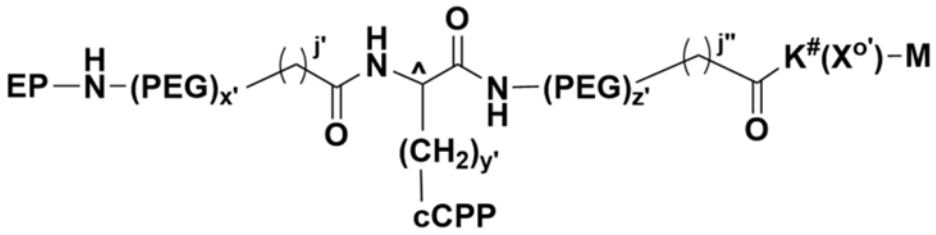
Formula (D):



(D).

22. The EEV of claim 6, comprising Formula (E):

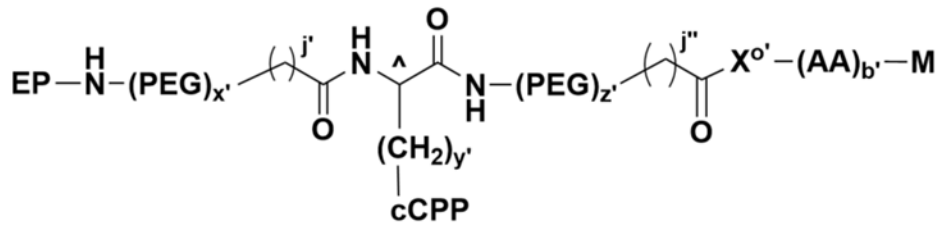
Formula (E):



(E).

23. The EEV of claim 6, comprising Formula (F):

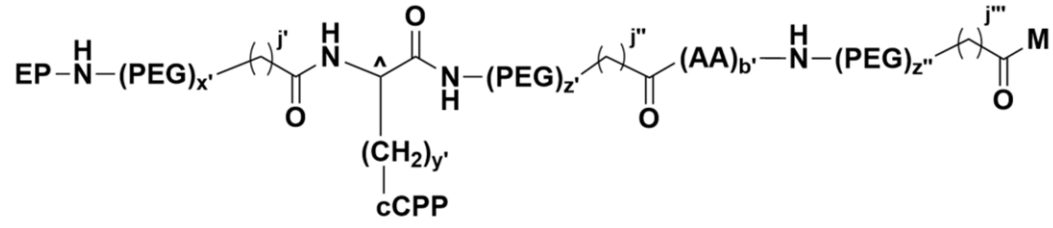
Formula (F):



(F).

24. The EEV of claim 7, comprising Formula (G):

Formula (G):



(G);

wherein:

x′ is 2;

j′ is 1 or 2;

z′ is 2, 4, or 8;

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

z″ is 2, 4 or 8;

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

(AA)b′ comprises an amino acid (AA) component comprising at least one amino acid residue; and

b′ is an integer from 0 to 12.

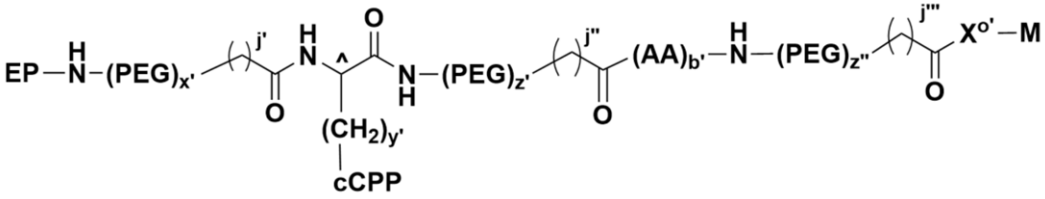
25. The EEV of claim 24, selected from:

Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_2-RFQILY-PEG_2-OH.
Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_2-RF-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-R-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-F-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RRF-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RFR-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RGRRG-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RFRRF-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RBRRBR-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-KKKRK-PEG_4-OH
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RF-PEG_4-OH
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RF-PEG_4-K(N_3)-NH_2
Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_4-RF-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_4-RF-PEG_4-OH
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_4-RF-PEG_4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_8-RF-PEG4-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_8-RF-PEG4-OH
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_8-RF-PEG4-OH
Ac-KKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_8-RF-PEG_4-OH
Ac-KRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_8-RF-PEG_4-OH

Ac-RK-PEG$_2$-K(cyclo[FGFGRGRQ])-PEG$_8$-RF-PEG$_4$-OH
Ac-RF-PEG$_2$-K(cyclo[FGFGRGRQ])-PEG$_8$-RF-PEG$_4$-OH
Ac-RF-PEG$_2$-K(cyclo[FGFGRGRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-RF-PEG$_2$-K(cyclo[FFFGRGRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKFRK-PEG$_2$-K(cyclo[FGFGRGRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKFRK-PEG$_2$-K(cyclo[FFFGRGRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FGRGFGRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FRFRFRFQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FGFLRLRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FGFVRVRQ]-PEG$_2$-RF-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FfFGRGRQ])-PEG$_2$-KRR-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FfFGRGRQ])-PEG$_2$-KKRR-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FfFGRGRQ])-PEG$_2$-KKKRR-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FfFGRGRQ])-PEG$_2$-KRK-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FfFGRGRQ])-PEG$_2$-KKRK-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FfFGRGRQ])-PEG$_2$-KKKRK-PEG$_4$-OH
Ac-KKKRK-PEG$_2$-K(cyclo[FGFGRGRQ])-PEG$_2$-RF-PEG$_4$-OH
Ac-KKRK-PEG$_2$-K(cyclo[FGFGRGRQ])-PEG$_2$-RF-PEG$_4$-OH
Ac-KRK-PEG$_2$-K(cyclo[FGFGRGRQ])-PEG$_2$-RF-PEG$_4$-OH, and
Ac-RK-PEG$_2$-K(cyclo[FGFGRGRQ])-PEG$_2$-RF-PEG$_4$-OH.

26. The EEV of claim 7, comprising Formula (H):

Formula (H):



(H)

wherein:

x′ is 2;

j′ is 1 or 2;

z′ is 2, 4, or 8;

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

z″ is 2, 4, or 8;

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

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

(AA)b′ and (AA)b″ are each an amino acid (AA) component comprising at least one amino acid residue;

b′ is an integer from 0 to 12; and

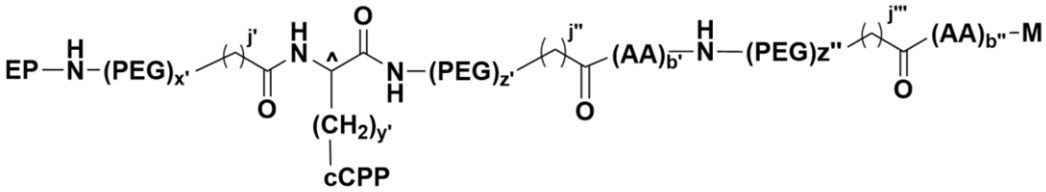
b″ is an integer from 0 to 12.

27. The EEV of claim 26, selected from:

Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_2-RF-PEG_2-Bip-OH.
Ac-PKKKRKV-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RF-PEG_4-Bip-OH
Ac-PKKKRKV-PEG_2-K(cyclo[FfFGRGRQ])-PEG_2-RF-PEG_4-Bip-OH; and
Ac-KKKRK-PEG_2-K(cyclo[FGFGRGRQ])-PEG_2-RF-PEG_4-Bip-OH

28. The EEV of claim 7, comprising Formula (I):

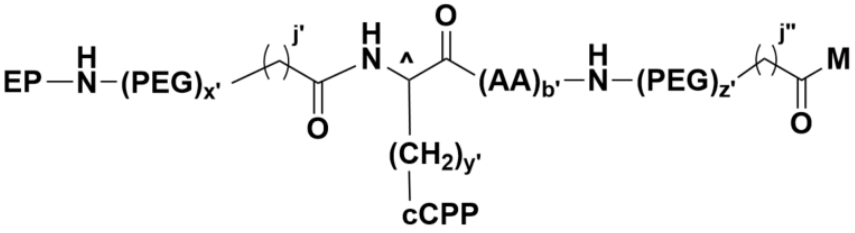
Formula (I):



(I).

29. The EEV of claim 7, comprising Formula (K):

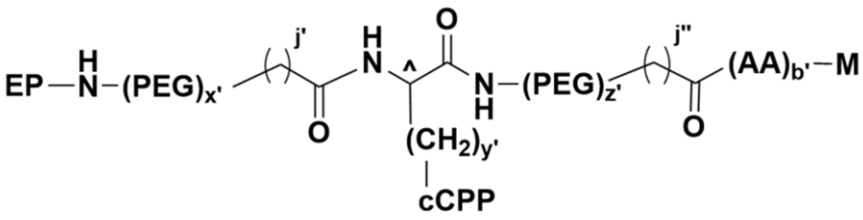
Formula (K):



(K).

30. The EEV of claim 7, comprising formula (L):

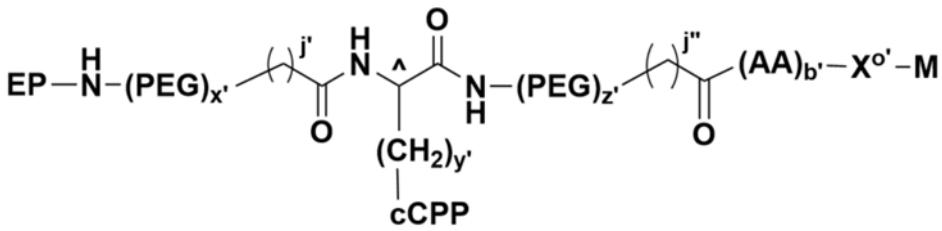
Formula (L):



(L).

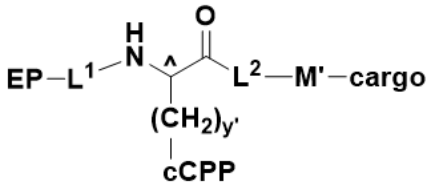
31. The EEV of claim 7, comprising Formula (M):

Formula (M):



(M).

32. An EEV-cargo conjugate comprising the EEV of any of claims 1-31 conjugated to a cargo, wherein the EEV-cargo conjugate has the structure of Formula (A-1):



(A-1)

wherein M' is a bonding group.

33. The EEV-cargo conjugate of claim 32, wherein the cargo is a peptide, oligonucleotide, a small molecule, or a combination thereof.

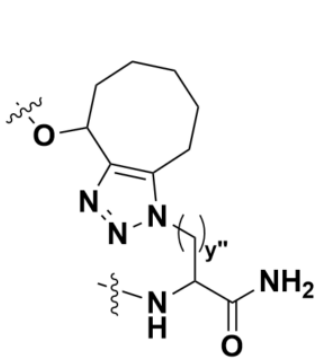
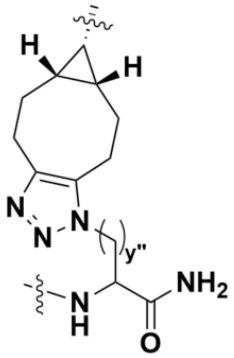
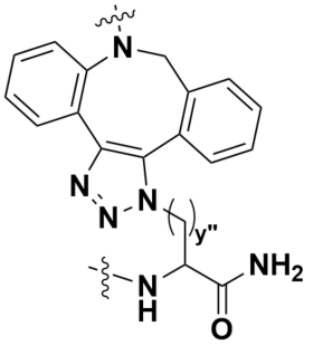
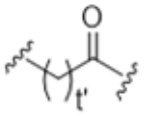
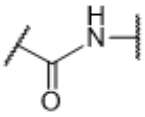
34. The EEV-cargo conjugate of claim 33, wherein the cargo is an oligonucleotide.

35. The EEV-cargo conjugate of claim 33 or 34, wherein the oligonucleotide is an antisense

oligonucleotide.

36. The EEV-cargo conjugate of claim 33 or 34, wherein the oligonucleotide is a phosphorodiamidate morpholino oligonucleotide (PMO).

37. The EEV-cargo conjugate of any of claims 33-36, wherein M’ is covalently bound to the 5' end of the oligonucleotide, the 3’ end of the oligonucleotide, or the backbone of the oligonucleotide.

38. The EEV-cargo conjugate of any of claims 32-37, wherein M′ comprises: -N(H), C(O), -O-, ; ; ; , or , wherein y″ is an integer from 1 to 4 and t′ is 0 to 10.

39. A pharmaceutical composition comprising the EEV-cargo conjugate of any of claims 32-38 and a pharmaceutically acceptable carrier.

40. Use of the pharmaceutical composition of claim 39 or the EEV-cargo conjugate of any of claims 32-38 in the manufacture of a medicament for treating a disease in a subject in need thereof.

41. The use of claim 40, wherein the medicament is to be administered via parenteral administration.

42. The use of claim 41, wherein parenteral administration is selected from: subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, intrasternal, intrathecal and intracerebroventricular administration.

43. The pharmaceutical composition of claim 39 or the EEV-cargo conjugate of any of claims 32-38 for use in therapy.