

CLAIMS:

1. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, the shuttle agent being:

- (1) a peptide at least 17, 18, 19, or 20 amino acids in length comprising
- (2) an amphipathic alpha-helical motif having
- (3) a positively-charged hydrophilic outer face, and a hydrophobic outer face,

wherein at least five of the following parameters (4) to (15) are respected:

- (4) the hydrophobic outer face comprises a highly hydrophobic core consisting of spatially adjacent L, I, F, V, W, and/or M amino acids representing 12 to 50% of the amino acids of the peptide, based on an open cylindrical representation of the alpha-helix having 3.6 residues per turn;
- (5) the peptide has a hydrophobic moment (μ) of 3.5 to 11;
- (6) the peptide has a predicted net charge of at least +4 at physiological pH;
- (7) the peptide has an isoelectric point (pI) of 8 to 13;
- (8) the peptide is composed of 35% to 65% of any combination of the amino acids: A, C, G, I, L, M, F, P, W, Y, and V;
- (9) the peptide is composed of 0% to 30% of any combination of the amino acids: N, Q, S, and T;
- (10) the peptide is composed of 35% to 85% of any combination of the amino acids: A, L, K, or R;
- (11) the peptide is composed of 15% to 45% of any combination of the amino acids: A and L, provided there being at least 5% of L in the peptide;
- (12) the peptide is composed of 20% to 45% of any combination of the amino acids: K and R;
- (13) the peptide is composed of 0% to 10% of any combination of the amino acids: D and E;
- (14) the difference between the percentage of A and L residues in the peptide (% A+ L), and the percentage of K and R residues in the peptide (K + R), is less than or equal to 10%; and
- (15) the peptide is composed of 10% to 45% of any combination of the amino acids: Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T and H,

wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent by at least 3, 3.5, 4, 4.5, 5,

5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold over a corresponding negative control lacking said shuttle agent, and/or enables a transduction efficiency of at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells;
and/or

wherein the shuttle agent increases the transduction efficiency of GFP-NLS by at least 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold over a corresponding negative control lacking said shuttle agent, and/or enables a transduction efficiency of at least 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of GFP-NLS, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells; and

wherein the shuttle agent comprises or consists of:

- (a) the amino acid sequence of **SEQ ID NO: 35** or any one of **SEQ ID NOs: 1 to 34 or 36 to 50, 58 to 78, 80 to 107, 109 to 139, 141 to 146, 149 to 161, 163 to 169, 171, 174 to 234, 236 to 240, 242 to 260, 262 to 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344;**
- (b) an amino acid sequence that differs from the amino acid sequence of (a) by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids excluding any linker domains; or
- (c) an amino acid sequence that is at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of (a), calculated excluding any linker domains.

2. The synthetic peptide shuttle agent of claim 1, wherein:

- (a) the shuttle agent respects at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or respects all of parameters (4) to (15);
- (b) the shuttle agent is a peptide having a minimum length of 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids, and a maximum length of 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 60, 65, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids;
- (c) said amphipathic alpha-helical motif has a hydrophobic moment (μ) between a lower limit of 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and an upper limit of 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, or 11.0;
- (d) said amphipathic alpha-helical motif comprises a positively-charged hydrophilic outer face comprising: (i) at least two, three, or four adjacent positively-charged K and/or R residues upon helical wheel projection; and/or (ii) a segment of six adjacent residues comprising three to five K and/or R residues upon helical wheel projection, based on an alpha helix having angle of rotation between consecutive amino acids of 100 degrees and/or an alpha-helix having 3.6 residues per turn;
- (e) said amphipathic alpha-helical motif comprises a hydrophobic outer face comprising: (i) at least two adjacent L residues upon helical wheel projection; and/or (ii) a segment of ten adjacent residues comprising at least five hydrophobic residues selected from: L, I, F, V, W, and M, upon helical wheel projection, based on an alpha helix having angle of rotation between consecutive amino acids of 100 degrees and/or an alpha-helix having 3.6 residues per turn;
- (f) said hydrophobic outer face comprises a highly hydrophobic core consisting of spatially adjacent L, I, F, V, W, and/or M amino acids representing from 12.5%, 13%, 13.5%, 14%, 14.5%, 15%, 15.5%, 16%, 16.5%, 17%, 17.5%, 18%, 18.5%, 19%, 19.5%, or 20%, to 25%, 30%, 35%, 40%, or 45% of the amino acids of the shuttle agent;
- (g) the shuttle agent has a hydrophobic moment (μ) between a lower limit of 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, and an upper limit of 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.1, 10.2, 10.3, 10.4, or 10.5;
- (h) the shuttle agent has a predicted net charge of between +4, +5, +6, +7, +8, +9, to +10, +11, +12, +13, +14, or +15;

- (i) the shuttle agent has a predicted pI of 10 to 13; or
- (j) any combination of (a) to (i).

3. The synthetic peptide shuttle agent of claim 1 or 2, wherein said shuttle agent respects at least one, at least two, at least three, at least four, at least five, at least six, or all of the following parameters:

- (8) the shuttle agent is composed of 36% to 64%, 37% to 63%, 38% to 62%, 39% to 61%, or 40% to 60% of any combination of the amino acids: A, C, G, I, L, M, F, P, W, Y, and V;
- (9) the shuttle agent is composed of 1% to 29%, 2% to 28%, 3% to 27%, 4% to 26%, 5% to 25%, 6% to 24%, 7% to 23%, 8% to 22%, 9% to 21%, or 10% to 20% of any combination of the amino acids: N, Q, S, and T;
- (10) the shuttle agent is composed of 36% to 80%, 37% to 75%, 38% to 70%, 39% to 65%, or 40% to 60% of any combination of the amino acids: A, L, K, or R;
- (11) the shuttle agent is composed of 15% to 40%, 20% to 40%, 20 to 35%, or 20 to 30% of any combination of the amino acids: A and L;
- (12) the shuttle agent is composed of 20% to 40%, 20 to 35%, or 20 to 30% of any combination of the amino acids: K and R;
- (13) the shuttle agent is composed of 5 to 10% of any combination of the amino acids: D and E;
- (14) the difference between the percentage of A and L residues in the shuttle agent (% A + L), and the percentage of K and R residues in the shuttle agent (K + R), is less than or equal to 9%, 8%, 7%, 6%, or 5%; and
- (15) the shuttle agent is composed of 15 to 40%, 20% to 35%, or 20% to 30% of any combination of the amino acids: Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T, and H.

4. The synthetic peptide shuttle agent of any one of claims 1 to 3, wherein said shuttle agent:

- (i) comprises a histidine-rich domain, optionally wherein the histidine-rich domain is: as defined in claim 8 (a) positioned towards the N terminus and/or towards the C terminus of the shuttle agent; (b) is a stretch of at least 3, at least 4, at least 5, or at least 6 amino acids comprising at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% histidine residues; and/or comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 consecutive histidine residues; or (c) both (a) and (b);

- (ii) comprises a flexible linker domain rich in serine and/or glycine residues; or
- (iii) both (i) and (ii).

5. The synthetic peptide shuttle agent of any one of claims 1 to 4, wherein the synthetic peptide: is a cyclic peptide; comprises one or more D-amino acids; and/or further comprises a chemical modification to one or more amino acids, wherein the chemical modification does not destroy the transduction activity of the synthetic peptide shuttle agent.

6. The synthetic peptide shuttle agent of claim 5, wherein the chemical modification is at the N and/or C terminus of the shuttle agent; and/or wherein the chemical modification is the addition of an acetyl group, a cysteamide group, or a fatty acid.

7. A synthetic peptide shuttle agent having transduction activity for both proteinaceous and non-proteinaceous cargoes in target eukaryotic cells, wherein the shuttle agent comprises or consists of:

- the amino acid sequence as defined in claim 1(a); or
- an amino acid sequence that differs from the amino acid sequence as defined in claim 1(a) by only conservative amino acid substitutions;

wherein shuttle agent: increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent by at least 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold over a corresponding negative control lacking said shuttle agent; and/or enables a transduction efficiency of at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells.

8. The synthetic peptide shuttle agent of claim 7, wherein each conservative amino acid substitution is selected from an amino acid within the same amino acid class, the amino acid class being: Aliphatic: G, A, V, L, and I; Hydroxyl or sulfur/selenium-containing: S, C, U, T, and M; Aromatic: F, Y, and W; Basic: H, K, and R; Acidic and their amides: D, E, N, and Q.

9. A synthetic peptide shuttle agent variant having transduction activity for proteinaceous and/or non-proteinaceous cargoes in target eukaryotic cells, the synthetic peptide shuttle agent variant being identical to the synthetic peptide shuttle agent as defined in any one of claims 1 to 8, except having at least one amino acid being replaced with a corresponding synthetic amino acid having a side chain of similar physiochemical properties as the amino acid being replaced, wherein the shuttle agent variant increases the transduction efficiency of said cargo in the target eukaryotic cells, as compared to in the absence of the shuttle agent variant, wherein the synthetic amino acid replacement:
- (a) replaces a basic amino acids with any one of: α -aminoglycine, α,γ -diaminobutyric acid, ornithine, α,β -diaminopropionic acid, 2,6-diamino-4-hexynoic acid, β -(1-piperazinyl)-alanine, 4,5-dehydro-lysine, δ -hydroxylysine, ω,ω -dimethylarginine, homoarginine, ω,ω' -dimethylarginine, ω -methylarginine, β -(2-quinolyl)-alanine, 4-aminopiperidine-4-carboxylic acid, α -methylhistidine, 2,5-diiodohistidine, 1-methylhistidine, 3-methylhistidine, spinacine, 4-aminophenylalanine, 3-aminotyrosine, β -(2-pyridyl)-alanine, or β -(3-pyridyl)-alanine;
 - (b) replaces a non-polar (hydrophobic) amino acid with any one of: dehydro-alanine, β -fluoroalanine, β -chloroalanine, β -iodoalanine, α -aminobutyric acid, α -aminoisobutyric acid, β -cyclopropylalanine, azetidine-2-carboxylic acid, α -allylglycine, propargylglycine, tert-butylalanine, β -(2-thiazolyl)-alanine, thiaproline, 3,4-dehydroproline, tert-butylglycine, β -cyclopentylalanine, β -cyclohexylalanine, α -methylproline, norvaline, α -methylvaline, penicillamine, β , β -dicyclohexylalanine, 4-fluoroproline, 1-aminocyclopentanecarboxylic acid, pipecolic acid, 4,5-dehydroleucine, allo-isoleucine, norleucine, α -methylleucine, cyclohexylglycine, cis-octahydroindole-2-carboxylic acid, β -(2-thienyl)-alanine, phenylglycine, α -methylphenylalanine, homophenylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, β -(3-benzothienyl)-alanine, 4-nitrophenylalanine, 4-bromophenylalanine, 4-tert-butylphenylalanine, α -methyltryptophan, β -(2-naphthyl)-alanine, β -(1-naphthyl)-alanine, 4-iodophenylalanine, 3-fluorophenylalanine, 4-fluorophenylalanine, 4-methyltryptophan, 4-chlorophenylalanine, 3,4-dichloro-phenylalanine, 2,6-difluoro-phenylalanine, n-in-methyltryptophan, 1,2,3,4-tetrahydronorharman-3-carboxylic acid, β,β -diphenylalanine, 4-methylphenylalanine, 4-phenylphenylalanine, 2,3,4,5,6-pentafluoro-phenylalanine, or 4-benzoylphenylalanine;
 - (c) replaces a polar, uncharged amino acid with any one of: β -cyanoalanine, β -ureidoalanine, homocysteine, allo-threonine, pyroglutamic acid, 2-oxothiazolidine-4-

carboxylic acid, citrulline, thiocitrulline, homocitrulline, hydroxyproline, 3,4-dihydroxyphenylalanine, β -(1,2,4-triazol-1-yl)-alanine, 2-mercaptohistidine, β -(3,4-dihydroxyphenyl)-serine, β -(2-thienyl)-serine, 4-azidophenylalanine, 4-cyanophenylalanine, 3-hydroxymethyltyrosine, 3-iodotyrosine, 3-nitrotyrosine, 3,5-dinitrotyrosine, 3,5-dibromotyrosine, 3,5-diiodotyrosine, 7-hydroxy-1,2,3,4-tetrahydroiso-quinoline-3-carboxylic acid, 5-hydroxytryptophan, thyronine, β -(7-methoxycoumarin-4-yl)-alanine, or 4-(7-hydroxy-4-coumarinyl)-aminobutyric acid; and/or

- (d) replaces an acidic amino acid with any one of: γ -hydroxyglutamic acid, γ -methyleneglutamic acid, γ -carboxyglutamic acid, α -aminoadipic acid, 2-aminoheptanedioic acid, α -aminosuberic acid, 4-carboxyphenylalanine, cysteic acid, 4-phosphonophenylalanine, or 4-sulfomethylphenylalanine.

10. The synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9:

- when used in an *in vitro* or *ex vivo* method for increasing the transduction efficiency of a proteinaceous and/or non-proteinaceous cargo into target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used at a concentration sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant; or
- for use in therapy, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant transduces a therapeutically active proteinaceous and/or non-proteinaceous cargo to the cytosol and/or nucleus of target eukaryotic cells, wherein the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant is used at a concentration sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent.

11. An *in vitro* or *ex vivo* method for proteinaceous and/or non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with the cargo and a concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9 sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of said synthetic peptide shuttle agent.

12. A composition for use in therapy, the composition comprising the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9 formulated with a therapeutically active proteinaceous and/or non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

13. Use of a composition in the manufacture of a medicament for treating a disease treatable by intracellular delivery of a therapeutically active proteinaceous and/or non-proteinaceous cargo, wherein the composition comprises the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9 formulated with the therapeutically active proteinaceous and/or non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent.

14. A process for producing a candidate synthetic peptide shuttle agent expected to have transduction activity for a cargo of interest in target eukaryotic cells, the method comprising synthesizing a peptide which is:

- (1) a peptide at least 17, 18, 19, or 20 amino acids in length comprising
- (2) an amphipathic alpha-helical motif having
- (3) a positively-charged hydrophilic outer face, and a hydrophobic outer face,

wherein at least five of the following parameters (4) to (15) are respected:

- (4) the hydrophobic outer face comprises a highly hydrophobic core consisting of spatially adjacent L, I, F, V, W, and/or M amino acids representing 12 to 50% of the amino acids of the peptide, based on an open cylindrical representation of the alpha-helix having 3.6 residues per turn;
- (5) the peptide has a hydrophobic moment (μ) of 3.5 to 11;
- (6) the peptide has a predicted net charge of at least +4 at physiological pH;
- (7) the peptide has an isoelectric point (pI) of 8 to 13;

- (8) the peptide is composed of 35% to 65% of any combination of the amino acids: A, C, G, I, L, M, F, P, W, Y, and V;
- (9) the peptide is composed of 0% to 30% of any combination of the amino acids: N, Q, S, and T;
- (10) the peptide is composed of 35% to 85% of any combination of the amino acids: A, L, K, or R;
- (11) the peptide is composed of 15% to 45% of any combination of the amino acids: A and L, provided there being at least 5% of L in the peptide;
- (12) the peptide is composed of 20% to 45% of any combination of the amino acids: K and R;
- (13) the peptide is composed of 0% to 10% of any combination of the amino acids: D and E;
- (14) the difference between the percentage of A and L residues in the peptide (% A+ L), and the percentage of K and R residues in the peptide (K + R), is less than or equal to 10%; and
- (15) the peptide is composed of 10% to 45% of any combination of the amino acids: Q, Y, W, P, I, S, G, V, F, E, D, C, M, N, T and H,

wherein the shuttle agent increases the transduction efficiency of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent by at least 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold over a corresponding negative control lacking said shuttle agent, and/or enables a transduction efficiency of at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of propidium iodide or other membrane-impermeable fluorescent DNA intercalating agent, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells; and/or

wherein the shuttle agent increases the transduction efficiency of GFP-NLS by at least 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10-fold over a corresponding negative control lacking said shuttle agent, and/or enables a transduction efficiency of at least 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%,

42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60% of GFP-NLS, in a eukaryotic cell line model suitable for assessing cargo transduction in said target eukaryotic cells; and

wherein the shuttle agent comprises or consists of:

- (a) the amino acid sequence of **SEQ ID NO: 35** or any one of **SEQ ID NOs: 1 to 34 or 36 to 50, 58 to 78, 80 to 107, 109 to 139, 141 to 146, 149 to 161, 163 to 169, 171, 174 to 234, 236 to 240, 242 to 260, 262 to 285, 287 to 294, 296 to 300, 302 to 308, 310, 311, 313 to 324, 326 to 332, 338 to 342, or 344;**
- (b) an amino acid sequence that differs from the amino acid sequence of (a) by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids; or
- (c) an amino acid sequence that is at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of (a), calculated excluding any linker domains.

15. An *in vitro* or *ex vivo* method for non-proteinaceous cargo transduction, the method comprising contacting target eukaryotic cells with a non-proteinaceous cargo and a concentration of a synthetic peptide shuttle agent sufficient to increase the transduction efficiency of said non-proteinaceous cargo, as compared to in the absence of said synthetic peptide shuttle agent, wherein the non-proteinaceous cargo:

- (a) is an organic compound;
- (b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;
- (c) is a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target;
- (d) is not a biopolymer, such as a polynucleotide or a polysaccharide;
- (e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or
- (f) any combination of (a) to (e).

16. The method of claim 15, wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.

17. A synthetic peptide shuttle agent:

- when used in an *in vitro* or *ex vivo* method for increasing the transduction efficiency of a non-proteinaceous cargo into target eukaryotic cells, wherein the synthetic peptide shuttle agent is used at a concentration sufficient to increase the transduction efficiency and cytosolic and/or nuclear delivery of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent; or
- when used in therapy, wherein the synthetic peptide shuttle agent transduces a therapeutically active non-proteinaceous cargo to the cytosol and/or nucleus of target eukaryotic cells, wherein the synthetic peptide shuttle agent is used at a concentration sufficient to increase the transduction efficiency of the cargo into the target eukaryotic cells, as compared to in the absence of the synthetic peptide shuttle agent;

wherein the non-proteinaceous cargo:

- (a) is an organic compound;
- (b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;
- (c) is a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target;
- (d) is not a biopolymer, such as a polynucleotide or a polysaccharide;
- (e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or
- (f) any combination of (a) to (e).

18. The synthetic peptide shuttle agent for use of claim 17, wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.

19. A composition for use in therapy, the composition comprising a synthetic peptide shuttle agent formulated with a therapeutically active non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and

cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent, and wherein the non-proteinaceous cargo:

- (a) is an organic compound;
- (b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;
- (c) is a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target;
- (d) is not a biopolymer, such as a polynucleotide or a polysaccharide;
- (e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or
- (f) any combination of (a) to (e).

20. The composition for the use of claim 19, wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.

21. Use of a composition in the manufacture of a medicament for treating a disease treatable by intracellular delivery of a therapeutically active non-proteinaceous cargo, wherein the composition comprises a synthetic peptide shuttle agent formulated with the therapeutically active non-proteinaceous cargo to be transduced into target eukaryotic cells by the synthetic peptide shuttle agent, wherein the concentration of the synthetic peptide shuttle agent in the composition is sufficient to increase the transduction efficiency and cytosolic delivery of the cargo into said target eukaryotic cells upon administration, as compared to in the absence of said synthetic peptide shuttle agent, and wherein the non-proteinaceous cargo:

- (a) is an organic compound;
- (b) has a molecular weight of less than 10 000, 9000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, or 1000 Da, or between 50 to 5000, 50 to 4000, 50 to 3000, 50 to 2000, or 50 to 1000 Da;
- (c) is a small molecule, such as a small molecule drug that binds to an intracellular biological or therapeutic target;
- (d) is not a biopolymer, such as a polynucleotide or a polysaccharide;
- (e) is not covalently linked to the synthetic peptide shuttle agent at the moment of transduction; or

(f) any combination of (a) to (e).

22. The use of claim 21, wherein the synthetic peptide shuttle agent is the synthetic peptide shuttle agent or synthetic peptide shuttle agent variant as defined in any one of claims 1 to 9.