CLAIMS

1. A composition comprising:

(a) a membrane impermeable cargo that binds or is to be delivered to an intracellular biological target; and

(b) a bioconjugate for mediating cytosolic/nuclear or intracellular delivery of said cargo, the bioconjugate comprising a synthetic peptide shuttle agent conjugated to a biocompatible non-anionic hydrophilic polymer,

wherein:

- the biocompatible non-anionic hydrophilic polymer is further conjugated to the membrane impermeable cargo via a cleavable or non-cleavable linkage;
- the concentration of the bioconjugate in the composition is sufficient to achieve increased delivery of the cargo to the intracellular biological target, as compared to a corresponding composition comprising an unconjugated synthetic peptide shuttle agent;
- the synthetic peptide shuttle agent comprises a core amphipathic alpha-helical motif at least 12 amino acids long having a solvent-exposed surface comprising a discrete positively-charged hydrophilic face and a discrete hydrophobic face (shuttle agent core motif); and
- the cargo is or comprises a peptide, recombinant protein, nucleoprotein, polysaccharide, small molecule, a charge-neutral polynucleotide analog moiety having a phosphorodiamidate backbone, an amide backbone, a methylphosphonate backbone, a neutral phosphotriester backbone, a sulfone backbone, or a triazole backbone, a cationic polynucleotide analog moiety, or any combination thereof.

2. The composition of claim 1, wherein the biocompatible non-anionic polymer is conjugated to the synthetic peptide shuttle agent N- and/or C-terminal with respect to said shuttle agent core motif.

3. The composition of claim 1 or 2, wherein conjugation of the biocompatible non-anionic hydrophilic polymer to the shuttle agent:

(i) raises the minimum effective concentration of the shuttle agent as compared to a corresponding unconjugated shuttle agent;

(ii) reduces the cytotoxicity of the shuttle agent as compared to a corresponding unconjugated shuttle agent;

(iii) broadens the effective concentration window of the shuttle agent as compared to a corresponding unconjugated shuttle agent;

(iv) alters the *in vivo* biodistribution of the shuttle agent and/or cargo as compared to a corresponding unconjugated shuttle agent; or

(v) any combination of (i) to (iv).

4. The composition of any one of claims 1 to 3, wherein the concentration of the bioconjugate in the composition is at least 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 μM.

5. The composition of any one of claims 1 to 4, wherein the biocompatible non-anionic hydrophilic polymer has a linear, branched, hyper-branched, or dendritic structure.

6. The composition of any one of claims 1 to 5, wherein the biocompatible non-anionic hydrophilic polymer is a polyether moiety, a polyester moiety, a polyoxazoline moiety, a polyvinylpyrrolidone moiety, a polyglycerol moiety, a polysaccharide moiety or any non-anionic derivative thereof, a hydrophilic peptide or polypeptide linker moiety, a polysiloxane moiety, a polylysine moiety, a charge-neutral polynucleotide analog moiety having a phosphorodiamidate backbone, an amide backbone, a methylphosphonate backbone, a neutral phosphotriester backbone, a sulfone backbone, or a triazole backbone; or a cationic polynucleotide analog moiety having an aminoalkylated phosphoramidate backbone, a guanidinium backbone, an S-methylthiourea backbone, a nucleosyl amino acid (NAA) backbone, or any combination thereof.

7. The composition of any one of claims 1 to 6, wherein the biocompatible non-anionic hydrophilic polymer comprises a polyethylene glycol (PEG) moiety and/or a polyester moiety.

8. The composition of any one of claims 1 to 7, wherein biocompatible non-anionic polymer:

(a) has a mass of at least 1-, 2-, 3-, 4-, 5-, 6-, 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-, or 40-fold of the mass of the synthetic peptide shuttle agent;

(b) has a mass of between 1-, 2-, 3-, 4-, 5-fold to 6-, 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-, or 40-fold of the mass of the synthetic peptide shuttle agent;

(c) has a mass of between about 1 to 80 kDa, 1 to 70 kDa, 1 to 60 kDa, 1 to 50 kDa, 1 to 40 kDa, 2 to 80 kDa, 2 to 70 kDa, 2 to 60 kDa, 2 to 50 kDa, 2 to 40 kDa, 3 to 80 kDa, 3 to 70 kDa, 3 to 60 kDa, 3 to 50 kDa, 3 to 40 kDa, 4 to 80 kDa, 4 to 70 kDa, 4 to 60 kDa, 4 to 50 kDa, 4 to 40 kDa, 5 to 80 kDa, 5 to 70 kDa, 5 to 60 kDa, 5 to 50 kDa, 5 to 40 kDa, 5 to 35 kDa, 10 to 35 kDa, 10 to 30 kDa, 10 to 25 kDa, or 10 to 20 kDa; or

(d) any combination of (a) to (c).

9. The composition of any one of claims 1 to 8, wherein the biocompatible non-anionic hydrophilic polymer is conjugated to the synthetic peptide shuttle agent via a cleavable or non-cleavable linkage.

10. The composition of any one of claims 1 to 9, wherein the bioconjugate is a multimer comprising at least two synthetic peptide shuttle agents tethered together via said biocompatible non-anionic hydrophilic polymer.

11. The composition of claim 10, wherein:

(a) the multimer tethers together at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 synthetic peptide shuttle agents; tethers together up to 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, 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, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, or 256 synthetic peptide shuttle agents; and/or tethers together up to 2^n synthetic peptide shuttle agents, wherein n is any integer from 2 to 8; and/or

(b) the synthetic peptide shuttle agent monomer concentration in the composition is at least 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410,

420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1500, 2000, 2500, or 3000 μM.

12. The composition of claim 10 or 11, wherein the multimer comprises a branched, hyper-branched, or dendritic PEG or polyester core.

13. The composition of any one of claims 10 to 12, wherein the biocompatible non-anionic hydrophilic polymer comprises cleavable linkages enabling untethering of synthetic peptide shuttle agents following administration.

14. The composition of any one of claims 1 to 13, wherein the cargo is covalently linked to the synthetic peptide shuttle agent(s) and/or to the biocompatible non-anionic hydrophilic polymer via a cleavable bond such that the cargo detaches therefrom through cleavage of said bond following administration when exposed to the reducing cellular environment, and/or but prior to, simultaneously with, or shortly after being delivered intracellularly.

15. The composition of any one of claims 1 to 14, wherein the cargo is or comprises a therapeutic cargo and/or a diagnostic cargo.

16. The composition of any one of claims 1 to 15, wherein the cargo is or comprises: a recombinant protein which is an enzyme, an antibody or antibody conjugate or antigen-binding fragment thereof, a transcription factor, a hormone, a growth factor; a nucleoprotein cargo which is a deoxyribonucleoprotein (DNP) or ribonucleoprotein (RNP) cargo.

17. The composition of any one of claims 1 to 15, the cargo is or comprises an antisense synthetic oligonucleotide (ASO).

18. The composition of any one of claims 1 to 15, wherein the cargo is or comprises: a phosphorodiamidate morpholino oligomer (PMO), a peptide nucleic acid (PNA), a methylphosphonate oligomer, a short interfering ribonucleic neutral oligonucleotide (siRNN).

19. The composition of any one of claims 1 to 18, wherein said shuttle agent core motif is sufficient to increase cytosolic/nuclear intracellular transduction of said cargo and comprises: a discrete positively-charged hydrophilic face harboring a cluster of positively charged residues on one side of the helix defining a positively charged angle of 40° to 160°, 40° to 140°, 60° to 140°, or 60° to 120° in Schiffer-Edmundson helical wheel representation; and/or a discrete hydrophobic face harboring a cluster of hydrophobic amino acid residues on an opposing side of the helix defining a hydrophobic angle of 140° to 280°, 160° to 260°, or 180° to 240° in Schiffer-Edmundson helical wheel representation.

20. The composition of claim 19, wherein:

(a) at least 20%, 30%, 40%, or 50% of the residues in the hydrophobic cluster are hydrophobic residues selected from phenylalanine, isoleucine, tryptophan, leucine, valine, methionine, tyrosine, cysteine, glycine, and alanine; or selected from phenylalanine, isoleucine, tryptophan, and/or leucine;

(b) at least 20%, 30%, 40%, or 50% of the residues in the positively charged cluster are positively charged residues selected from lysine and arginine;

(c) the shuttle agent core motif has a hydrophobic moment (μH) of at least 3.0, 3.1, 3.2, 3.3, 3.4, 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, or 5.5;

(d) the shuttle agent core motif has a maximal length of 14, 15, 16, 17, 18, 19, or 20 residues; or

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

21. The composition of any one of claims 1 to 20, wherein the synthetic peptide shuttle agent is a peptide of between 15, 16, 17, 18, 19 or 20 to 150 amino acids in length, wherein any combination of at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or all of following parameters are respected:

- the peptide is soluble in aqueous solution and has a grand average of hydropathicity (GRAVY) index of less than -0.35, -0.40, -0.45, -0.50, -0.55, or -0.60);
- the hydrophobic face comprises a 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;
- the peptide has a hydrophobic moment (μH) of 3.5 to 11;
- the peptide has a predicted net charge of +3, +4, +5, +6, +7, +8, +9 to +10, +11, +12, +13, +14, or +15 at physiological pH;
- the peptide has an isoelectric point (pI) of 8 to 13 or 10 to 13;

- 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;
- the peptide is composed of 0% to 30% of any combination of the amino acids: N, Q, S, and T;
- the peptide is composed of 35% to 85% of any combination of the amino acids: A, L, K, or R;
- 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;
- the peptide is composed of 20% to 45% of any combination of the amino acids: K and R;
- the peptide is composed of 0% to 10% of any combination of the amino acids: D and E;
- 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
- 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, and preferably less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of D and/or E.

22. The composition of any one of claims 1 to 21, wherein said synthetic peptide shuttle agent comprises a flexible linker domain rich in serine and/or glycine residues.

23. The composition of any one of claims 1 to 22, wherein the shuttle agent comprises an endosome leakage domain (ELD).

24. The composition of any one of claims 1 to 23 for use in therapy, wherein the cargo is a therapeutic cargo and the composition is formulated to be administered *in vivo*.

25. The composition as defined in any one of claims 1 to 23 for use in therapy, wherein the cargo is a therapeutic cargo and the composition is formulated to be administered:

(a) intravenously or parenterally, or intranasally; or

(b) to target organs or tissues contacting or proximal to bodily fluids and/or secretions; or

(c) both (a) and (b).

26. The composition as defined in any one of claims 1 to 23 for use in therapy, wherein the cargo is a therapeutic cargo, or for the manufacture of a medicament for treating a disease or disorder ameliorated by cytosolic/nuclear and/or intracellular delivery of the cargo in a subject.

27. The composition as defined in any one of claims 1 to 23, wherein the composition is formulated to be intranasally administered, wherein the cargo is a therapeutic cargo for treating or preventing a lung or respiratory disease or disorder.

28. The composition of claim 27, further comprising a mucolytic agent, an anti-inflammatory agent, a bronchodilator, an antibiotic, or any combination thereof.

29. The composition of claim 27 or 28, formulated for inhalation via a nebulizer or an inhaler.